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A direct comparison of the diagnostic efficacy of alternative scaffold-based radiopharmaceuticals [^{99m}Tc]Tc-ADAPT6 and [^{99m}Tc]Tc-(HE)3-G3 in patients with HER2-positive breast cancer

Bragina O.D.[1, 2], Deyev S.M.[2, 3], Garbukov E.Yu.[1], Goldberg V.E.[1], Chernov V.I.[1, 2], Tolmachev V.M.[2, 4]

[1] *Cancer Research Institute, Tomsk National Research Medical Center (NRMC) of the Russian Academy of Sciences 5, Kooperativny Str., Tomsk, 634009, Russian Federation*

[2] *National Research Tomsk Polytechnic University 30, Lenina Av., Tomsk, 634050, Russian Federation*

[3] *Shemyakin – Ovchinnikov Institute of Bioorganic Chemistry of the Russian Academy of Sciences 16/10, Miklukho-Maklaya Str., Moscow, 117997, Russian Federation*

[4] *Uppsala University 7, Dag Hammarskjöldsväg, Segerstedthuset, Uppsala, Sweden*

ABSTRACT

Aim. To perform a direct comparison of the diagnostic efficacy of [^{99m}Tc]Tc-ADAPT6 and [^{99m}Tc]Tc-$(HE)_3$-G3 in HER2-positive breast cancer patients before the systemic treatment.

Materials and methods. The study included 11 patients with HER2-positive breast cancer (T1–4N0–2M0–1) before the initiation of systemic treatment. All patients underwent a radionuclide examination with [^{99m}Tc]Tc-ADAPT6 and [^{99m}Tc]Tc-$(HE)_3$-G3 with the interval of 3–4 days. Single-photon emission computed tomography (SPECT) /computed tomography (CT) was performed 2 and 4 hours after [^{99m}Tc]Tc-ADAPT6 and [^{99m}Tc]Tc-$(HE)_3$-G3 administration, respectively.

Results. The analysis of [^{99m}Tc]Tc-ADAPT6 and [^{99m}Tc]Tc-$(HE)_3$-G3 distribution showed their high uptake in the kidneys and liver. Breast tumors were visualized in all cases. The average tumor uptake of [99mTc]Tc-ADAPT6 was 4.7 ± 2.1, which was significantly higher than in the [99mTc]Tc-(HE)3-G3 injection (3.5 ± 1.7) ($p < 0.005$, paired *t*-test). The tumor-to-background ratio (15.2 ± 7.4 and 19.6 ± 12.4, respectively) had no statistical differences in both cases ($p > 0.05$, paired *t*-test). Liver metastases were visualized in patients 1 and 5 and corresponded to the projection of metastases according to contrast-enhanced abdominal CT. The accumulation of [^{99m}Tc]Tc-ADAPT6 and [^{99m}Tc]Tc-(HE)3-G3 in the projection of metastases in both cases was significantly higher compared to the primary tumor (1.3 and 1.7 times higher in patient 1; 2.2 and 3.5 times higher in patient 5, respectively).

Conclusion. Both [^{99m}Tc]Tc-ADAPT6 and [^{99m}Tc]Tc-(HE)3-G3 demonstrated the diagnostic efficacy in visualizing a primary HER2-positive tumor in breast cancer patients. However, [99mTc]Tc-ADEPT6 had higher accumulation values, which makes it a more promising diagnostic agent.

Keywords: breast cancer, SPECT / CT, ADAPT6, DARPinG3, HER2/neu

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

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Conformity with the principles of ethics. The study was approved by the Bioethics Committee at Cancer Research Institute of Tomsk NRMC (Protocol No. 6 of 04.03.2022). All patients signed an informed consent to participate in the study.

✉ *Bragina Olga D.,* bragina_od@mail.ru

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Прямое сравнение диагностической эффективности радиофармацевтических препаратов на основе альтернативных каркасных протеинов [^{99m}Tc]Tc-ADAPT6 и [^{99m}Tc]Tc-(HE)$_3$-G3 у больных HER2-позитивным раком молочной железы

Брагина О.Д.[1, 2], Деев С.М.[2, 3], Гарбуков Е.Ю.[1], Гольдберг В.Е.[1], Чернов В.И.[1, 2], Толмачев В.М.[2, 4]

[1] Научно-исследовательский институт (НИИ) онкологии, Томский национальный исследовательский медицинский центр (НИМЦ) Российской академии наук
Россия, 634009, г. Томск, пер. Кооперативный, 5

[2] Научно-исследовательский центр (НИЦ) «Онкотераностика», Национальный исследовательский Томский политехнический университет (НИ ТПУ)
Россия, 634050, г. Томск, пр. Ленина, 30

[3] Институт биоорганической химии (ИБХ) им. академиков М.М. Шемякина и Ю.А. Овчинникова Российской академии наук (РАН)
Россия, 117997, Москва, ГСП-7, ул. Миклухо-Маклая, 16/10

[4] Уппсальский университет
Швеция, Уппсала, Segerstedthuset, DagHammarskjöldsväg, 7

РЕЗЮМЕ

Цель: проведение прямого сравнения диагностической эффективности препаратов [^{99m}Tc]Tc-ADAPT6 и [^{99m}Tc]Tc-(HE)$_3$-G3 у больных HER2-позитивным раком молочной железы до начала системного лечения.

Материалы и методы. В исследование включены 11 больных HER2-позитивным раком молочной железы (T1–4N0–2M0–1) до начала системного лечения. Всем больным в интервале 3–4 дней выполнялись радионуклидные исследования с использованием препаратов [^{99m}Tc]Tc-ADAPT6 и [^{99m}Tc]Tc-(HE)$_3$-G3. Однофотонная эмиссионная компьютерная томография/компьютерная томография (КТ) проводилась через 2 ч для препарата [^{99m}Tc]Tc-ADAPT6 и через 4 ч для [^{99m}Tc]Tc-(HE)$_3$-G3.

Результаты. При анализе распределения обоих препаратов больший захват нормальными тканями был отмечен в почках и печени. Опухоли молочных желез визуализировались всех случаях. Средний захват опухолью при использовании препарата [^{99m}Tc]Tc-ADAPT6 составил 4,7 ± 2,1, что было значительно выше, чем при [^{99m}Tc]Tc-(HE)$_3$-G3 (3,5 ± 1,7) ($p < 0,005$, парный *t*-тест). Соотношение опухоль/фон (15,2 ± 7,4 и 19,6 ± 12,4 соответственно) в обоих случаях не имело статистических различий ($p > 0,05$, парный *t*-тест). Метастазы в печень визуализированы у пациенток № 1 и 5, что соответствовало проекции метастазов по данным КТ органов брюшной полости, выполненной с контрастированием. Аккумуляция [^{99m}Tc]Tc-ADAPT6 и [^{99m}Tc]Tc-(HE)$_3$-G3 в проекции метастазов у обеих больных была значительно выше по сравнению с первичной опухолью (в 1,3 и 1,7 раза у пациентки № 1; в 2,2 и 3,5 раза у пациентки № 5 соответственно).

Заключение. Препараты[^{99m}Tc]Tc-ADAPT6 и [^{99m}Tc]Tc-(HE)$_3$-G3 продемонстрировали свою эффективность в отношении визуализации первичныхHER2-позитивных опухолей молочных желез. При этом аккумуляция [^{99m}Tc]Tc-ADAPT6 имела более высокие показатели накопления, что делает это соединение более перспективным диагностическим агентом.

Ключевые слова: рак молочной железы, однофотонная эмиссионная компьютерная томография, компьютерная томография ОФЭКТ/КТ, DARPinG3, ADAPT6, HER2/neu

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 биоэтическим комитетом НИИ онкологии Томского НИМЦ (протокол № 6 от 04.03.2022).

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INTRODUCTION

Overexpression of the human epidermal growth factor receptor 2 (HER2 / neu) occurs in 20–25% of breast cancer patients and indicates a need for targeted therapy, which significantly improves overall and relapse-free survival rates [1, 2]. Despite the availability and prevalence of commonly used immunohistochemistry (IHC) and fluorescent *in situ* hybridization (FISH), there are still problems that significantly limit the assessment of the HER2 / neu status in patients with breast cancer. In particular, the impossibility of performing a simultaneous assessment of tumor spread and a molecular analysis of identified sites remains obvious and is due to difficulties or the impossibility of performing a core biopsy [3, 4].

One of the solutions to these problems is targeted radionuclide diagnosis [5], which is currently being actively studied and uses alternative scaffold proteins with optimal characteristics as a "targeting" module for delivering radioisotopes to tumor cells [6–8]. Thus, the results of phase I clinical trials of [^{99m}Tc]Tc-ADAPT6 (ClinicalTrials.gov Identifier: NCT03991260) and [^{99m}Tc]Tc-(HE)3-G3 (ClinicalTrials.gov ID: NCT04277338) in breast cancer patients demonstrated good tolerability and absence of adverse reactions both at the time of injection and for the entire follow-up periods. Also, in both cases, differences in the accumulation of radiopharmaceuticals in patients with HER2-positive and HER2-negative breast tumors were shown ($p < 0.05$, Mann – Whitney test), and optimal dosages and study periods after the injection of labeled proteins were revealed: 500 μ and 2 hours for [^{99m}Tc]Tc-ADAPT6 and 3,000 μ and 4 hours for [^{99m}Tc]Tc-(HE)3-G3 [9, 10].

The results of the preclinical comparative analysis of [^{99m}Tc]Tc-ADAPT6 and [^{99m}Tc]Tc-$(HE)_3$-G3 showed high uptake of both radiopharmaceuticals by the HER2-positive SKOV-3 cell line compared to the HER2-negative MDA-MB-468 cell line [11]. The aim of the study was to perform a direct comparison of the diagnostic efficacy of [^{99m}Tc]Tc-ADAPT6 and [^{99m}Tc]Tc-(HE)3-G3 in HER2-positive breast cancer patients before the initiation of systemic treatment.

MATERIALS AND METHODS

The study was approved by the Bioethics Committee at Cancer Research Institute of Tomsk NRMC (Protocol No. 6 of 04.03.2022) and registered before recruitment of patients (ClinicalTrials.gov Identifier: NCT05376644). The study included 11 breast cancer patients (T1–4N0–3M0–1) with overexpression of HER2 / neu in the primary tumor before the initiation of chemo / targeted therapy (Table 1). All patients signed an informed consent to participate in the study.

In all patients, the HER2/neu expression status was established according to the immunohistochemistry (IHC) of the biopsy material obtained from the primary tumor according to the American Society of Clinical Oncology (ASCO) / College of American Pathologists (CAP) guidelines (2018). Tumors were classified as HER2-positive if an IHC score was 3+. The study was carried out according to the standard methodology. Lesions of the lymph nodes in all patients were confirmed by the results of the histologic examination.

Before treatment, all patients underwent mammography (Giotto Image), osteoscintigraphy with ^{99m}Tc pyrophosphate (Siemens Symbia Intevo Bold), computed tomography (CT) of the chest (Siemens Somatom Emotions 16 ECO), and ultrasound

Table 1

Characteristics of breast cancer patients before the administration of [^{99m}Tc]Tc-ADAPT6 and [^{99m}Tc]Tc-(HE)3-G3

Patient number	Age, years	HER2 in primary breast tumor (IHC)	ER, PR, Ki67 expression in primary breast tumor	Clinical stage before tumor visualization
1	61	3+ (IHC)	ER +; PR +; Ki67 40%	IV (T4N3M1)
2	48	3+ (IHC)	ER +; PR +; Ki67 18%	IIB (T2N1M0)
3	26	3+ (IHC)	ER +; PR +; Ki67 45%	IIB (T2N1M0)
4	49	3+ (IHC)	ER +; PR +; Ki67 20%	I (T1N0M0)
5	41	3+ (IHC)	ER +; PR +; Ki67 45%	IV (T1N1M1)
6	65	3+ (IHC)	ER +; PR +; Ki67 60%	IIB (T2N1M0)
7	59	3+ (IHC)	ER –; PR –; Ki67 55%	IIA (T1N1M0)
8	55	3+ (IHC)	ER –; PR –; Ki67 18%	IIB (T2N1M0)
9	38	3+ (IHC)	ER +; PR + Ki67 25%	IIB (T2N1M0)
10	65	3+ (IHC)	ER –; PR –; Ki67 18%	I (T1N0M0)
11	63	3+ (IHC)	ER +; PR + Ki67 10%	I (T1N0M0)

Note: ER – estrogen receptor, PR – progesterone receptor, Ki67 – marker of cell proliferation.

examination of the mammary glands, regional lymph nodes, and liver (GE LOGIQ E9). Patients 1 and 5 additionally underwent contrast-enhanced abdominal CT. The size of the primary tumor and metastatic lymph nodes was determined according to the ultrasound findings.

Radionuclide studies. Radiopharmaceuticals [^{99m}Tc]Tc-ADAPT6 and [^{99m}Tc]Tc-(HE)3-G3 were prepared using the aseptic technique at the Department of Radionuclide Therapy and Diagnosis of Cancer Research Institute of Tomsk NRMC immediately before the study. Labeling was carried out according to the methods described earlier [12, 13]. The radiochemical yield was 97 ± 1% for [^{99m}Tc]Tc-ADAPT6 and 97 ± 2% for [^{99m}Tc]Tc-(HE)3-G3. The protein dose was 500 μ for [^{99m}Tc]Tc-ADAPT6 and 3,000 μ for [^{99m}Tc]Tc-(HE)3-G3. Consecutive intravenous injections of ready-made compounds [^{99m}Tc]Tc-ADAPT6 and [^{99m}Tc]Tc-(HE)3-G3 were performed in all patients with an interval of 3–4 days.

All patients underwent single-photon emission computed tomography (SPECT) / computed tomography (CT) of the chest using a Siemens Symbia Intevo Bold scanner equipped with a high-resolution low-energy collimator. SPECT / CT scans were performed 2 hours after the injection of [^{99m}Tc]Tc-ADAPT6 and 4 hours after the injection of [^{99m}Tc]Tc-(HE)3-G3. The images were reconstructed using the xSPECT Reconstruction Protocol (Siemens) based on the Ordered Subset Conjugate Gradient (OSCG) method. A 3D Gaussian filter with a 10-mm FWHM (soft tissue) was used. The images were processed using the Syngo.via software package (Siemens).

Maximum standardized uptake values (SUVmax) normalized for the body surface were calculated for the primary tumors and the liver. SUVmax were also determined in the contralateral symmetrical areas of the breast to calculate the tumor-to-background ratio, as well as in areas of the liver free from metastases to calculate the liver / metastasis ratio. To assess the uptake of the radiopharmaceuticals in healthy organs, which may contribute to the occurrence of background in typical metastatic sites, SUVmax were also measured in unaffected lymph nodes, lungs (S3 of the right lung in the projection of the aortic arch), and bones (five thoracic vertebrae).

The data were presented as $M \pm m$, where M is the mean and m is a standard deviation. A paired t-test was used to compare the uptake values and derived parameters. The differences were statistically significant at $p < 0.05$.

RESULTS

According to the results of IHC, all 11 patients were diagnosed with HER2-positive breast cancer (3+). Liver metastases were detected in two patients (patients 1 and 5). When analyzing the distribution of both radiopharmaceuticals, their greater uptake by healthy tissues was noted in the kidneys and the liver, while a greater uptake in the liver was detected using [^{99m}Tc]Tc-(HE)3-G3 (the distribution is shown in Fig. 1). The uptake of [^{99m}Tc]Tc-ADAPT6 (SUVmax 0.3 ± 0.1) in the contralateral breast area was significantly higher than that of [^{99m}Tc]Tc-(HE)3-G3 (SUVmax 0.2 ± 0.1) ($p < 0.01$, paired t-test). At the same time, the uptake of both radiopharmaceuticals

did not differ in the unaffected lymph nodes ($p > 0.05$, paired *t*-test). The accumulation of the radiopharmaceuticals in unaltered lung tissue was 0.4 ± 0.2 for [^{99m}Tc]Tc-ADAPT6 and 0.4 ± 0.1 for [^{99m}Tc]Tc-(HE)3-G3; in bones – 0.6 ± 0.2 and 0.9 ± 0.5, which had no significant difference according to the statistical analysis ($p > 0.05$, paired *t*-test) (Fig. 2). Accumulation of the radiopharmaceutical was also visualized in the salivary and thyroid glands.

Breast tumors were visualized using both [^{99m}Tc]Tc-ADAPT6 and [^{99m}Tc]Tc-(HE)3-G3. The average tumor uptake of [^{99m}Tc]Tc-ADAPT6 was 4.7 ± 2.1, which was significantly higher than for [^{99m}Tc]Tc-(HE)3-G3 (3.5 ± 1.7) ($p < 0.005$, paired *t*-test). At the same time, the tumor-to-background ratio (15.2 ± 7.4 and 19.6 ± 12.4, respectively) when using both compounds showed no significant differences ($p > 0.05$, paired *t*-test) (Fig. 3).

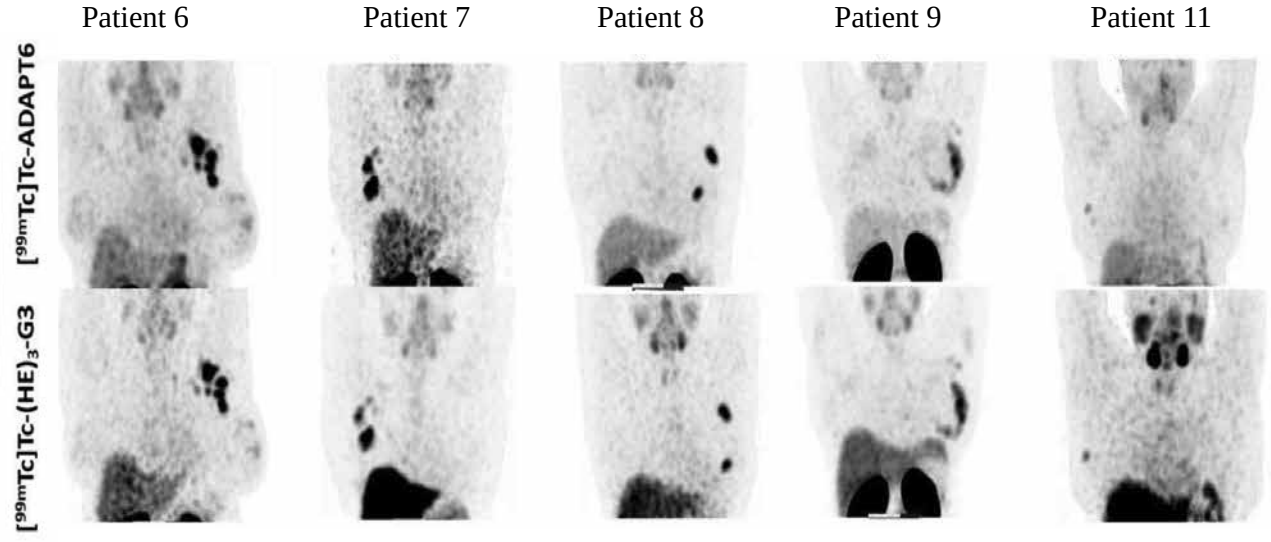

Fig. 1. Comparison of SPECT / CT using [^{99m}Tc]Tc-ADAPT6 and [^{99m}Tc]Tc-(HE)3-G3 in patients with HER2-positive breast cancer (SUV is 6.8 in all images)

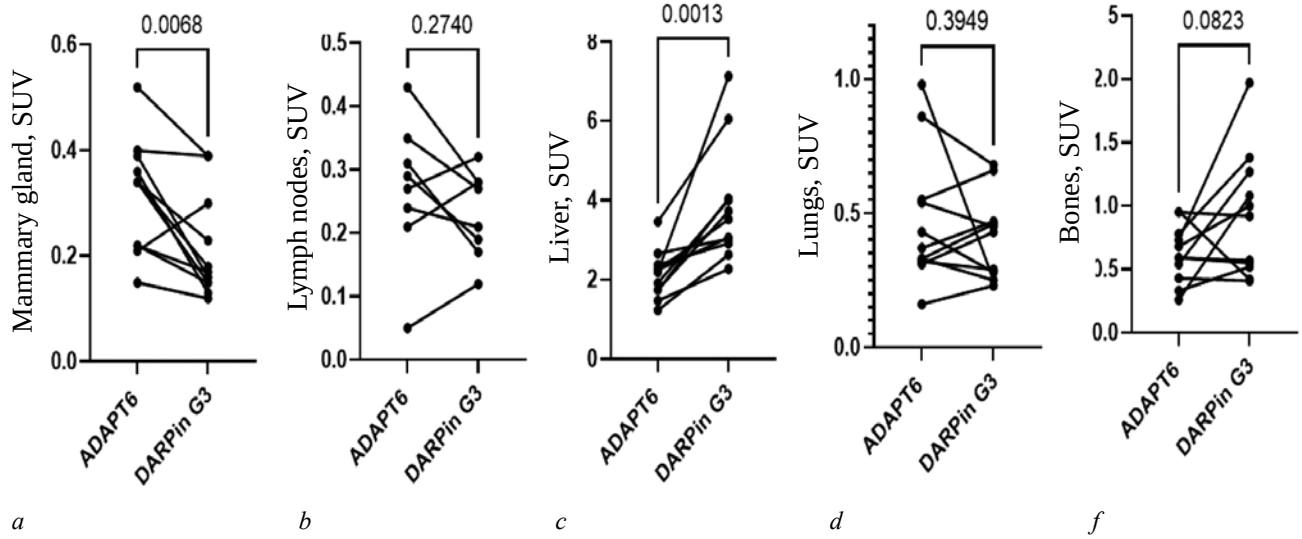

Fig. 2. Accumulation (SUV) of [^{99m}Tc]Tc-ADAPT6 and [^{99m}Tc]Tc-(HE)3-G3 in healthy tissues: *a* – mammary gland; *b* – unaffected lymph nodes; *c* – liver; *d* – lungs (S3 of the right lung in the projection of the aortic arch); *e* – bones (fifth thoracic vertebra)

Also, when using both [^{99m}Tc]Tc-ADAPT6 and [^{99m}Tc]Tc-(HE)3-G3, metastatic lymph nodes were detected in all patients (Fig. 4).

Liver metastases were visualized in patients 1 and 5 using both [^{99m}Tc]Tc-ADAPT6 and [^{99m}Tc]Tc-(HE)3-G3 and corresponded to the projection of metastases according to contrast-enhanced abdominal CT (Fig. 5). In both cases, morphological verification of the foci was not performed due to refusal of the patients to undergo a biopsy. Interestingly, the accumulation of [^{99m}Tc]Tc-ADAPT6 and [^{99m}Tc]Tc-(HE)3-G3 in the projection of metastases in both patients was significantly higher compared to the primary tumor (1.3 and 1.7 times in patient 1; 2.2 and 3.5 times in patient 5, respectively).

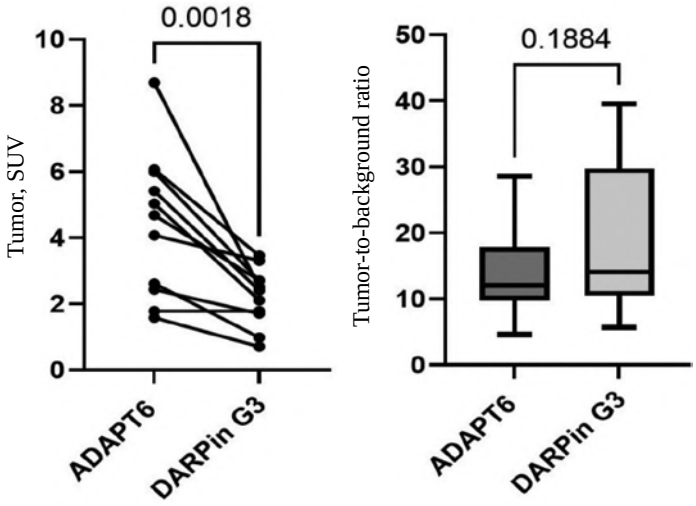

Fig. 3. Accumulation (SUV) of [^{99m}Tc]Tc-ADAPT6 and [^{99m}Tc]Tc-(HE)3-G3 in primary tumors and contralateral symmetrical areas of the breast in patients with HER2-positive breast cancer

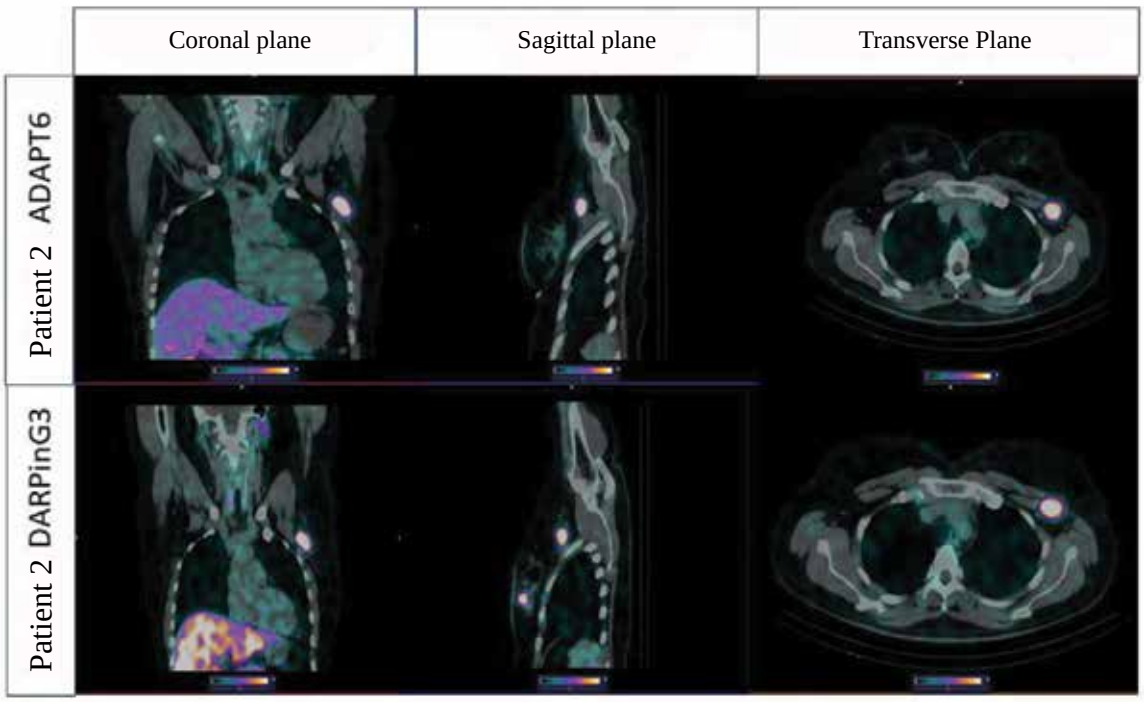

Fig. 4. Accumulation of [^{99m}Tc]Tc-ADAPT6 and [^{99m}Tc]Tc-(HE)3-G3 in metastatic lymph nodes in HER2-positive breast cancer patient. 2

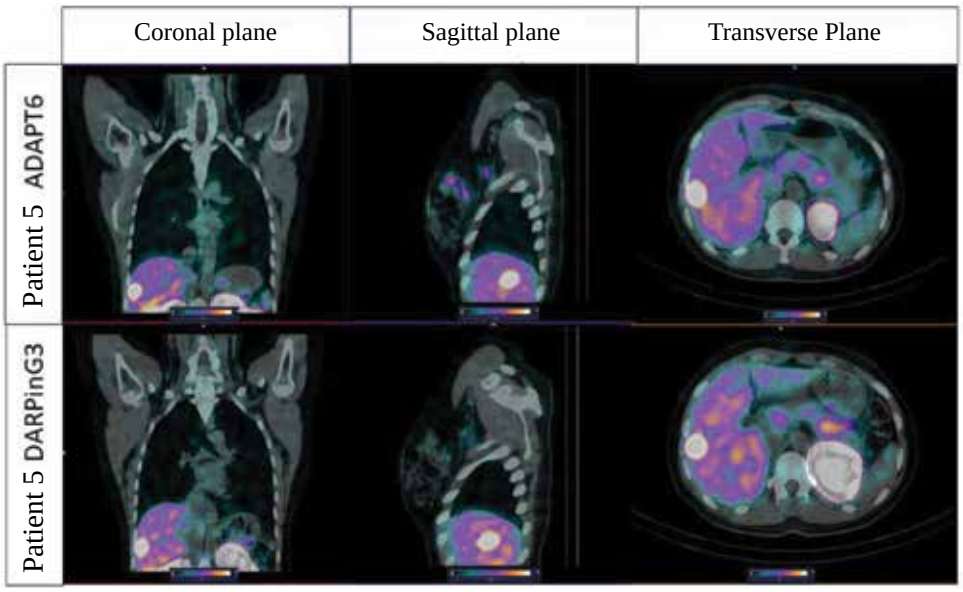

Fig. 5. Accumulation of [^{99m}Tc] Tc-ADAPT6 and [^{99m}Tc]Tc-(HE)3-G3 in primary tumors and liver metastases in patients with HER2-positive breast cancer

DISCUSSION

Performed phase I clinical trials of [^{99m}Tc] Tc-ADAPT6 and [^{99m}Tc]Tc-(HE)3-G3 clearly demonstrated the possibility of their use not only as primary tumor imaging agents, but also as markers that can effectively assess the HER2 / neu status in breast cancer patients [9, 10]. Taking into account the results of both analyses, a direct comparison of both [^{99m}Tc] Tc-ADAPT6 and [^{99m}Tc]Tc-(HE)3-G3 was naturally required by a complex of preclinical and clinical studies. Summarizing the data obtained, [^{99m}Tc]Tc-ADAPT6 showed its greatest efficacy in assessing the HER2 / neu status in breast cancer patients and, therefore, in selecting patients for targeted therapy. At the same time, [^{99m}Tc]Tc-(HE)3-G3 showed its sensitivity to a change (decrease) in the HER2 / neu expression in response to targeted therapy with trastuzumab, and, therefore, it can be used for monitoring an early response to systemic treatment [11].

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Authors' contribution

Bragina O.D., Chernov V.I., Deyev S.M., Tolmachev V.M., Garbukov E.Yu., Goldberg V.E. – conception and design, analysis and interpretation of the data, justification of the manuscript, critical revision of the manuscript for important intellectual content, final approval of the manuscript for publication.

Authors' information

Bragina Olga D. – Dr. Sci. (Med.), Oncologist, Principal Researcher, Department of Nuclear therapy and Diagnosis, Cancer Research Institute, Tomsk NRMC; Senior Researcher, Oncotheranostics Research Center, National Research Tomsk Polytechnic University, Tomsk, bragina_od@mail.ru, http://orcid.org/0000-0001-5281-7758

Deyev Sergei M. – Dr. Sci. (Biology), Professor, Academician of RAS, Head of Molecular Immunology Department, Shemyakin – Ovchinnikov Institute of Bioorganic Chemistry of the Russian Academy of Sciences, Moscow, deev_sm@tpu.ru, http://orcid.org/0000-0002-3952-0631

Garbukov Eugenii Yu. – Cand. Sci. (Med.), Senior Researcher, General Oncology Department, Cancer Research Institute, Tomsk NRMC, Tomsk, jrmaximum9@gmail.com, http://orcid.org/0000-0002-6016-7078

Goldberg Victor E. – Dr. Sci. (Med.), Professor, Head of the Chemotherapy Department, Cancer Research Institute, Tomsk NRMC, Tomsk, goldbergve@mail.ru, http://orcid.org/0000-0003-4753-5283

Chernov Vladimir I. – Dr. Sci. (Med.), Professor, Corresponding Member of RAS, Head of the Department of Nuclear Therapy and Diagnosis, Cancer Research Institute, Tomsk NRMC, Tomsk, chernov@tnimc.ru, http://orcid.org/0000-0002-5524-9546

Tolmachev Vladimir.M. – Dr. Sci. (Med.), Professor., Head of the Laboratory for Immunology, Genetics, and Pathology, Uppsala University, Uppsala, Sweden; Head of the Oncotheranostics Research Center, National Research Tomsk Polytechnic University, Tomsk, Vladimir.tolmachev@igp.uu.se, http://orcid.org/0000-0002-6122-1734

(✉) **Bragina Olga D.,** bragina_od@mail.ru

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Clinical and prognostic value of leptin resistance in the hospital period of myocardial infarction

Gorbatovskaya E.E.[1], Gruzdeva O.V.[1, 2], Dyleva Ya.A.[1], Belik E.V.[1], Uchasova E.G.[1], Tarasov R.S.[1], Kashtalap V.V.[1, 2]

[1] *Research Institute for Complex Issues of Cardiovascular Diseases*
6, Sosnoviy Blvd, Kemerovo, 650002, Russian Federation

[2] *Kemerovo State Medical University*
22a, Voroshilova Str., Kemerovo, 650056, Russian Federation

ABSTRACT

Aim. To evaluate the prevalence of leptin resistance (LR) and its clinical and prognostic value in association with metabolic disorders and features of the proinflammatory state in the hospital period of myocardial infarction.

Materials and methods. The study included 114 men diagnosed with ST segment elevation myocardial infarction (MI). On day 1 and 12 of MI, the levels of leptin and leptin receptor were measured in patients, and the free leptin index (FLI) was calculated. Leptin resistance (LR) was recorded at leptin > 6.45 ng / ml and FLI > 25. A comparative analysis of clinical and anamnestic characteristics, biochemical parameters, and cardiovascular prognosis was carried out between patients with and without LR. Statistical data processing was carried out using the Statistica 10.0 software package and SPSS 17.0 for Windows**.**

Results. The prevalence of LR in the hospital period of MI was 64%. LR was associated with cardiovascular pathology in the family history, arterial hypertension, dyslipidemia, and obesity. The presence of LR was accompanied by a significant increase in the level of glucose, free fatty acids (FFA), and interleukin (IL)-6 on day 1 of MI and by a significant rise in insulin, C-peptide, tumor necrosis factor (TNF)-alpha, and plasminogen activator inhibitor-1 (PAI-1) throughout the hospital stay. Patients with LR were characterized by multi-vessel and more severe lesions of the coronary bed and were more often subject to early post-infarction angina, recurrent MI, rhythm and conduction disturbances during hospital stay for MI.

Conclusion. Patients with MI are characterized by high prevalence of LR during the hospital stay. LR is associated with cardiovascular risk factors, metabolic disorders, formation of insulin resistance, and increased proinflammatory and prothrombogenic factors. The identified features in the presence of LR probably contribute to the development of adverse cardiovascular events in the hospital period of MI.

Keywords: leptin, leptin resistance, hospital period, myocardial infarction

Conflict of interests. 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 the Research Institute for Complex Issues of Cardiovascular Diseases.

✉ *Gorbatovskaya Evgeniya E.,* eugenia.tarasowa@yandex.ru

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Клинико-прогностическая значимость лептинорезистентности в госпитальном периоде инфаркта миокарда

Горбатовская Е.Е.[1], Груздева О.В.[1, 2], Дылева Ю.А.[1], Белик Е.В.[1], Учасова Е.Г.[1], Тарасов Р.С.[1], Кашталап В.В.[1, 2]

[1] Научно-исследовательский институт комплексных проблем сердечно-сосудистых заболеваний (НИИ КПССЗ)
Россия, 650002, г. Кемерово, Сосновый бульвар, 6

[2] Кемеровский государственный медицинский университет (КемГМУ)
Россия, 650056, г. Кемерово, ул. Ворошилова, 22а

РЕЗЮМЕ

Цель: оценить распространенность лептинорезистентности (ЛР) и ее клинико-прогностическую значимость во взаимосвязи с метаболическими нарушениями и особенностями провоспалительного статуса в госпитальном периоде инфаркта миокарда (ИМ).

Материалы и методы. В исследование включены 114 мужчин с установленным диагнозом ИМ с подъемом сегмента ST. Пациентам на 1-е и 12-е сут ИМ измеряли концентрацию лептина, рецептора лептина, рассчитывали индекс свободного лептина (ИСЛ). Лептинорезистентность фиксировали при уровне лептина более 6,45 нг/мл и ИСЛ более 25. Проведен сравнительный анализ клинико-анамнестических характеристик, биохимических показателей и кардиоваскулярного прогноза между пациентами с наличием ЛР и без ЛР. Статистическую обработку данных проводили с использованием программного пакета Statistica 10.0 и SPSS 17.0 for Windows.

Результаты. Распространенность ЛР в госпитальном периоде ИМ составила 64%. Лептинорезистентность ассоциирована с факторами риска сердечно-сосудистых заболевания (ССЗ) – наследственная отягощенность по сердечно-сосудистой патологии, артериальная гипертензия, дислипидемия, ожирение. У пациентов с ЛР наблюдались равные доли поражения передней и задней стенки левого желудочка. Наличие ЛР сопровождалось статистически значимым увеличением содержанием глюкозы, свободных жирных кислот и интерлейкина-6 в 1-е сут ИМ, инсулина, С-пептида, фактора некроза опухоли альфа и ингибитора активатора плазминогена 1-го типа на протяжении всего госпитального периода. Пациенты с ЛР характеризовались многососудистым и более тяжелым поражением коронарного русла, были чаще подвержены ранней постинфарктной стенокардии, рецидиву ИМ, нарушениям ритма и проводимости в госпитальном периоде ИМ.

Заключение. Для пациентов с ИМ характерна высокая распространенность ЛР в госпитальном периоде. Лептинорезистентность ассоциирована с факторами риска ССЗ, нарушениями метаболизма, формированием инсулинорезистентности, усилением провоспалительных и протромбогенных факторов. Выявленные особенности при наличии ЛР, вероятно, могут способствовать развитию неблагоприятных кардиоваскулярных событий в госпитальном периоде ИМ.

Ключевые слова: лептин, рецептор лептина, лептинорезистентность, госпитальный период, инфаркт миокарда

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

Источник финансирования. Исследование выполнено в рамках фундаментальной темы НИИ КПССЗ № 0419-2022-0002 «Разработка инновационных моделей управления риском развития болезней системы кровообращения с учетом коморбидности на основе изучения фундаментальных, клинических, эпидемиологических механизмов и организационных технологий медицинской помощи в условиях промышленного региона Сибири».

Соответствие принципам этики. Все пациенты подписали информированное согласие на участие в исследовании. Исследование одобрено локальным этическим комитетом НИИ КПССЗ.

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INTRODUCTION

Leptin has historically been one of the most important adipokines, playing a key role in the regulation of energy metabolism. In addition to the main effect, leptin exerts many pleiotropic effects, including those on the cardiovascular system (CVS). The role of leptin in cardiovascular disease (CVD) has been widely discussed over the years. Elevated leptin levels have potentially atherogenic, thrombotic, and angiogenic effects on the CVS. High concentrations of leptin stimulate proliferation and hypertrophy of smooth muscle cells in the vessel wall and accumulation of cholesterol esters in foam cells. They also enhance the proinflammatory activity of interleukin (IL)-6 and activate platelet aggregation [1]. Leptin-induced aldosterone secretion contributes to hypertension and endothelial dysfunction [2]. However, most of the evidence is obtained from cellular and animal models, so the role of leptin in human CVS and the fact whether leptin directly affects cardiac function or acts through a leptin-regulated neurohumoral pathway remain unclear.

Relatively recently, the phenomenon of leptin resistance (LR) has been discussed in modern literature. LR is a condition that develops as a result of a defect in intracellular signaling at the level of the leptin receptor or in the context of a decrease in leptin transport across the blood – brain barrier. As a result, leptin is not able to exert physiological effects, despite its elevated level [3]. In CVS, LR has an adverse effect on the cardiovascular response to stress and promotes cardiac remodeling [4]. To date, a lack of precise criteria for assessing the presence of LR makes studying this phenomenon difficult. Therefore, data on the contribution of LR to the development and prognosis of CVD, and, in particular, myocardial infarction (MI), are scarce; moreover, they are extremely contradictory.

The aim of the study was to evaluate the prevalence of LR and its clinical and prognostic value in association with metabolic disorders and features of the proinflammatory state at the in-hospital phase of MI.

MATERIALS AND METHODS

The study was conducted in accordance with the principles set forth in the Declaration of Helsinki and approved by the local Ethics Committee at the Research Institute for Complex Problems of Cardiovascular Diseases. The study included 114 men diagnosed with ST segment elevation myocardial infarction (STEMI). The average age of the patients was 60.0 [56.0; 70.0] years. The exclusion criteria were age over 75 years, as well as the presence of concomitant clinical conditions such as anemia, type 1 and type 2 diabetes, cancers, autoimmune diseases, and kidney and liver failure. All patients signed an informed consent to participate in the study.

In the examined individuals, the prevailing anamnestic risk factors for MI were arterial hypertension (AH), smoking, angina pectoris in the medical history, as well as cardiovascular pathology in the family history. More than 60% of patients were overweight and with varying degrees of obesity. The features of the developed MI included the predominance of Q wave MI and equal damage to the anterior and posterior walls of the left ventricle (LV). Preserved left ventricular ejection fraction (EF) was registered in about 65% of patients with MI (Table 1).

Table 1

Clinical and anamnestic characteristics of the examined patients, *n* = 114

Parameter	Absolute value	Relative value, %
Age, years, *Me* [Q_1; Q_3]	60.0 [56.0; 70.0]	–
Medical history		
Cardiovascular pathology in the family history	52	45.6
Smoking	58	50.8
Arterial hypertension	102	89.9
Dyslipidemia	20	17,5
Clinical manifestations of angina pectoris before MI	54	47.4
Previous myocardial infarction	31	27.2
Chronic heart failure	9	7.9
Body mass index (BMI)		
– under 25 kg / m^2; – 25.0–29.9 kg / m^2; – 30.0–39.9 kg / m^2	38 54 22	33.3 47.4 19.3
Coronary artery disease		
One-vessel coronary artery disease	64	56.1
Double-vessel coronary artery disease	35	30.7
Multivessel coronary artery disease	15	13.2

Table 1 (continued)

Parameter	Absolute value	Relative value, %
Myocardial infarction (MI)		
Q wave MI	96	84.2
Non-Q wave MI	18	15.8
Localization:		
– Anterior wall of the LV	50	43.9
– Posterior wall of the LV	55	48.2
– Posterior wall of the LV and right ventricle (RV)	9	7.9
Left ventricular ejection fraction (LVEF)		
≤ 50%	74	64.9%
40–49%	34	29.8%
> 40%	6	5.3%

On days 1 and 12 of MI, the levels of leptin and leptin receptor were determined in all patients by the enzyme immunoassay using standard commercial test systems (BioVendor, Czech Republic; eBioscience, Austria). The free leptin index (FLI) was calculated as the ratio of total leptin concentration (ng / ml) to the soluble leptin receptor concentration (ng / ml) multiplied by 100. LR was recorded at leptin levels > 6.45 ng / ml and FLI > 25 [5].

A comparative analysis of metabolic, proinflammatory, and adipokine profile parameters between patients with and without LR was carried out. The assessment of the glucose level, lipid profile (total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDLC), low-density lipoprotein cholesterol (LDLC)), and C-reactive protein (CRP) in the blood serum was carried out using standard commercial test systems from Thermo Fisher Scientific on an automated biochemistry analyzer Konelab 30i (Finland). The content of C-peptide, insulin, IL-6, plasminogen activator inhibitor-1 (PAI-1), tumor necrosis factor-alpha (TNF-α), and C-reactive protein (CRP) in the blood serum was determined using the enzyme-linked immunosorbent assay using standard commercial tests systems (BioVendor, Czech Republic; eBioscience, Austria; Cloud-Clone Corp. USA) according to the manufacturer's protocol. To assess insulin resistance (IR), the QUICKI index (Quantitative Insulin Sensitivity Check Index) was calculated: QUICKI = 1/[log(I0) + log(G0)], where I0 is basal plasma glucose (mmol / l), and G0 is basal insulin (μIU / ml). The severity of IR was assessed according to A. Katz et al. [6]. The mean QUICKI value of 0.382 ± 0.007 indicated normal tissue sensitivity to insulin; QUICKI values equal to 0.331 ± 0.010 and 0.304 ± 0.007 indicated moderate and severe tissue IR, respectively.

Coronary angiography was performed by the Judkins technique on the Innova angiographic system (USA). Xenetix-350 was used as a radiopaque agent. The SYNTAX Score was used to assess the severity of coronary lesions. An unfavorable outcome during an early in-hospital phase was registered with the development of early post-infarction angina, recurrent acute myocardial infarction (AMI), and life-threatening cardiac arrhythmias and conduction disorders.

As reperfusion therapy, all patients underwent primary percutaneous coronary intervention with stenting of the infarct-related artery. Continuous 24-hour infusion of nitroglycerin and heparin at standard doses with control of hemodynamic parameters and activated partial thromboplastin clotting time (aPTT) was performed. During the in-hospital phase of MI, we used β-blockers (in 100% of patients), angiotensin-converting enzyme inhibitors (89.4%), calcium channel blockers (88.5%), diuretics (31.7%), nitrates (17.3%), aspirin (98%), heparin (100%), clopidogrel (100%), and statins (100%).

Statistical data processing was carried out using Statistica 10.0 and SPSS 17.0 for Windows. The Kolmogorov – Smirnov test was used to check normality of data distribution. The data were presented as the median and the interquartile range *Me* [Q_1; Q_3]. The Mann – Whitney test was used to compare two independent samples. The differences were considered statistically significant at $p < 0.05$. The frequency analysis was carried out using 2x2 contingency tables. The logistic regression analysis with the calculation of odds ratio (OR) and 95% confidence interval (CI) was performed. In all statistical procedures, the differences were considered statistically significant at $p < 0.05$.

RESULTS

In patients with MI, during the entire observation period, an increased level of leptin was registered relative to the reference interval of 2.0–5.6 ng / ml. Thus, on days 1 and 12 of MI, the leptin concentration in patients with MI was 11.6 [6.6; 20.5] ng / ml and 11.5 [5.4; 13.9] ng / ml, respectively. The content of the leptin receptor did not go beyond the established reference interval and amounted to 40.8 [28.8; 46.1] ng / ml on day 1 of MI and 34.8 [27.1; 46.6] ng / ml on day 12. FLI was 32.7 [14.3; 70.5] on day 1 and 31.9 [16.2; 64.5] on day 12. At the time of patient division into groups with and without MI, the prevalence of LR was 64%.

LR in patients with myocardial infarction was associated with the presence of CVD risk factors: cardiovascular pathology in the family history, AH, and dyslipidemia. Patients in both groups were overweight, however, patients with LR were characterized by different degrees of obesity, in contrast to patients without LR. In patients with LR, equal damage to the anterior and posterior wall of the LV was observed, in patients without LR, equal damage to the posterior wall of the LV was noted. Q wave MI was significantly more common in the group of patients with LR. Patients with LR were characterized by an intermediate and critical decrease in LVEF (Table 2).

A comparative analysis of the metabolic profile revealed a significant increase in the level of glucose (p = 0.02), insulin (p = 0.02), and C-peptide (p = 0.03) on day 1 of MI, a rise in insulin (p = 0.01) and C-peptide (p = 0.03) on day 12 of the disease, and a decrease in the QUICKI index (p = 0.03) throughout the entire hospital stay in patients with LR compared to patients without LR (Table 3).

Table 2

Clinical and anamnestic characteristics of patients with and without leptin resistance, abs. (%)

Parameter	Patients with LR, n = 73	Patients without LR, n = 41	p
Medical history			
Cardiovascular pathology in the family history	39 (53.4%)	13 (31.7%)	0.02
Smoking	32 (43.8%)	18 (39%)	0.52
Arterial hypertension	72 (98.6%)	30 (73.2%)	0.01
Dyslipidemia	16 (21.9%)	4 (9.8%)	0.001
Clinical manifestations of angina pectoris before MI	35 (47.9%)	19 (46.3%)	0.61
Previous myocardial infarction	20 (27.4%)	11 (26.8)	0.82
Chronic heart failure	6 (8.2%)	3 (7.3%)	0.63
Body mass index (BMI)			
– under 25 kg / m^2; – 25.0–29.9 kg / m^2; – 30.0–39.9 kg / m^2	15 (20.6%) 29 (39.7%) 29 (39.7%)	21 (51.2%) 20 (49.8%) 0 (0%)	0.01 0.08 0.001
Myocardial infarction (MI)			
Q wave MI Non-Q wave MI	64 (87.7%) 9 (12.3%)	32 (78.1%) 9 (21.9%)	0.04 0.02
Localization of MI: – Anterior wall of the LV; – Posterior wall of the LV; – Posterior wall of the LV and RV	 34 (46.6%) 30 (41.1%) 9 (12.3%)	 16 (39%) 25 (61%) 0 (0%)	 0.03 0.01 0.001
Left ventricular ejection fraction (LVEF)			
≥ 50% 40–49% < 40%	40 (54.8%) 28 (38.4%) 5 (6.8%)	34 (83.0%) 6 (14.6%) 1 (2.4%)	0.001 0.02 0.01

Table 3

Comparative characteristics of carbohydrate and lipid metabolism parameters and proinflammatory and prothrombotic state in patients with and without leptin resistance, *Me* [Q_1; Q_3]

Parameter	Patients with LR		Patients without LR	
	Day 1	Day 12	Day 1	Day 12
Glucose, mmol / l	6.7 [5.6; 8.6]	6.1 [5.4; 7.2]	5.9 [5.4; 6.9]	5.9 [5.2; 7.2]
Insulin, μIU / ml	10.9* [5.8; 18.7]	12.2* [4.7; 19.5]	7.5 [2.8; 12.8]	5.3 [3.2; 10.2]
C-peptide, ng / ml	1.23* [0.62; 2.1]	1.2 * [0.58; 1.89]	0.99 [0.56; 1.47]	0.99 [0.56; 1.47]
QUICKI index	0.34* [0.31; 0.39]	0.31 * [0.29; 0.39]	0.38 [0.33; 0.45]	0.39 [0.35; 0.50]
FFA, mmol / l	1.64* [1.21; 1.94]	0.64 [0.5; 1.1]	1.1 [0.8; 1.26]	0.5 [0.43; 0.79]
CRP, mg / ml	25.0 [8.3; 52.4]	8.3 [5.0; 15.0]	20.0 [9.6; 29.0]	7.0 [3.0; 25.0]
IL-6, pg / ml	17.5* [13.3; 25.8]	9.6 [3.3; 11.0]	12.7 [10.9; 20.0]	10.4 [4.8; 16.3]
TNF-α, pg / ml	20.6* [1.4; 23.3]	19.8* [1.9; 24.9]	1.92 [0.7; 11.5]	2.36 [0.84; 12.4]
PAI-1, ng/ml	127.8* [39.9; 153.6]	88.65* [32.4; 148.0]	89.1 [72.0; 140.8]	60.14 [30.43;72.62]

* statistically significant differences between groups of patients with and without LR, $p < 0.05$.

In the group of patients with LR, 45 people (61.8%) had moderate and severe IR, in the group of patients without LR, this phenomenon was observed in 12 patients (29.2%). The correlation analysis revealed a significant direct correlation between the level of insulin on day 12 of MI and FLI (r = 0.509, p = 0.02), as well as an inverse correlation between the QUICKI index on day 12 and FLI (r = –0.367, p = 0.01).

Among the lipid metabolism parameters in the in-hospital phase of MI, only the level of free fatty acids (FFA) on day 1 of the disease was higher in the group of patients with LR than in the group without LR (p = 0.03).

In patients with MI, the level of CRP was higher than normal values throughout the observation period, however, there were no significant differences between the study groups. In patients with LR on day 1 of the disease, an increase in the concentration of IL-6 (by 1.4 times) and TNFα (by 10.7 times) was observed; on day 12, a rise in the level of TNFα (by 8.3 times) was noted compared to patients without LR. The values of PAI-1 in the group of patients with LR were significantly higher throughout the entire in-hospital phase of MI compared to the group without LR (Table 3).

Patients with LR were characterized by both moderate and severe lesions of the coronary bed. In patients without LR, only a minor lesion of the coronary artery was found (Table 4).

LR in patients with MI was more often associated with multivessel coronary artery disease (Figure).

Table 4

Comparative characteristics of patients with and without LR, depending on the severity of coronary lesions according to the SYNTAX score, abs. (%)

Severity of coronary lesion	Patients with LR	Patients without LR
Minor lesion (≤ 22 points)	55 (75.4%)	41 (100%)
Moderate lesion (23–32 points)	9 (12.3%)	0 (0%)
Severe lesion (> 32 points)	9 (12.3%)	0 (0%)

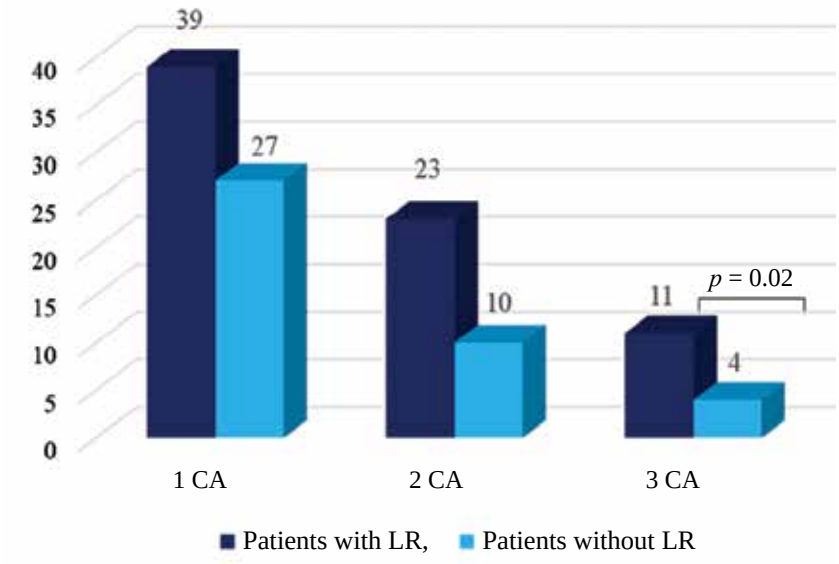

Figure. Characteristics of coronary bed lesions

The logistic regression analysis revealed the most significant parameters for verification of LR in patients with MI during the in-hospital phase of the disease. The closest relationship with LR was revealed for BMI, the number of affected arteries, and LVEF.

Among the carbohydrate and lipid metabolism parameters, a rise in the content of glucose in the blood serum on day 1 of the disease by 1 mmol / l increased the chances of detecting LR by 2.2 times, FFA – by 6.3 times. Among the markers of

proinflammatory and prothrombotic state, the closest relationship with LR was determined for IL-6 on day 1 of MI (Table 5).

Table 5

Markers of the proinflammatory and prothrombotic state in MI patients with LR

Parameter	OR	95% CI	*p*
BMI, kg / m²	2.34	1.07–5.20	0.03
Number of affected arteries	6.21	1.24–31.24	0.03
LVEF, %	0.53	0.23–0.84	0.01
Glucose on day 1, mmol / l	2.21	1.07–4.57	0.03
FFA on day 1, mmol / l	6.35	1.332–30.22	0.02
IL-6 on day 1, pg / ml	1.19	1.09–1.20	0.03

When analyzing adverse outcomes in the in-hospital phase of MI, it was found that patients with LR are more susceptible to cardiovascular events. Early post-infarction angina and recurrent AMI developed in 10.9% (8 / 73) and 6.8% (5 / 73) of patients, in patients with LR; in patients without LR, these complications were not registered. Conduction disorders were significantly (p = 0.03) more often recorded in patients with LR. This complication occurred in 34.2% (25 / 73) of patients with LR and in 12.2% (5 / 41) of patients without LR. On the whole, the incidence of adverse events in the in-hospital phase of MI in patients with LR was greater than in patients without LR. Thus, in the group of patients with LR, it was 44.4%, while in patients without LR, it was 12.2% (p = 0.01).

According to the logistic regression analysis, the prognostic value in relation to the risk of adverse cardiovascular events in the in-hospital phase of MI was determined for FLI both on days 1 and 12 of the disease, for BMI and the levels of FFA and IL-6 – on day 1 of MI, and for TNFα – on day 12 of MI (Table 6).

Table 6

The risk of developing adverse cardiovascular events in patients in the in-hospital phase of MI

Parameter	OR	95% CI	*p*
BMI, kg / m²	1.66	1.03–2.69	0.03
FLI on day 1	1.17	1.03–1.26	0.03
FLI on day 12	1.18	1.01–1.37	0.04
FFA on day 1, mmol / l	2.91	1.18–4.74	0.02
IL-6 on day 1, pg / ml	1.15	1.01–1.32	0.04
TNF-α on day 12, pg / ml	1.42	1.0–2.02	0.04

DISCUSSION

Currently, data on studying the incidence of LR in MI are extremely scarce in the scientific literature. However, the relevance of this issue is undeniable, since the assessment of LR in CVD will reduce the risk of developing cardiovascular complications in the future. In addition, the issue of choosing criteria for the LR assessment remains unresolved. To date, there is no generally accepted method to establish the presence of LR, as there is no single rule for its selection. One of the approaches to the diagnosis of LR is the presence of elevated leptin levels.

Many authors believe that hyperleptinemia is evidence of insensitivity to leptin and serves as an indirect sign of LR [7]. It is assumed that a high level of leptin is due to impaired interaction between leptin and its receptors, as a result of which LR develops. However, elevated levels of leptin can only characterize the concentration of the hormone which production increases without reflecting changes that led to hyperleptinemia [4]. The second approach is to evaluate the FLI. This method makes it possible to assess the relationship between leptin and the receptor and reflects the functional activity of leptin [8]. Both approaches were used in the present study. Thus, according to our data, the incidence of LR in the in-hospital phase of MI was 64%.

High prevalence of LR may be due to the inclusion of more than 60% of overweight and obese patients in the study. LR is characterized by impaired sensitivity of leptin receptors in the hypothalamus and peripheral tissues, which leads to a decrease in the feeling of satiety, excessive intake of nutrients, and an increase in body fat, expressed in higher BMI [9].

In the present study, we analyzed the relationship between LR and metabolic, proinflammatory, and adipokine profiles in patients with MI. When studying the carbohydrate metabolism parameters, hyperglycemia was detected on day 1 of the disease in patients of both groups. The pathogenetic mechanisms underlying hyperglycemia in the acute phase of MI have not been fully elucidated. Blood glucose levels can be transiently elevated either as a stress response to an acute process or as a result of inflammatory and adrenergic adaptation to ischemic injury leading to release of catecholamines, steroids, and induction of glycogenolysis [10]. However, patients with LR had a significantly higher glucose level on day 1 after MI compared to patients without LR. One explanation to this phenomenon in LR may be the inability of leptin to exert a physiological hypoglycemic effect. Leptin lowers blood glucose levels both through the central nervous system (by binding to its receptor on GABAergic neurons,

in particular pro-opiomelanocortin (POMC) neurons and agouti-related proteins (AgRP) in the hypothalamus) and through a direct effect on peripheral tissues – the pancreas, muscles, and the liver [11]. As a result, glucagon synthesis is suppressed, glucose uptake is increased, and glucose production by the liver is inhibited. However, in LR, the transmission of intracellular signals is impaired either at the level of the leptin receptor or against the background of a decrease in leptin transport through the blood – brain barrier [12]. Consequently, sensitivity of tissues to leptin is reduced despite its abundant amount, leading to an increased content of glucose in the blood plasma.

According to the scientific literature, leptin is involved in the regulation of sensitivity to insulin, whereas the presence of LR can serve as one of the triggers for the development of IR. More than 60% of patients with LR and about 30% of patients without LR had moderate and severe IR according to the QUICKI index. Apparently, high prevalence of IR in the LR group is associated with a significantly higher insulin level in these patients. Under physiological conditions, leptin inhibits biosynthesis and secretion of insulin by pancreatic β-cells. When sensitivity to leptin is impaired, its physiological effect disappears, leading to increased insulin synthesis despite hyperleptinemia. In addition, it has been experimentally shown that insulin stimulates the production and secretion of leptin by fat cells, thereby maintaining a high level of leptin [13] and forming a "vicious circle". The above assumption is confirmed by the results of the correlation analysis – the FLI had a direct relationship with insulin concentrations and an inverse relationship with the values of the QUICKI index on day 12 after the MI onset.

Decreased sensitivity to insulin observed in MI also affected FFA metabolism [14]. According to our data, an increased level of FFA was observed on day 1 after MI in both groups, while LR was associated with a more pronounced increase in the content of FFA in the blood. At early stages of IR formation, the amount of FFA increases due to a loss of the inhibitory effect of insulin on lipolysis in adipocytes. The incidence of IR in the group of patients with LR was 2.1 times higher than in the group of patients without LR, which apparently explains the significant increase in the concentration of FFA in this group of patients. The association of IR and FFA is confirmed by the data of the correlation analysis. An inverse relationship was found between the QUICKI index and the content of FFA on day 1 after MI (r = –0.424, p = 0.02). On day 12 after MI, the content of FFA decreased in both studied groups, which is probably due to increased utilization of FFA by the myocardium, which are necessary for synthesis of ATP, the main substrate for energy production for cardiomyocytes.

Patients of both groups were characterized by an increase in markers of proinflammatory and prothrombotic state throughout the entire observation period. Acute myocardial ischemia causes cellular damage and death of various components of the myocardium – cardiomyocytes, endothelial cells, fibroblasts, and interstitium. This, in turn, initiates an acute inflammatory response and leads to release of various proinflammatory mediators that induce recruitment of inflammatory cells to the area of MI and enhance the inflammatory response after MI [15]. High content of PAI-1 in the in-hospital phase of MI can be maintained by TNFα, which is one of the most powerful activators of PAI-1 synthesis [16]. However, patients with LR were characterized by a significant increase in the concentration of IL-6, TNFα, and PAI-1 in the blood serum compared to patients without LR.

One possible explanation to this phenomenon may be a high concentration of leptin observed in LR. Hyperleptinemia functionally activates circulating monocytes and dendritic cells and stimulates their proliferation. As a result, increased production of IL-6 and TNF-α is induced [17]. High concentrations of leptin also activate B lymphocytes via the JAK2 / STAT3 signaling pathways, causing the secretion of IL-6 and TNFα. Binding of leptin to the leptin receptor on B lymphocytes results in the formation of a receptor complex that allows JAK2 to be activated by phosphorylation. Activated JAK2 phosphorylates several tyrosine residues and provides a docking site for STAT3. STAT3 translocates to the nucleus and modulates the transcription of genes, including proinflammatory cytokine genes [18]. In addition, a high level of leptin probably enhances the expression of PAI-1 in vascular endothelial cells through the activation of ERK1/2, resulting in its increased secretion [19].

The identified changes in body reactivity in LR, being potentially atherogenic and thrombotic, probably contribute to the development and progression of atherosclerotic lesions of the coronary arteries [20]. The results obtained confirm this assumption. Thus, patients with LR had a higher degree of coronary damage according to the SYNTAX Score. Multivessel

coronary artery disease was also more common in the group of patients with LR compared to patients without LR.

Extensive and profound myocardial injury, characterized by predominant damage to the anterior wall of the LV and Q-wave MI, was most common in the group of patients with LR. Hyperleptinemia disrupts NO-dependent vasorelaxation induced by acetylcholine, reducing blood flow to tissues and contributing to the aggravation of ischemic heart damage. Elevated leptin levels may act synergistically with other factors such as inflammation. In particular, the response of the hypothalamic – pituitary – adrenal axis to inflammation causes activation of the sympathetic nervous system, leading to coronary vasoconstriction, affecting both macro- and microcirculation and contributing to more profound myocardial damage. In addition, it was found that IR increases sensitivity of the myocardium to ischemia, thereby leading to a decrease in left ventricular contractility. Thus, according to our data, patients with LR were significantly more likely to have reduced LVEF compared to patients without LR.

When carrying out the logistic regression analysis, we found a possibility of verifying LR in patients during the in-hospital phase of MI using BMI, the number of affected arteries, LVEF, as well as the concentration of glucose, FFA, and IL-6 on day 1 after MI. Patients with LR were more likely to experience adverse cardiovascular events compared to patients without LR. Patients with LR in the early in-hospital phase of MI were characterized by the development of early post-infarction angina and recurrent AMI. These complications did not occur in the group of patients without LR.

One of the reasons for the development of early post-infarction angina and recurrent AMI is a stenosing lesion of other branches of the coronary arteries. The development of early post-infarction angina and recurrent AMI in patients with LR may be explained both by elevated leptin levels throughout the in-hospital phase of MI and by the revealed changes in metabolism that accompany LR. Leptin has a direct effect on platelet aggregation, since the leptin receptor is also present on platelets. Thus, in LR characterized by elevated leptin levels, ADP-induced platelet aggregation increases [21]. According to our study, LR increases the content of prothrombotic and antifibrinolytic protein – PAI-1.

PAI-1 has antiprotease activity and is the main physiological inhibitor of tissue and urokinase-type plasminogen activators. Elevated levels of PAI-1 lead to inhibition of intravascular fibrinolysis, which may potentiate atherothrombosis. LR is also accompanied by a pronounced increase in proinflammatory cytokines with proatherogenic effects. The proatherogenic effect of TNFα on the endothelium is due to its role in the production of reactive oxygen species, a decrease in the bioavailability of NO, and an increase in the permeability of the endothelium for components and cells of the circulating blood. As a result, the above effects observed in LR can provoke the development of arterial thrombosis and, as a result, early post-infection angina and recurrent AMI.

Rhythm and conduction disorders were typical of patients with LR. For patients with STEMI, the most common complication of arrhythmia is atrial fibrillation (AF). Currently, there are studies proving the role of LR in the development of A.F. Anaszewicz et al. conducted a comparative study of 80 patients with AF and 169 patients without AF and confirmed that patients with AF have higher levels of leptin. In addition, a 1 pg / ml rise in the blood leptin concentration increased the risk of AF by an average of 2%. [22]. The observed changes in the body reactivity in LR might lead to more pronounced ischemia and myocardial necrosis, thereby contributing to the morphological and electrophysiological changes necessary for the development of AF.

According to the results of our study, an increase in the FLI, one of the criteria for LR, may increase the odds for developing cardiovascular complications in the in-hospital phase of MI. In addition, according to the data of the logistic regression analysis, the prognosis can be affected by BMI, FFA, and IL-6 on day 1 of MI and by TNF-α on day 12 of MI, an increase in which is observed in patients with LR.

CONCLUSION

Patients with MI are characterized by high prevalence of LR in the in-hospital phase of MI. LR is associated with CVD risk factors, metabolic disorders, IR formation, and increased markers of the proinflammatory and prothrombotic state. The identified features in the presence of LR may contribute to the development of adverse cardiovascular events in the in-hospital phase of MI.

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Authors' contribution

Gorbatovskaya E.E. – analysis of the data, drafting of the article. Gruzdeva O.V. – conception and design, drafting of the article. Dyleva Ya.A., Belik E.V., Uchasova E.G. – collection and processing of the material. Tarasov R.S. – collection and processing of the material, analysis of the data. Kashtalap V.V. – critical revision of the manuscript for important intellectual content.

Authors' information

Gorbatovskaya Evgeniya E. – Post-Graduate Student, Research Laboratory Assistant, Laboratory for Homeostasis Research, Research Institute of Complex Problems of Cardiovascular Diseases, Kemerovo, eugenia.tarasowa@yandex.ru, https://orcid.org/0000-0002-0500-2449

Gruzdeva Olga V. – Dr. Sci. (Med.), Professor of the Russian Academy of Sciences, Head of the Laboratory for Homeostasis Research, Research Institute of Complex Problems of Cardiovascular Diseases, Kemerovo, o_gruzdeva@mail.ru, https://orcid.org/0000-0002-7780-829X

Dyleva Yulia A. – Cand. Sci. (Med.), Senior Researcher, Laboratory for Homeostasis Research, Research Institute of Complex Problems of Cardiovascular Diseases, Kemerovo, dyleva87@yandex.ru, https://orcid.org/0000-0002-6890-3287

Belik Ekaterina V. – Cand. Sci. (Med.), Researcher, Laboratory for Homeostasis Research, Research Institute of Complex Problems of Cardiovascular Diseases, Kemerovo, sionina.ev@mail.ru, https://orcid.org/0000-0003-3996-3325

Uchasova Evgeniya G. – Cand. Sci. (Med.), Senior Researcher, Laboratory for Homeostasis Research, Research Institute of Complex Problems of Cardiovascular Diseases, Kemerovo, evg.uchasova@yandex.ru, https://orcid.org/0000-0003-4321-8977

Tarasov Roman S. – Dr. Sci. (Med.), Associate Professor, Head of the Laboratory for Endovascular and Reconstructive Cardiovascular Surgery, Research Institute for Complex Issues of Cardiovascular Diseases, Kemerovo, roman.tarasov@mail.ru, https://orcid.org/0000-0003-3882-709X

Kashtalap Vasily V. – Dr. Sci. (Med.), Associate Professor, Head of the Department of Clinical Cardiology, Research Institute for Complex Issues of Cardiovascular Diseases, Kemerovo, v_kash@mail.ru, https://orcid.org/0000-0003-3729-616X

(✉) **Gorbatovskaya Evgeniya E.,** eugenia.tarasowa@yandex.ru

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Prognostic value of humoral markers in patients with anthracycline-related cardiac dysfunction

Grakova E.V.[1], Kopeva K.V.[1], Shilov S.N.[2], Berezikova E.N.[2], Bobyleva E.T.[2], Kalyuzhin V.V.[3], Teplyakov A.T.[1]

[1] Cardiology Research Institute, Tomsk National Research Medical Center (NRMC) of the Russian Academy of Sciences (RAS)
111a, Kievskaya Str., Tomsk, 634012, Russian Federation

[2] Novosibirsk State Medical University
52, Krasny Av., Novosibirsk, 630091, Russian Federation

[3] Siberian State Medical University
2, Moscow Trakt, Tomsk, 634050, Russian Federation

ABSTRACT

Aim. To carry out a 12-month study on the prognostic role of humoral markers responsible for the main mechanisms of initiation of cardiotoxic myocardial damage (endothelin-1, soluble Fas-L, N-terminal pro-brain natriuretic peptide (NT-proBNP), tumor necrosis factor-α, interleukin (IL)-1β, matrix metalloproteinase (MMP)-2 and MMP-9, soluble form of the ST2 protein (sST2), a tissue inhibitor of metalloproteinase-1, and tetranectin) in assessing the risk of progression of anthracycline-related left ventricular dysfunction.

Materials and methods. The study included a total of 114 women aged 48.0 (46.0; 52.0) years without concomitant cardiovascular diseases and risk factors who received chemotherapy with anthracyclines in the past. The levels of serum biomarkers were determined using the enzyme immunoassay. Transthoracic echocardiography was performed at baseline and at 12 months of follow-up.

Results. After 12 months of follow-up, all patients were retrospectively divided into 2 groups: group 1 (n = 54) included patients with an unfavorable course of anthracycline-related cardiac dysfunction (ARCD), group 2 (n = 60) encompassed patients with a favorable course of the disease. According to the ROC analysis, MMP-2 ≥ 338.8 pg / ml (sensitivity 57%, specificity 78%; AUC = 0.629; p = 0.025), MMP-9 ≥ 22.18 pg / ml (sensitivity 89%, specificity 87%; AUC = 0.886; p < 0.001), sST2 ≥ 32.4 ng / ml (sensitivity 64%, specificity 70.5%; AUC = 0.691; p = 0.002), and tetranectin ≤ 15.4 pg / ml (sensitivity 69%, specificity 72%; AUC = 0.764; p < 0.001) were identified as predictors of an adverse course of ARCD. When comparing ROC curves, it was found that the concentration of MMP-9 (p = 0.002) was the most significant predictor of the progression of ARCD.

Conclusion. MMP-2 and -9, soluble ST2, and tetranectin can be considered as non-invasive markers for assessing the risk of ARCD progression. At the same time, an increased level of MMP-9 is the most significant predictor of ARCD progression.

Keywords: left ventricular dysfunction, anthracyclines, humoral markers, prognosis

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

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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 the Cardiology Research Institute of Tomsk NRMC (Protocol No. 177 of 30.10.2018).

✉ *Kopeva Kristina V.,* Kristin-kop@inbox.ru

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Прогностическая роль гуморальных маркеров у больных с дисфункций левого желудочка, индуцированной приемом антрациклинов

Гракова Е.В.[1], Копьева К.В.[1], Шилов С.Н.[2], Бобылева Е.Т.[2], Березикова Е.Н.[2], Калюжин В.В.[3], Тепляков А.Т.[1]

[1] Научно-исследовательский институт (НИИ) кардиологии, Томский национальный исследовательский медицинский центр (НИМЦ) Российской академии наук
Россия, 634012, Томск, ул. Киевская, 111а

[2] Новосибирский государственный медицинский университет (НГМУ)
Россия, 630091, Новосибирск, Красный пр., 52

[3] Сибирский государственный медицинский университет (СибГМУ)
Россия, 634050, Томск, Московский тракт, 2

РЕЗЮМЕ

Цель. В ходе 12-месячного исследования изучить прогностическую роль гуморальных маркеров, ответственных за основные механизмы инициирования кардиотоксического повреждения миокарда (эндотелин-1, растворимый Fas-L, NT-proBNP, фактор некроза опухоли альфа, интерлейкин-1β, матриксные металлопротеиназы-2 (ММП-2) и -9 (ММП-9), растворимая форма белка ST2 (sST2), тканевой ингибитор металлопротеиназы-1 и тетранектин), в оценке риска прогрессирования дисфункции левого желудочка (ЛЖ), индуцированной приемом антрациклинов.

Материалы и методы. Обследованы 114 женщин в возрасте 48,0 (46,0; 52,0) лет без сопутствующих сердечно-сосудистых заболеваний и факторов риска, получавших в анамнезе химиотерапевтическое лечение антрациклинами. Уровни биомаркеров в сыворотке крови определяли с помощью иммуноферментного анализа. Трансторакальная эхокардиография была выполнена исходно и через 12 мес наблюдения.

Результаты. Через 12 мес все пациентки были ретроспективно разделены на две группы: 1-ю группу (n = 54) составили больные с неблагоприятным течением дисфункции ЛЖ, индуцированной приемом антрациклинами, 2-ю группу (n = 60) – с благоприятным. По данным ROC-анализа, концентрации ММП-2 ≥ 338,8 пг/мл (чувствительность 57%, специфичность 78%; AUC = 0,629; p = 0,025), ММП-9 ≥ ≥ 22,18 пг/мл (чувствительность 89%, специфичность 87%; AUC = 0,886; p < 0,001), растворимой формы белка ST2 ≥ 32,4 нг/мл (чувствительность 64%, специфичность 70,5%; AUC = 0,691; p = 0,002) и тетранектина ≤ 15,4 пг/мл (чувствительность 69%, специфичность 72%; AUC = 0,764; p < 0,001) были идентифицированы как предикторы неблагоприятного течения дисфункции ЛЖ. При сравнении ROC-кривых установлено, что концентрация ММП-9 (p = 0,002) была наиболее значимым предиктором.

Заключение. Матриксные ММП-2 и -9, растворимый ST2 и тетранектин могут быть рассмотрены как неинвазивные маркеры для оценки риска прогрессирования дисфункции ЛЖ, индуцированной приемом антрациклинов. При этом повышенный уровень матриксной ММП-9 является наиболее значимым предиктором прогрессирования дисфункции ЛЖ, индуцированной приемом антрациклинов.

Ключевые слова: дисфункция левого желудочка, антрациклины, гуморальные маркеры, прогноз

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 локальным этическим комитетом НИИ кардиологии Томского НИМЦ (протокол № 177 от 30.10.2018).

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INTRODUCTION

Currently, several anticancer drugs are most commonly used to treat neoplasms. These drugs are highly effective, but at the same time have a potentially high risk of developing cardiotoxicity: cyclophosphamide, doxorubicin, trastuzumab, fluorouracil, cisplatin, and immunosuppressive drugs (that block cytotoxic T-lymphocyte-associated protein 4, programmed cell death protein-1, and programmed cell death receptor ligand) [1]. At the same time, anthracyclines are some of the main components in treatment regimens for patients with breast cancer (BC), leukemia, lymphoma, and sarcoma, however, anthracycline-induced cardiac dysfunction remains a common clinical problem, jeopardizing the effectiveness of anticancer therapy [2].

Subclinical damage to myocardial cells exerted by anthracyclines can manifest itself by asymptomatic anthracycline-related cardiac dysfunction (ARCD), as well as by symptomatic heart failure [2]. It has been established that the risk of developing ARCD increases with an increase in the administered cumulative dose of anthracyclines, the age of patients, previous or concomitant radiation therapy, and the presence of cardiovascular pathology or risk factors [3, 4]. Cardiomyocytes are considered as the main cellular target of the toxic effect of anthracyclines on the heart due to the effect of doxorubicin on the mitochondrial redox cycle, which leads to their death and progression of cardiac dysfunction [5].

Recently, other cell types, such as cardiovascular progenitor cells, cardiac fibroblasts, and endothelial cells, have been identified as potential cellular targets, creating a more complex and intriguing scenario in the pathogenesis of ARCD [6]. It is discussed that the mechanisms of this pathology are implemented through mitochondrial dysfunction (mitochondrial NADH dehydrogenase), changes in iron homeostasis, generation of oxidative stress with the help of NRF2 and reactive oxygen species (ROS) by nitric oxide mediated by neuronal NO synthase, development of endothelial dysfunction, stimulation of apoptosis (induction of p53 protein), pyroptosis (activation of proinflammatory molecules), and various caspases, as well as through induction of the signaling pathway influencing interstitial and perivascular fibrosis with the participation of matrix metalloproteinases (MMPs) and transforming growth factor-β [1, 6–10]. However, the role of these factors in further progression of ARCD has not yet been determined.

The aim of this research was to carry out a comprehensive study on the prognostic role of humoral markers responsible for the main mechanisms of initiation of cardiotoxic myocardial damage (endothelin-1, soluble Fas-L, N-terminal pro-brain natriuretic peptide (NT-proBNP), tumor necrosis factor-α, interleukin (IL)-1β, MMP-2 and MMP-9, soluble form of the ST2 protein (sST2), a tissue inhibitor of metalloproteinase-1 (TIMP-1), and tetranectin) in assessing the risk of ARCD progression during a 12-month follow-up.

MATERIALS AND METHODS

The study was approved by the local Ethics Committee at Cardiology Research Institute of Tomsk NRMC (Protocol No. 177 of 30.10.2018). All patients signed an informed consent to participate in the study.

It was a prospective, observational, single center study. From December 2020 to September 2021,

114 women aged 48.0 (46.0; 52.0) years who met the inclusion / exclusion criteria were consecutively included in the study.

Inclusion criteria: 1) women with BC without cardiovascular disease in the medical history; 2) previous polychemotherapy to treat BC: a combination of doxorubicin and cyclophosphamide (AC regimen), or a combination of doxorubicin, cyclophosphamide, and docetaxel (TAS regimen); 3) cancer in remission; 4) newly diagnosed ARCD (symptomatic or asymptomatic); 5) a signed informed consent.

Exclusion criteria: 1) type 1 and type 2 diabetes; 2) coronary artery disease; 3) arterial hypertension; 4) valvular defects and cardiomyopathy of any etiology; 5) heart failure with an alternative cause of manifestation; 6) glomerular filtration rate (CKD-EPI) < 50 ml / min / m^2; 7) Child – Pugh class C liver failure; 8) hemoglobin level < 100 g / l; 9) chronic alcohol abuse or mental disorders; 10) previous pulmonary embolism with pulmonary hypertension (systolic pressure in the right ventricle ≥ 45 mm Hg); 11) severe form of bronchial asthma and chronic obstructive pulmonary disease; 12) pathology of the thyroid gland; 13) pathology of the reproductive system.

The cumulative dose of doxorubicin was 300–360 mg / m^2, and all patients underwent radiation therapy. All patients underwent a 6-minute walk test to assess the functional class (FC) of heart failure. ARCD was diagnosed in accordance with the European guidelines on cardio-oncology (2022) [11]. According to the criteria, 36 patients had symptomatic ARCD or FC I–III heart failure, and 78 patients had asymptomatic ARCD.

Echocardiography. The Philips Affiniti 70 enhanced imaging ultrasound machine was used to perform two-dimensional (2D, B-real time) transthoracic echocardiography. All studies were performed by one highly qualified specialist at baseline and at 12 months of follow-up. In the analysis of echocardiography parameters, linear dimensions of the heart were assessed (measurements at the basal, middle, and apical levels): thickness of the interventricular septum (IVS); posterior wall thickness (PWT) of the left ventricle (LV); end-systolic dimension (ESD), and end-diastolic dimension (EDD) of the LV. Left ventricular ejection fraction (LVEF) was calculated using the Simpson's rule.

Biochemistry test. Blood sampling was performed by venipuncture, and serum samples obtained after centrifugation were stored at –24 °C with one freeze – thaw cycle. Serum biomarker levels were determined using the enzyme immunoassay – NT-proBNP (Biomedica immunoassays, Austria), MMP-2 and MMP-9 (eBioscience, USA), TIMP-1 (Biomedica immunoassays, Austria), sST2 (Presage® ST2 assay, Critical Diagnostics, USA), tetranectin (eBioscience, USA), endothelin-1 (BG Medicine, Waltham, USA), soluble Fas-L (Human ELISA Kit, USA), IL-1β (Boster Biological Technology, USA), and tumor necrosis factor (TNF)α (TNFSF1A Immunoassay, Minneapolis, USA).

Adverse cardiovascular events and follow-up. Adverse cardiovascular events were hospitalizations due to progression of symptoms of heart failure, a decrease in the functional class of heart failure by 1 or more (according to NYHA), an asymptomatic decrease in LVEF by 10 or more absolute units (%), emergence of symptoms / signs of heart failure. After detection of LV dysfunction and inclusion in the study, all patients were prescribed optimal drug therapy. After adjustment of treatment, follow-up began. At 12 months of follow-up, following a patients' visit to the clinic, we collected and analyzed data on the presence of adverse events and the time of their onset, changes in drug therapy over this period, and the clinical status of patients. Echocardiography was performed to assess the asymptomatic decrease in LVEF.

Statistical analysis. Statistical processing of the study results was carried out using the STATISTICA 10.0 and MedCalc 11.5.0.0 software packages. The data were presented as the median and the interquartile range *Me* ($Q_{25;}$ Q_{75}). To test statistical hypotheses when comparing two independent samples, the Mann – Whitney *U*-test was used. The Wilcoxon signed rank test was used to compare two dependent samples. For qualitative variables, contingency tables were analyzed using the Pearson's $\chi 2$ test. To identify predictors of an unfavorable course of the disease, the ROC analysis was used with the construction of characteristic curves and the calculation of area under the curve (AUC). The univariate regression analysis with the calculation of odds ratio (OR) and 95% confidence interval (CI) was used to evaluate the effect of biomarker levels on the risk of developing adverse cardiovascular events. The critical significance level *p* for all statistical procedures was taken equal to 0.05.

RESULTS

Initially, we examined 114 women aged 48.0 (46.0; 52.0) years without concomitant cardiovascular diseases and risk factors who previously received chemotherapy with anthracyclines. After 12 months of follow-up, all patients were retrospectively divided into 2 groups: group 1 (n = 54) included patients with an unfavorable course of ARCD, group 2 (n = 51) encompassed individuals with a favorable course of the disease. Baseline clinical and demographic characteristics of patients did not differ between the groups, except for the levels of MMP-2, MMP-9, sST2, and tetranectin (Table 1). In patients in group 1, MMP-2 concentrations were higher by 8% (p = 0.017), MMP-9 – by 15.7% (p < 0.001), sST2 – by 26.9% (p < 0.001), while tetranectin was lower by 24.5% (p < 0.001).

Table 1

Baseline clinical and demographic characteristics, *Me* (Q_{25}; Q_{75})

Parameter	Group 1, n = 54	Group 2, n = 60	p
Age, years	48 (46; 50)	50 (48; 52)	0.918
CD of doxorubicin, mg / m^2	360 (300; 360)	360 (300; 360)	0.817
Body mass index, kg / m^2	24.7 (21.8; 25.8)	23.0 (21.1; 25.6)	0.781
Polychemotherapy regimen, n (%): – AC; – TAC	 29 (53.7) 25 (46.3)	 36 (60.0) 24 (40.0)	 0.747 0.516
Stage of breast cancer, n (%): – 2A–2B; – 3A–3B	 34 (62.9) 20 (37.1)	 39 (65.0) 21 (35.0)	 0.712 0.716
Heart rate, beats / min	75 (68; 83)	72 (69; 81)	0.615
Systolic blood pressure, mm Hg	115 (112; 124)	115 (110; 120)	0.981
Diastolic blood pressure, mm Hg	70 (68; 79)	72 (69; 80)	0.761
Smoking, n (%)	7 (12.9)	9 (15.0)	0.153
COPD, n (%)	4 (7.4)	5 (8.3)	0.614
Menopause, n (%)	40 (74.1)	43 (71.1)	0.515
GFR, ml / min / m^2	89 (78; 96)	88 (76; 98)	0.192
6MWT, m	554 (451; 574)	558 (461; 598)	0.291
Total cholesterol, mmol / l	5.2 (4.85; 5.7)	5.25 (4.8; 5.7)	0.616
Potassium, mmol / l	4.2 (3.9; 4.7)	4.3 (3.96; 4.56)	0.761
Hemoglobin, g / l	109.5 (100; 117)	108.5 (101; 117.5)	0.173
NT-proBNP, pg / ml	324.7 (263.05; 378.2)	316.6 (260.1; 377.7)	0.832
MMP-2, ng / ml	376.8 (329.5; 426.7)	348.1 (295.3; 381.7)	0.017
MMP-9, ng / ml	23.6 (21.4; 24.6)	19.9 (19.4; 20.7)	< 0.001
TIMP-1, ng / ml	1,191 (998.3; 1,651.1)	1,087 (912; 1,429.1)	0.412
Soluble ST2, ng / ml	41.2 (32.1; 47.6)	30.1 (27.3; 34.9)	< 0.001
Tetranectin, ng / ml	13.9 (12.7; 16.8)	18.4 (16.9; 20.7)	< 0.001
Endothelin-1, ng / ml	6.96 (5.34; 7.61)	6.32 (4.79; 7.03)	0.756
Soluble Fas-L, ng / ml	117.9 (103; 137.5)	109.1 (99.7; 128.3)	0.376
Interleukin-1β, ng / ml	5.4 (4.7; 6.3)	5.9 (4.9; 6.1)	0.541
Tumor necrosis factor-α, ng / ml	5.3 (4.9; 6.2)	5.6 (4.8; 6.7)	0.172

Note: AC-regimen – a combination of doxorubicin and cyclophosphamide; TAC-regimen – a combination of doxorubicin, cyclophosphamide, and docetaxel; CD – cumulative dose; MMP – matrix metalloproteinase; GFR – glomerular filtration rate (according to the CKD-EPI equation); TIMP-1 – tissue inhibitor of metalloproteinase-1; 6MWT – 6-minute walk test; COPD – chronic obstructive pulmonary disease.

Baseline echocardiography parameters were also comparable in both groups. However, after 12 months of follow-up, in group 1, LVEF significantly (p < 0.001) decreased by 8% from 50 (48; 51) to 46 (39; 49.5)%; ESD increased by 3.0% (p = 0.037), EDD rose by 4.0% (p = 0.001), the size of the left atrium increased by 3.1% (0.049), the 6-minute walk test distance decreased (p = 0.045) by 5.1%. In group 2, LVEF significantly (p = 0.005) increased by 7.4% from 50 (47; 53) to 54 (51; 55)%.

Table 2

Dynamics of echocardiography parameters and 6-minute walk test distance, *Me* (Q_{25}; Q_{75})

Parameter	Baseline		*p*	At 12 months		*p*
	Group 1, *n* = 54	Group 2, *n* = 60		Group 1, *n* = 54	Group 2, *n* = 60	
LVEF, %	50 (48; 51)	50 (47; 53)	0.699	46 (39; 49.5)	54 (51; 55)	<0.001
LA, mm	31 (30; 35)	30 (29; 33.3)	<0.001	32 (31; 37)	31 (29.5; 34)	<0.001
EDD, mm	50 (48; 51.0)	48 (45; 50.5)	0.079	52 (48; 54)	48 (45; 49)	<0.001
ESD, mm	36 (34; 38)	36 (33; 38.5)	0.889	37 (36; 39)	34 (32; 36)	<0.001
IVS, mm	10.5 (10; 11)	10.5 (10; 11)	0.783	11 (10; 11)	10.5 (10; 11)	0.041
PWS, mm	11 (10; 12)	11 (10; 12)	0.076	11 (10; 12)	11 (10; 12)	0.008
GLS, %	–16.1 (–14.8; –18.3)	–15.9 (–13.6; –17.8)	0.162	–14.5 (–13.1; –17.2)	–15.1 (–13.3; –17.9)	<0.001
6MWT, m	412 (364; 466)	429 (356; 470)	0.617	391 (332; 412)	476 (400; 517)	<0.001

Note: GLS - global longitudinal strain of the left ventricle; EDD – end-diastolic dimension; ESD – end-systolic dimension; LA – left atrium; IVS – interventricular septum; PWS – posterior wall thickness of the left ventricle; 6MWT – 6-minute walk test; LVEF – left ventricular ejection fraction.

Following the ROC analysis, the concentrations of MMP-2 ≥ 338.8 pg / ml (sensitivity 57%, specificity 78%; AUC = 0.629; $p = 0.025$), MMP-9 ≥ 22.18 pg / ml (sensitivity 89%, specificity 87%; AUC = 0.886; $p < 0.001$), sST2 ≥ 32.4 ng / ml (sensitivity 64%, specificity 70.5%; AUC = 0.691; $p = 0.002$), and tetranectin ≤ 15.4 ng / ml (sensitivity 69%, specificity 72%; AUC = 0.764; $p < 0.001$) were identified as predictors of an adverse course of ARCD during 12 months of follow-up (Fig. 1). When comparing the ROC curves, it was found that the level of MMP-9 ($p = 0.002$) was a more significant predictor (Fig. 2).

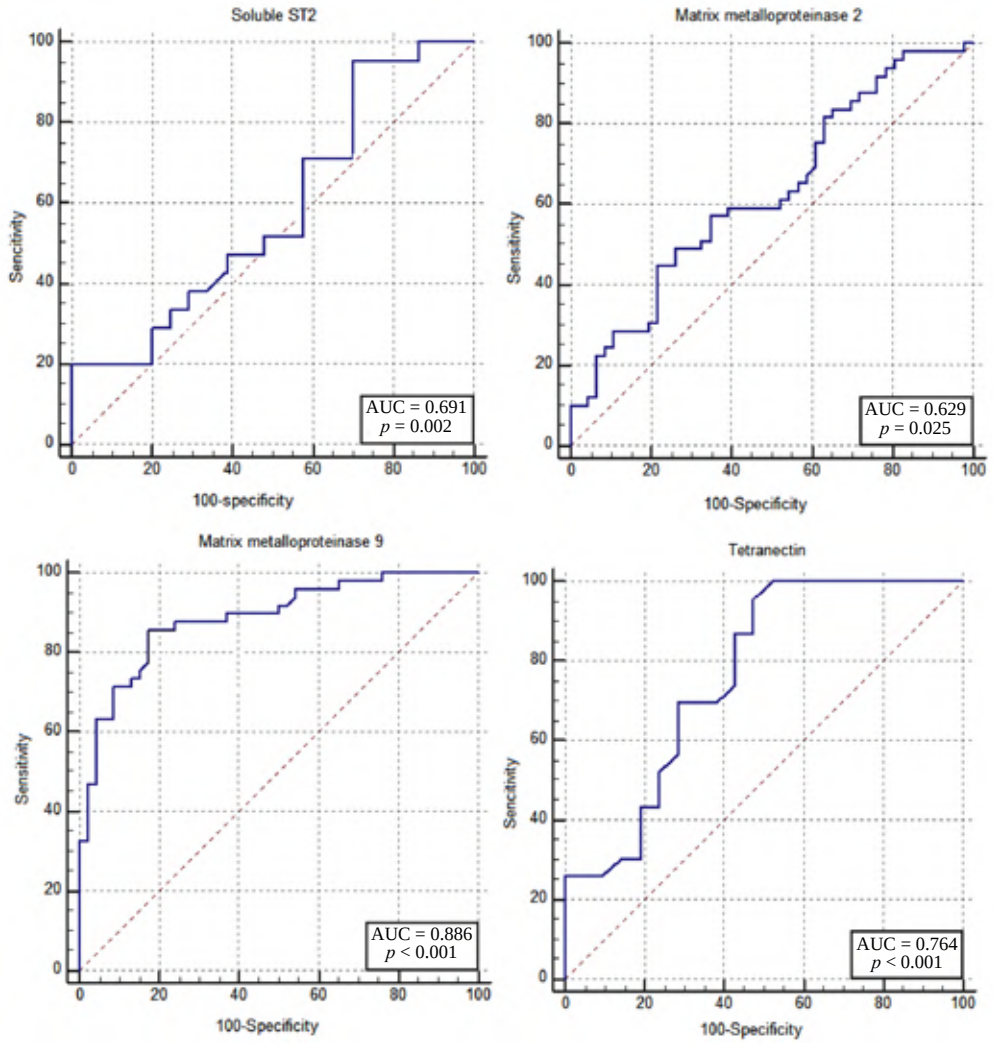

Fig. 1. Sensitivity and specificity of sST2, MMP-2, MMP-9, and tetranectin levels in the risk stratification for an adverse course of ARCD during 12-month follow-up

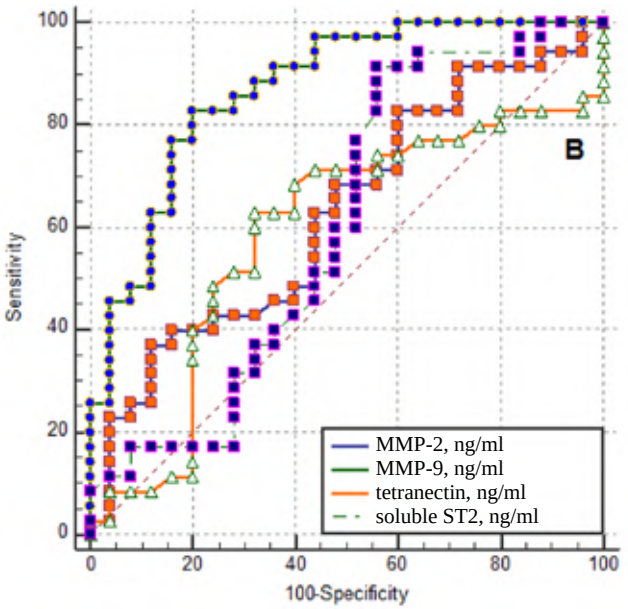

Fig. 2. Comparison of the ROC curves for concentrations of humoral markers in assessing the risk of ARCD progression during 12-month follow-up

Based on the univariate regression analysis, it was found that MMP-2 overexpression ≥ 338.8 ng / ml (OR 1.92; 95% CI 1.09–3.93; $p = 0.003$), MMP-9 ≥ 22.18 ng / ml (OR 4.76; 95% CI 2.98–14.54; $p < 0.0001$), sST2 ≥ 32.4 ng / ml (OR 2.01; 95% CI 1.54–4.18; $p = 0.012$), and a decrease in tetranectin expression ≤ 15.4 ng / ml (OR 2.98; 95% CI 1.23–4.97; $p = 0.001$) were associated with the progression of ARCD during the 12-month follow-up (Table 3).

Table 3

Results of the univariate regression analysis

Variable	OR	95% CI	*p*
MMP-2 (<338.8 / ≥ 338.8 ng / ml)	1.92	1.09–3.93	0.003
MMP-9 (<22.18/ ≥ 22.18 ng /ml)	4.76	2.98–14.54	<0.0001
sST2 (<32.4/ ≥ 32.4 ng /ml)	2.01	1.54–4.18	0.012
Tetranectin (>15.4/ ≤ 15.4 ng / ml)	2.98	1.23–4.97	0.001

DISCUSSION

Doxorubicin, first isolated in the early 1960s, remains one of the most effective anthracycline antibiotics with antitumor activity against BC [2]. However, its use has dose-dependent cardiovascular toxic effects, which lead to changes in cardiomyocytes, vessels, and endothelium, which can potentially lead to the development of severe and irreversible LV dysfunction [2–4, 11]. Some of the triggers for the formation of anthracycline-induced damage to the myocardium are generation of ROS, development of endothelial dysfunction, and inhibition of topoisomerase 2β in cardiomyocytes. Inhibition of topoisomerase 2β by doxorubicin in cardiomyocytes leads to damage to mitochondria and activation of the internal p53-mediated and external Fas-L pathways of apoptosis [12].

In contrast to the triggers of ARCD initiation, triggers of its further progression are myocardial fibrosis and tissue hypoxia, which is most likely provoked by endothelial dysfunction, development of perivascular fibrosis, and induction of apoptosis of cardiomyocytes [13]. It has been proven that myocytolysis, focal myocardial necrosis, focal myocardial fibrosis, and diffuse interstitial pulmonary fibrosis are significantly associated with the use of anthracyclines [14]. Myocardial fibrosis, previously considered as a non-specific sign, is now a major component of anthracycline-induced cardiac remodeling, even after low cumulative doses [15]. Ultimately, direct death of cardiomyocytes and subsequent fibrosis contribute to cardiac dysfunction and a decreased cellular response to hypoxia [16]. In our study, we found that biomarkers of fibrosis, such as sST2, MMP-2, MMP-9, and tetranectin, were involved in further progression of ARCD.

MMPs are present in a healthy heart in an inactive form. MMP activation in patients with heart failure, especially activation of gelatinases MMP-2 and MMP-9, is associated with adverse LV remodeling and dilatation [17]. MMP-2 and MMP-9 are secreted by cardiac fibroblasts, cardiomyocytes, and endothelial and immune cells [18], and their expression can be increased during oxidative stress, endothelial dysfunction, and inflammation [19–21]. Doxorubicin causes a significant increase in the generation of ROS and a rapid rise in the expression and activation of MMPs, which explains the presence and activity of MMP-2 and MMP-9 in ARCD [20]. MMP-2 and probably also MMP-9 are stimulated by oxidative stress at both transcriptional and post-translational levels.

First, oxidative stress enhances MMP-2 transcription, including *de novo* expression of intracellular N-terminal truncated MMP-2, through an alternative promoter in the first intron [22]. Second, intracellular MMP-2 is directly activated by peroxynitrite via S-glutathiolation, opening its catalytic site [23]. MMP-2 is best known not only for proteolyzing extracellular matrix proteins, but it is also active inside cardiomyocytes, where it cleaves sarcomeric proteins [23, 24].

Changes in the extracellular matrix and pronounced transcriptional activation of some specific MMPs in ARCD have been demonstrated in several animal models [14, 25]. In rats, the effects of doxorubicin were associated with stimulation of plasma MMP-2 and MMP-9 activity and tissue expression of MMP-2, which was associated with stimulation of AKT1 activation, superoxide dismutase inhibition, increased superoxide levels, induction of iNOS expression, and caspase-3 activation. [25]. In a model of non-ischemic anthracycline-induced chronic cardiomyopathy in rabbits, the immunohistochemical analysis revealed increased MMP-2 expression in both cardiomyocytes and fibroblasts [14]. An increase in MMP-2, MMP-7, and MMP-9 and a rise in the levels of TIMP-3 and TIMP-4 were noted in the group of children receiving high doses of anthracycline [26].

Our study demonstrated that patients with an unfavorable course of ARCD had higher MMP-2 and MMP-9 levels than patients with favorable outcomes. According to the ROC analysis, concentrations of MMP-2 $\geq$ 338.8 pg / ml (AUC = 0.629; p = 0.025) and MMP-9 $\geq$ 22.18 pg / ml (AUC = 0.886; $p < 0.001$) were identified as predictors of an unfavorable course of ARCD. At the same time, it was found that the concentrations of MMP-9 (p = 0.002) were more significant predictors. These data prove that MMPs are undoubtedly involved in the pathogenesis of ARCD.

Tetranectin, a potential new biomarker for heart failure, is expressed in the myocardium and is associated with cardiac fibrosis. It is suggested that tetranectin is involved in tissue remodeling due to its ability to stimulate plasminogen activation and expression in developing tissues, such as bones and muscles [27]. It was also found in endothelial and epithelial tissues, especially in cells with a high storage function, such as parietal cells and absorptive cells of surface epithelium in the small intestine, exocrine gland ducts, and pseudostratified columnar epithelium in the airways. Mesenchymal cells also exhibit a positive staining reaction for tetranectin, which is most prominent in mast cells, but is also present in some lymphocytes, plasma cells, macrophages, granulocytes, striated and smooth muscle cells, and fibroblasts [28, 29].

For many years, this biomarker has been evaluated in cancer patients and found to be present in the extracellular matrix in some human carcinomas (tumors of the breast, colon, and ovaries), whereas low plasma tetranectin levels have been associated with an increased risk of cancer progression and mortality [30]. In the case of ovarian cancer, a decrease in plasma tetranectin was a stronger predictor of a poor prognosis than a cancer stage [28]. It was shown that serum tetranectin concentrations decrease not only in cancer, but also in non-cardiovascular conditions (sepsis, inflammatory diseases) [31]. Recently, tetranectin has been found to be a potential new diagnostic biomarker for heart failure that accumulates in the myocardium and is associated with cardiac fibrosis. The results of the study showed significant expression of tetranectin in the human myocardium and its correlation with the degree of tissue fibrosis, possibly due to its role in extracellular matrix remodeling [32].

K. McDonald et al. were the first to put forward and prove the hypothesis that a decrease in the level of circulating tetranectin indicates its accumulation in the myocardium to combat myocardial interstitial fibrosis. Therefore, it is possible that a decrease in circulating tetranectin may predispose to the development of heart failure [32]. In another study, higher plasma tetranectin levels were inversely correlated with the risk of atherosclerosis [33]. Y. Chen et al. reported lower serum tetranectin levels in patients with coronary artery disease compared to healthy subjects and hypothesized that atherosclerosis-associated endothelial injury may lead to accumulation of tetranectin in the intima in complexes of atherosclerotic plaques with lipoprotein (a) and / or fibrin, thus reducing its serum levels [34]. We demonstrated that circulating tetranectin levels were reduced to a greater extent in patients with an unfavorable course of ARCD compared to patients with a favorable course of the disease ($p < 0.001$); and its decrease $\leq$ 15.4 ng / ml (AUC = 0.764; $p < 0.001$) was identified as a predictor of an adverse course of ARCD during the 12-month follow-up.

One of the main biomarkers that signals the presence and severity of adverse cardiac remodeling and tissue fibrosis that occur with myocardial infarction, acute coronary syndrome, or progression of chronic heart failure is sST2 [35]. In patients with ARCD, sST2 levels most likely reflect periarteriolar fibrosis, which may result from endothelial dysfunction. ROS generation, apoptosis, and endothelial dysfunction may contribute to periarteriolar fibrosis and microvascular rarefaction, leading to sST2 overexpression [36]. Following the ROC analysis, the concentration of sST2 $\geq$ 32.4 ng / ml (AUC = 0.691; p = 0.002) was also identified as a predictor of an unfavorable course of ARCD during 12 months of follow-up.

The best option for predicting the development of LV dysfunction from the point of view of a cardiologist is to develop multi-biomarker panels, which would be used in specially designed system algorithms [37].

CONCLUSION

Thus, it was found that overexpression of MMP-2, MMP-9, and sST2 and hypoexpression of tetranectin can be considered as non-invasive markers for assessing the risk of ARCD progression. The levels of MMP-9 are the most significant predictors of ARCD progression ($p = 0.002$).

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Authors' contribution

Grakova E.V. – conception and design of the study, coordination of the research, drafting of the article, final approval of the manuscript for publication. Kopeva K.V. – statistical processing of the data, critical revision of the manuscript for important intellectual content, final approval of the manuscript for publication. Shilov S.N. – compilation of the database, critical revision of the manuscript for important intellectual content, final approval of the manuscript for publication. Bobyleva E.T. – review of the literature, acquisition and interpretation of the clinical data, compilation of the database, final approval of the manuscript for publication. Berezikova E.N – acquisition and interpretation of the clinical data, compilation of the database, final approval of the manuscript for publication. Kalyuzhin V.V. – review of the literature, interpretation of the data, drafting of the article, final approval of the manuscript for publication. Teplyakov A.T. – coordination of the research, drafting of the article, final approval of the manuscript for publication

Authors' information

Grakova Elena V. – Dr. Sci. (Med.), Leading Researcher, Department of Myocardial Pathology, Cardiology Research Institute, Tomsk NRMC of RAS, Tomsk, gev@cardio-tomsk.ru, http://orcid.org/0000-0003-4019-3735

Kopeva Kristina V. – Cand. Sci. (Med.), Researcher, Department of Myocardial Pathology, Cardiology Research Institute, Tomsk NRMC of RAS, Tomsk, kristin-kop@inbox.ru, http://orcid.org/0000-0002-2285-6438

Shilov Sergey N. – Dr. Sci. (Med.), Associate Professor, Pathological Physiology and Clinical Pathophysiology Department, Novosibirsk State Medical University, Novosibirsk, newsib54@gmail.com, http://orcid.org/0000-0002-7777-6419

Bobyleva Elena T. – Teaching Assistant, Policlinic Therapy and General Medical Practice Department, Novosibirsk State Medical University, Novosibirsk, lalala777elena@yandex.ru, http://orcid.org/0000-0002-4223-3457

Berezikova Ekaterina N. – Dr. Sci. (Med.), Associate Professor, Policlinic Therapy and General Medical Practice Department, Novosibirsk State Medical University, Novosibirsk, berezikova@ngs.ru, http://orcid.org/0000-0002-9630-0213

Kalyuzhin Vadim V. – Dr. Sci. (Med.), Professor, Head of the Advanced Therapy Division with a Course in Rehabilitation, Physiotherapy, and Sports Medicine, Siberian State Medical University, Tomsk, kalyuzhinvv@mail.ru, http://orcid.org/0000-0001-9640-2028

Teplyakov Alexander T. – Dr. Sci. (Med.), Professor, Principal Researcher, Cardiology Research Institute, Tomsk NRMC of RAS, Tomsk, vgelen1970@gmail.com, http://orcid.org/0000-0003-0721-0038

(✉) **Kopeva Kristina V.,** Kristin-kop@inbox.ru

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Association of *MnSOD* and *GPX1* gene polymorphisms with a risk of chronic dust-induced bronchitis

Zhukova A.G.[1, 2], Kazitskaya A.S.[1, 2], Yadykina T.K.[1], Gulyaeva O.N.[1]

[1] *Research Institute for Complex Problems of Hygiene and Occupational Diseases*
23, Kutuzova Str., Novokuznetsk, 654041, Russian Federation

[2] *Kuzbass Humanitarian Pedagogical Institute, Kemerovo State University*
23, Tsiolkovskogo Str., Novokuznetsk, 654041, Russian Federation

ABSTRACT

Aim. To assess the association of the *MnSOD* (rs4880) and *GPX1* (rs1050450) gene polymorphisms with a risk of developing chronic dust-induced bronchitis in workers of the coal mining industry.

Materials and methods. The study included 182 coal miners with prolonged exposure to high concentrations of coal dust, including 116 people with a previously established diagnosis of chronic dust-induced bronchitis (CDB) and 66 people without pathology of the bronchopulmonary system, working under the same sanitary and hygienic conditions. Polymorphisms of the *MnSOD* (rs4880) and *GPX1* (rs1050450) genes were studied using polymerase chain reaction.

Results. For the first time, we established a statistically significant association between the polymorphisms of the *MnSOD* (rs4880) and *GPX1* genes (rs1050450) and CDB. Thus, the chance of detecting the homozygous A/A (Val/Val) *MnSOD* genotype in miners with CDB was 2 times higher than in the comparison group (χ^2 – 5.42; p = 0.02; odds ratio (OR) 2.21; 95% confidence interval (CI) 1.13–4.33), while the chance of detecting the homozygous G/G (Pro/Pro) *GPX1* genotype in miners with CDB was almost 6 times higher than in the comparison group (χ2 – 21.47; p = 0.001; OR 5.89; 95% CI 2.65–13.08). It was found that the combination of AA/GG genotypes of the *MnSOD/GPX*1 genes was significantly associated with a 1.5-fold risk of developing CDB (χ^2 – 11.49; p < 0.001; relative risk (RR) 1.59; 95% CI 1.36–1.84), while the chance of detecting this combination of genotypes in miners with bronchopulmonary pathology was 15 times higher than in the comparison group (OR 15.09; 95% CI 1.99–114.64).

Conclusion. Carriage of homozygous genotypes A/A at the rs4880 *MnSOD* locus and G/G at the rs1050450 *GPX1* locus was shown to be a marker of genetic predisposition to the development of CDB. The combination of homozygous genotypes of the studied AA/GG *MnSOD/GPX1* genes indicated a 1.5-fold risk of developing CDB. Carrying one of the three combinations of the *MnSOD* and *GPX1* genotypes (GG/AA, AA/AA, and AG/AA) indicated resistance to the development of CDB.

Keywords: gene polymorphism, *MnSOD, GPX1*, coal and rock dust, chronic dust-induced bronchitis

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 at the Research Institute for Complex Problems of Hygiene and Occupational Diseases (Protocol No. 3, § 1 of 04.28.2022).

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✉ *Zhukova Anna G.,* nyura_g@mail.ru

Ассоциация полиморфизмов генов *MnSOD* и *GPX1* с риском развития хронического пылевого бронхита

Жукова А.Г.[1, 2], Казицкая А.С.[1, 2], Ядыкина Т.К.[1], Гуляева О.Н.[1]

[1] *Научно-исследовательский институт комплексных проблем гигиены и профессиональных заболеваний (НИИ КПГПЗ)*
Россия, 654041, г. Новокузнецк, ул. Кутузова, 23

[2] *Кузбасский гуманитарно-педагогический институт (КГПИ), Кемеровский государственный университет (КемГУ)*
Россия, 654041, г. Новокузнецк, ул. Циолковского, 23

РЕЗЮМЕ

Цель. Оценить связь полиморфных локусов генов *MnSOD* (rs4880) и *GPX1* (rs1050450) с риском развития хронического пылевого бронхита у работников основных профессий угледобывающей отрасли.

Материалы и методы. В исследование включены 182 работника угольных шахт с длительным воздействием высоких концентраций угольно-породной пыли, среди которых 116 человек с ранее установленным диагнозом «хронический пылевой бронхит» (ХПБ), 66 – лица без патологии бронхолегочной системы, работающие в тех же санитарно-гигиенических условиях. Полиморфизмы генов *MnSOD* (rs4880) и *GPX1* (rs1050450) изучали с помощью полимеразной цепной реакции.

Результаты. Впервые для полиморфизмов генов ферментов антиоксидантной защиты – *MnSOD* (rs4880) и *GPX1* (rs1050450) установлена статистически значимая ассоциация с ХПБ. Так, шанс обнаружить гомозиготный генотип A/A (Val/Val) *MnSOD* у шахтеров с ХПБ в 2 раза выше, чем в группе сравнения (χ^2 – 5,42; p = 0,02; отношение шансов (ОШ) 2,21; 95%-й доверительный интервал (95%-й ДИ) 1,13–4,33), тогда как шанс обнаружить гомозиготный генотип G/G (Pro/Pro) *GPX1* у шахтеров с ХПБ почти в 6 раз выше, чем в группе сравнения (χ^2 – 21,47; p = 0,001; ОШ 5,89; 95%-й ДИ 2,65–13,08). Выявлено, что сочетание генотипов AA/GG генов *MnSOD*/*GPX1* статистически значимо связано с полуторакратным риском развития ХПБ (χ^2 – 11,49; p < 0,001; относительный риск 1,59; 95%-й ДИ 1,36–1,84), тогда как шанс обнаружить это сочетание генотипов у шахтеров с патологией бронхолегочной системы в 15 раз выше, чем в группе сравнения (ОШ 15,09; 95%-й ДИ 1,99–114,64).

Заключение. Показано, что маркером генетической предрасположенности к развитию ХПБ является носительство гомозиготных генотипов A/A в локусе rs4880 *MnSOD* и G/G в локусе rs1050450 *GPX1*. Сочетание гомозиготных генотипов изученных генов AA/GG *MnSOD/GPX1* свидетельствует о полуторакратном риске развития ХПБ. Носительство одного из трех сочетаний генотипов генов *MnSOD* и *GPX1*: GG/AA, AA/AA и AG/AA свидетельствует о резистентности к формированию ХПБ.

Ключевые слова: полиморфизм генов, *MnSOD, GPX1*, угольно-породная пыль, хронический пылевой бронхит

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INTRODUCTION

Chronic dust-induced bronchitis (CDB) in miners is a special form of bronchial inflammation in response to exposure to high concentrations of coal dust with the development of diffuse atrophic and sclerotic changes, accompanied by impaired bronchial motility and respiratory failure [1, 2]. The key mechanism of the pathological response to long-term coal dust exposure is excessive activation of free radical processes (FRP) and changes in the activity of antioxidant defense enzymes [3–5].

Manganese superoxide dismutase (MnSOD) and glutathione peroxidase (GPx) are the key antioxidant enzymes which provide the first line of defense against oxidative stress [6–9]. Experiments on long-term exposure to coal dust showed changes in the expression of antioxidant defense components in different organs in rat models, including MnSOD, catalase, and GPx [10].

There are practically no studies that describe genetic variations in antioxidant defense in individuals with long-term exposure to coal dust at work. Among the antioxidant enzymes in CDB, the polymorphism of the glutathione-S-transferase (GSTT) gene family is the most studied one. The glutathione S-transferase gene family provides resistance of cells and tissues to toxic substances and products of lipid peroxidation. It has been shown that carriers of the *GSTT1*+ genotype which is responsible for normal production of the enzyme are most susceptible to the development of CDB, and carriers of the *GSTT1* null-genotype are resistant to CDB development [11, 12].

The aim of the study was to assess the association of the *MnSOD* (rs4880) and *GPX1* (rs1050450) gene polymorphisms with a risk of developing CDB in workers of the coal mining industry.

MATERIALS AND METHODS

Individuals employed in the main mining occupations in south Kuzbass mines (an underground tunneller, a stope miner, a mining machine operator) aged 39–58 years were examined in the clinic of the Research Institute for Complex Problems of Hygiene and Occupational Diseases. A total of 182 coal miners with long-term exposure to high concentrations of coal dust (exceeding the maximum permissible concentration by up to 35 times), including 116 miners previously diagnosed with chronic dust-induced bronchitis (CDB), were examined. Occupational bronchopulmonary pathology was diagnosed after the examination at the clinic of the Research Institute for Complex Hygiene Problems and Occupational Diseases by the medical expert board using federal guidelines.

The comparison group (66 workers) that underwent a preventive medical examination consisted of individuals without bronchopulmonary pathology, working under the same sanitary and hygienic conditions. The examined groups of miners are comparable in age and work experience, the difference between the groups is statistically insignificant ($p > 0.05$). The average work experience of miners with CDB was 24.39 ± 0.5 years, in the comparison group – 23.1 ± 1.2 years. The average age of miners with CDB was 48 ± 0.6 years, in the comparison group – 46 ± 0.7 years.

The following inclusion criteria were used in the study: Russian ethnicity; male gender; employment in the main occupations in the coal mines of south Kuzbass; at least 10 years of working underground; signed voluntary informed consent to participate in the study; clinically confirmed diagnosis of CDB for individuals included in the experimental group. The exclusion criteria were as follows: belonging to indigenous or settler descendant ethnic groups; mental disorders; malignant neoplasms and autoimmune diseases; refusal to sign an informed consent to participate in the study. For the comparison group, an additional exclusion criterion was any bronchopulmonary pathology, including both occupational and general somatic pathology.

For genetic studies, venous blood was taken on an empty stomach in vacutainers with K_3EDTA as an anticoagulant. Extraction of genomic DNA from blood cells was performed by phenol – chloroform extraction followed by ethanol precipitation [13]. Polymorphic variants of the genes were analyzed by real-time polymerase chain reaction using competitive TaqMan probes complementary to the polymorphic DNA sequence on DTprime-4 (DNA-Technology LLC, Moscow, Russian Federation). Test systems for molecular genetic analysis were developed by the Institute of Chemical Biology and Fundamental Medicine of the Siberian Branch of the Russian Academy of Sciences and synthesized by SibDNK LLC (Novosibirsk, Russian Federation). *MnSOD* (rs4880) and *GPX1* (rs1050450) gene polymorphisms were studied.

Statistical analysis of the results obtained was carried out using the IBM SPSS Statistics 22 software (license agreement No. 20/604/3–1 of 22.04.2016). The normality of the distribution of quantitative variables (age and work experience of patients) was

checked using kurtosis and asymmetry parameters. Quantitative variables were presented as the mean (*M*) and the standard error of the mean (*m*). Under normal distribution, Student's parametric *t*-test was used to compare two independent samples. The critical significance level (*p*) at which the null hypothesis would be rejected was 0.05.

The correspondence of the actual distribution of polymorphic variants of the *MnSOD* and *GPX1* genes to the theoretically expected one was determined according to the Hardy – Weinberg equilibrium. Pearson's χ^2 value was calculated to assess differences in the distribution of genotypes in patients with CDB and healthy individuals. The critical value of the significance level of differences was $p = 0.05$. The significance of differences in parameters was assessed by calculating the odds ratio (OR) and relative risk (RR) with the determination of the limits of the 95% confidence interval (CI). If the OR is more than 1, it means that the chances of finding a risk factor are higher in the group with an outcome (disease). If 95% CI does not include 1, that is, both limits are > 1 or < 1, a conclusion is made about the statistical significance of the identified association between the factor and the outcome at $p < 0.05$.

RESULTS AND DISCUSSION

The correspondence of the actual distribution of *MnSOD (*rs4880) and *GPX1 (*rs1050450) gene polymorphisms to the theoretically expected one was determined according to the Hardy – Weinberg equilibrium (Table 1). In the comparison group, the frequency of the A allele of the *GPX1* gene was two times higher than the frequency indicated in the dbSNP database of the NCBI open access information resource for the general population (0.6924 compared to 0.3219). However, no deviation of the obtained data from the expected frequencies was observed, the significance level was above 0.05 both for each genotype (χ^2 – 0.26–1.17–1.32; $p > 0.05$) and the total (χ^2 – 2.75; $p > 0.005$). For the *MnSOD* gene, no deviations from the frequency indicated in the dbSNP database were observed in both study groups.

The results obtained indicated that the Hardy – Weinberg equilibrium conditions were met (Table 1) with $p > 0.05$ for all studied genotypes, which made it possible to interpret the data obtained and conduct further analysis using the Pearson's χ^2 test to assess the distribution of genotypes between miners with CDB and individuals in the comparison group.

Table 1

Distribution of alleles and genotypes of the *MnSOD (*rs4880) and *GPX1 (*rs1050450) genes in the study groups

Group	Gene	Genotype	Absolute numbers	Genotype frequency, %	Allele frequency, %	Theoretically expected genotype frequency, %	Theoretically expected number of individuals with a given genotype	Heterozygosity	χ^2 Hardy–Weinberg
CDB	*MnSOD*	*A/A*	48	41.4	61.6	38.0	44	47.3	0.35
		A/G	47	40.5	–	47.3	55		1.13
		G/G	21	18.1	38.4	14.7	17		0.9
		Total	116	1	1	1	116		2.38
Comparison group		*A/A*	16	24.25	50.0	25.0	16.5	50.0	0.02
		A/G	34	51.5	–	50.0	33		0.03
		G/G	16	24.25	50.0	25.0	16.5		0.02
		Total	66	1	1	1	66		0.06
CDB	*GPX1*	*A/A*	14	12.9	32.1	10.3	11	43.6	0.67
		A/G	42	38.5	–	43.6	48		0.64
		G/G	53	48.6	67.9	46.1	50		0.15
		Total	109	1	1	1	109		1.46
Comparison group		*A/A*	34	52.3	69.2	47.9	31	42.6	0.26
		A/G	22	33.9	–	42.6	28		1.17
		G/G	9	13.8	30.8	9.5	6		1.32
		Total	65	1	1	1	65		2.75

*$p > 0.05$.

In the course of our study, the *MnSOD (*rs4880) and *GPX1 (*rs1050450) gene polymorphisms were considered separately. A molecular genetic study of *MnSOD* and *GPX1* gene polymorphisms revealed statistically significant differences between patients with CDB and the comparison group (Table 2). The chance of detecting the homozygous A/A genotype in *MnSOD (*rs4880) in miners with CDB was two

times higher than in the comparison group (χ^2 – 5.42; $p < 0.02$; OR – 2.21; 95% CI 1.13–4.33).

It has been shown that the A/A (Val/Val) genotype of the *MnSOD* gene causes a change in the secondary structure of the *MnSOD* signal sequence (from α-helix to β-sheet structure), resulting in a decrease in the transport of the antioxidant enzyme into mitochondria [14]. In addition, a change in the secondary structure reduces the activity of *MnSOD* by 30–40% and the efficiency of detoxification of superoxide anion radicals [6, 8, 15], which is one of the factors of excessive FRP activation. Thus, earlier rat model experiments focusing on long-term exposure to coal dust showed a decrease in SOD activity by 1.9 times and an increase in the sensitivity of lung tissue to FRP induction *in vitro* [10].

The study of the *GPX1 (*rs1050450) gene polymorphism showed that the chance of detecting the homozygous genotype G/G (Pro/Pro) in miners with CDB was almost 6 times higher than in the comparison group (χ^2 – 21.47; $p = 0.001$; OR – 5.89; 95% CI 2.65–13.08). Previously, carriers of the G/G (Pro/Pro) genotype had high activity of glutathione peroxidase (GPx1) and a high level of free radical oxidation products in blood plasma. In addition, the G/G (Pro/Pro) *GPX1* genotype provides a more intense antioxidant response to damaging effects compared to the G/A (Pro/Leu) and A/A (Leu/Leu) genotypes [9]. In turn, the homozygous genotype A/A is the genotype of resistance to the development of CDB, the frequency of which in the comparison group was 4 times higher than in miners – 52.31% and 12.85%, respectively.

Table 2

Distribution of *MnSOD* and *GPX1* genotypes in the comparison group and in patients with chronic dust-induced bronchitis

Gene	Genotype	Comparison group, abs. (%)	CDB, abs. (%)	χ^2; p	OR, 95% CI
MnSOD (rs4880)	*A/A* (Val/Val)	16 (24.24)	48 (41.38*)	5.42; 0.02	2.21; 1.13; 4.33
	A/G (Ala/Val)	34 (51.52)	47 (40.52)		0.64; 0.35; 1.18
	G/G (Ala/Ala)	16 (24.24)	21 (18.10)		0.69; 0.33; 1.44
GPX1 (rs1050450)	*G/G* (Pro/Pro)	9 (13.84)	53 (48.62*)	21.47; 0.001	5.89; 2.65; 13.08
	A/G (Pro/Leu)	22 (33.85)	42 (38.53)		0.82; 0.43; 1.55
	A/A (Leu/Leu)	34 (52.31)	14 (12.85*)		0.13; 0.06; 0.28

* – significance of differences compared to the comparison group.

Table 3

Combinations of *MnSOD (*rs4880) and *GPX1* (rs1050450) gene polymorphisms in coal industry employees

Group	Combination of genotypes			
	AA/GG	*GG/AA*	*AA/AA*	*AG/AA*
CDB patients	23	1	5	9
Comparison group	1	8	11	17
χ^2; p	11.49; $p < 0.001$	12.20; $p < 0.001$	8.91; $p = 0.003$	12.52; $p < 0.001$
OR 95% CI	15.09 1.99–114.64	0.06 0.01–0.48	0.21 [0.07–0.63	0.22 0.09–0.54
RR, 95% CI	1.59 1.36–1.85	0.16 0.03–1.04	0.46 0.22–0.95	0.49 0.29–0.84

Note: the table presents the combinations of genotypes that have a statistically significant association with the development of chronic dust-induced bronchitis and resistance to its development.

Next, we determined whether there were significant differences in combinations of genotypes of the *MnSOD*/*GPX1* antioxidant enzyme genes between coal miners with CDB and healthy individuals (Table 3). The analysis showed that the combination of AA/GG genotypes of the *MnSOD*/*GPX1* genes had a statistically significant association with a 1.5-fold risk of developing CDB (χ^2 – 11.49; $p < 0.001$; RR – 1.59; 95% CI 1.36–1.84), while the odds of detecting this combination of genotypes in miners with bronchopulmonary pathology were 15 times higher than in the comparison group (OR – 15.09; 95% CI 1.99–114.64). This may be due to a significant decrease in MnSOD activity and excessive activation of GPx1 and FRP in organs, in particular in the lungs [16]. Carriers of the combination of AA/GG

genotypes who have a high genetic risk of developing CDB should be advised to change their job, have a regular health checkup, and participate in preventive measures if they were previously exposed to coal dust.

Three combinations of polymorphic genotypes of the *MnSOD*/*GPX1* genes: GG/AA (χ^2 – 12.20; $p < 0.001$; OR – 0.06; 95% CI 0.01–0.48; RR – 0.16; 95% CI 0.03–1.04), AA/AA (χ^2 – 8.91; $p = 0.003$; OR – 0.21; 95% CI 0.07–0.63; RR – 0.46; 95% CI 0.22–0.95), and AG/AA (χ^2 – 12.52; $p < 0.001$; OR – 0.22; 95% CI 0.09–0.54; RR – 0.49; 95% CI 0.29–0.84) are associated with resistance to the development of CDB (Table 3). This may be due to preserved normal activity of the antioxidant defense enzymes MnSOD and GPx1 and maintenance of a normal redox balance.

CONCLUSION

Based on the obtained results, we can conclude that the carriage of homozygous genotypes A/A at the rs4880 *MnSOD* locus and G/G at the rs1050450 *GPX1* locus is a marker of genetic predisposition to the development of CDB, and their combination AA/GG in the *MnSOD/GPX1* genes indicates a 1.5-fold risk of developing CDB. The rs1050450 *GPX1* polymorphism homozygous for the A allele forms resistance to CDB development. Carrying one of the three combinations of genotypes in the *MnSOD* and *GPX1* genes (GG/AA, AA/AA and AG/AA) also indicates resistance to CDB.

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Authors' information

Zhukova Anna G. – Dr. Sci. (Biology), Associate Professor, Head of the Laboratory for Molecular Genetic and Experimental Research, Research Institute for Complex Hygiene Problems and Occupational Diseases; Head of the Department of Natural Sciences, Kuzbass Humanitarian Pedagogical Institute, Kemerovo State University, Novokuznetsk, nyura_g@mail.ru, https://orcid.org/0000-0002-4797-7842

Kazitskaya Anastasia S. – Cand. Sci. (Biology), Senior Researcher, Laboratory for Molecular Genetic and Experimental Research, Research Institute for Complex Hygiene Problems and Occupational Diseases; Associate Professor of the Department of Natural Sciences, Kuzbass Humanitarian Pedagogical Institute, Kemerovo State University, Novokuznetsk, anastasiya_kazitskaya@mail.ru, https://orcid.org/0000-0001-8292-4810

Yadykina Tatiana K. – Cand. Sci. (Biology), Leading Researcher, Laboratory for Molecular Genetic and Experimental Research, Research Institute for Complex Hygiene Problems and Occupational Diseases, Novokuznetsk, yadykina.tanya@yandex.ru, https://orcid.org/0000-0001-7008-1035

Gulyaeva Olga N. – Senior Researcher, Laboratory for Molecular Genetic and Experimental Research, Research Institute for Complex Hygiene Problems and Occupational Diseases, Novokuznetsk, Gulyaich1973@mail.ru, https://orcid.org/0000-0003-2225-6923

(✉) **Zhukova Anna G.,** nyura_g@mail.ru

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Constitutional risk factors for the development of glaucoma and cataracts in the Europioid population of Russia

Konenkov V.I.[1], Shevchenko A.V.[1], Prokofiev V.F.[1], Chernykh V.V.[2], Trunov A.N.[2]

[1] *Research Institute of Clinical and Experimental Lymphology – a branch of the Federal Research Center "Institute of Cytology and Genetics of the Siberian Branch of the Russian Academy of Sciences"*
2, Timakova Str., Novosibirsk, 630060, Russian Federation

[2] *S. Fyodorov Eye Microsurgery Federal State Institution, Novosibirsk branch*
10, Kolkhidskaya Str., Novosibirsk, 630096, Russian Federation

ABSTRACT

Aim. To identify endogenous risk factors for the development of glaucoma and cataracts based on the results of a comparative analysis of the nature of complex genetic trait distribution, including variants of genes for a number of cytokines and receptors for them, metalloproteinases, and their tissue inhibitors included in the genome of patients.

Materials and methods. The study included 501 people of the Caucasian race born and living in the Siberian region of Russia. They were divided into three groups of patients – patients with primary open-angle glaucoma (POAG) (n = 99), patients with senile cataract (n = 100), and the control group (n = 302) without ophthalmic pathology. Genotyping of the analyzed polymorphic loci was carried out by real-time PCR using the SYBRGreen I dye and TaqMan probes and by restriction fragment length polymorphism (RFLP) for different polymorphisms.

Results. The results of the study on the frequency of the analyzed genetic traits among patients with POAG compared to the control group showed the presence of combined genetic traits. The frequency of their detection in POAG was high and characterized by the two-digit value of the odds ratio, high values of specificity (99–100%), and high diagnostic coefficient. A direct comparison of the distribution of two ensembles of genes which protein products are involved in the extracellular matrix remodeling revealed a significant number of genetic traits characteristic of both diseases. This indicates significant differences in the implementation of the genetic predisposition to their development.

Conclusion. The data obtained indicate the possibility of developing reliable laboratory criteria (riskometers) for predicting predisposition to the development of POAG and early diagnosis at the stage of preclinical manifestations.

Keywords: primary open-angle glaucoma, cataract, extracellular matrix, *TGFB1 – TGFBR2*, MMP – TIMP, immunogenetics

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Conformity with the principles of ethics. All patients signed an informed consent to surgery, blood sampling, and the use of research data for scientific purposes. The study was approved by the Ethics Committee at the Research Institute of Clinical and Experimental Lymphology (Protocol No. 177 of 02.02.2003) and the Bioethics Committee at the Novosibirsk branch of S. Fyodorov Eye Microsurgery Federal State Institution (Protocol No. 2 of 2.09.2018).

✉ *Shevchenko Alla V.,* shalla64@mail.ru

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Конституциональные факторы риска первичной открытоугольной глаукомы и катаракты у европеоидного населения России

Коненков В.И.[1], Шевченко А.В.[1], Прокофьев В.Ф.[1], Трунов А.Н.[2], Черных В.В.[2]

[1] Научно-исследовательский институт клинической и экспериментальной лимфологии – филиал Федерального исследовательского центра «Институт цитологии и генетики Сибирского отделения Российской академии наук» (НИИКЭЛ – филиал ИЦиГ СО РАН)
Россия, 630060 г. Новосибирск, ул. Тимакова, 2

[2] Национальный медицинский исследовательский центр (НМИЦ) «МНТК "Микрохирургия глаза" им. акад. С.Н. Федорова», Новосибирский филиал
Россия, 630096, г. Новосибирск, ул. Колхидская, 10

РЕЗЮМЕ

Цель: выявление эндогенных факторов риска развития глаукомы и катаракты по результатам сравнительного анализа характера распределения комплексных генетических признаков, включающих в себя варианты генов ряда цитокинов и рецепторов к ним, металлопротеиназ и их тканевых ингибиторов, входящих в геном пациентов.

Материалы и методы. Обследован 501 человек европеоидного происхождения, родившихся и проживающих в сибирском регионе России, разделенных на три группы пациентов – с первичной открытоугольной глаукомой (ПОУГ) (n = 99), со старческой катарактой (n = 100), контрольная группа (n = 302) без офтальмопатологии. Генотипирование анализируемых полиморфных позиций осуществляли методами реал-тайм ПЦР с использованием интеркалирующего красителя SYBRGreen I, TaqMan зондов и методом рестриктного анализа длин продуктов амплификации (ПДРФ-анализ) – для разных полиморфных генов.

Результаты. Результаты исследования частот встречаемости анализируемых генетических признаков среди пациентов с ПОУГ относительно данных контрольной группы показали наличие комбинированных генетических признаков, частота выявления которых при ПОУГ высока и характеризуется двухзначными показателями отношения шансов, высокими значениями показателей специфичности 99–100% и высокими значениями величины диагностического коэффициента. Прямое сравнение характера распределения двух ансамблей генов, белковые продукты которых участвуют в процессах ремоделирования внеклеточного матрикса, выявило значительное количество генетических признаков, характерных как для одного, так и для другого заболевания, что свидетельствует о значительных различиях в реализации генетической предрасположенности к их развитию.

Заключение. Полученные данные свидетельствуют о принципиальной возможности разработки достоверных лабораторных критериев (рискометров) прогноза предрасположенности к развитию ПОУГ и ранней диагностики на стадии доклинических проявлений.

Ключевые слова: первичная открытоугольная глаукома, катаракта, внеклеточный матрикс, *TGFB1 – TGFBR2*, MMP – TIMP, иммуногенетика

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

Источник финансирования. Исследование проводилось в рамках государственного задания НИИКЭЛ – филиал ИЦиГ СО РАН FWNR-2022-0009 (регистрационный номер 122022800132-1) и НМИЦ «МНТК "Микрохирургия глаза" им. акад. С.Н. Федорова» «Разработка комплексной системы диагностики и мониторинга больных первичной открытоугольной глаукомы с оценкой состояния зрительных функций и эффективности лечения (медикаментозное, лазерное, хирургическое)» (регистрационный номер № 121072800028-3).

Соответствие принципам этики. Все пациенты подписали информированное согласие на проведение

операции, забор крови, а также использование данных исследования в научных целях. Исследование одобрено этическим комитетом НИИКЭЛ – филиал ИЦиГ СО РАН (протокол № 177 от 02.02.2003) и комитетом по биомедицинской этике Новосибирского филиала НМИЦ «МНТК "Микрохирургия глаза" им. акад. С.Н. Федорова» (протокол № 2 от 2.09.2018).

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INTRODUCTION

The extracellular matrix (ECM) is a well-organized 3-dimensional architectural network that plays an important structural and functional role in the organization and remodeling of tissues, as well as in the regulation of cellular processes [1]. The building blocks of these ultrastructures are collagens, proteoglycans and glycosaminoglycans, elastin and elastic fibers, laminins, fibronectin, and other proteins / glycoproteins [2]. ECM provides interaction between cells in organs and tissues, coordinating multiple commands for the transmission of intracellular and intercellular signals. As a consequence, ECM affects morphogenesis, development, and homeostasis of tissues through regulation of cellular physiology, growth, proliferation, differentiation, and adhesion. ECM undergoes intensive remodeling under pathological conditions, playing a key role in the progression of many diseases, including ophthalmic pathologies [3, 4].

Like most complexly organized physiological systems, the functional state of ECM is largely determined by genetic factors, the most important of which are structures of polymorphic sites in regulatory regions of genes of both ultrastructural ECM components and humoral factors affecting its activity (growth factors, cytokines, chemokines, matrix metalloproteinases, their tissue inhibitors, etc.). Combinations of structural variants in regulatory regions of these genes, usually located in promoter regions, determine the intensity of protein expression and the level of synthesis by producer cells [5]. These parameters define the concept of "quantitative trait loci", which are attracting increasing attention of researchers in the field of medical genetics [6]. Given the undoubtedly polygenic nature of a person's genetic predisposition to development of most diseases, studies on the association of pathological processes not so much with single candidate genes as with functionally related complexes of polymorphic genotypes is of the greatest interest.

The aim of the study was to identify endogenous risk factors for the development of glaucoma and cataracts based on the results of a comparative analysis of the nature of complex genetic trait distribution, including variants of genes for a number of cytokines and receptors for them, metalloproteinases, and their tissue inhibitors included in the genome of patients.

MATERIALS AND METHODS

The study was carried out in accordance with the principles of the Declaration of Helsinki "Ethical Principles of Medical Research Involving Human Subjects", the Federal Law of the Russian Federation No. 323 FZ of 21.11.2011 "On principles of health preservation of citizens of the Russian Federation", and requirements of the Federal Law No 152-FZ of 27.07.2006 (ed. of 21.07.2014) "On personal data" (with amendments that came into effect on 01.09.2015). The study included 501 people of the Caucasian race born and living in the Siberian region of Russia.

Following the ophthalmic examination (determination of visual acuity, binocular indirect ophthalmoscopy, perimetry, echoophthalmography, optical coherence tomography, measurement of intraocular pressure), they were divided into three groups of patients. Group 1 included 99 patients with a verified diagnosis of stage II (advanced) primary open-angle glaucoma (POAG) (ICD-10 code H40.1), 52 (52.53%) males and 47 (47.47%) females. The average age of patients in this group was 62.8 ± 4.3 years.

Group 2 encompassed 100 patients with senile (uncomplicated) cataract, 81 (81%) females and 19 (19%) males, 63.5 ± 0.4 years old. Exclusion criteria were inflammatory eye diseases, diabetic retinopathy, neovascular glaucoma, uveitis, hemophthalmos, verified autoimmune diseases and tumors, as well as diabetes mellitus without ocular complications. The control group (similar in age and ethnic composition to groups 1 and 2) included 302 people without ophthalmic pathology.

All patients underwent an immunogenetic examination at the Laboratory for Clinical Immunogenetics of the Research Institute of Clinical and Experimental Lymphology, a branch of ICIG SB RAS. Genotyping was carried out by real-time PCR using SYBRGreen I dye (Litech, Russia) for rs1800629, rs361525, rs1800630, rs1143627, rs2243250, rs1800872, rs1800896 and TaqMan probes for rs1800795, rs243865, and rs3918242 (Syntol, Russia); by restriction fragment length polymorphism (RFLP) for rs4073 [7], rs4898 [8], rs8179090 [9], rs3025058 [10], rs1800469 [11], Gene ID 7046, D50683, L07594 [12].

The comparison group was analyzed by 11 polymorphic sites (Table 1). Patients with glaucoma and cataract were examined for 8 cytokine genes: *IL8- A251T* (rs4073), *IL17A- A197G* (rs227593), *TGFB- C509T* (rs1800469), *TGFBRI, TGFBR II, TGFBR III* receptor genes (Gene ID 7046, D50683, L07594, respectively), metalloproteinase inhibitor genes *TIMP1 -C372T* (rs4898), *TIMP2 -G 418C* (rs8179090).

In the statistical analysis of the results of genetic studies, we calculated the frequency of genotypes and their combinations, the odds ratio (OR) and the 95% confidence interval (CI) [13]. The distribution of genotypes was tested by the Hardy – Weinberg equilibrium.

To evaluate the results obtained, in addition to the common methods of statistical processing, we used computational methods of bioinformatics based on the probability theory of pattern recognition, based on the Bayes theorem (inverse probability theorem or hypothesis theorem) and the modified Wald's sequential probability test – a heterogeneous sequential pattern recognition procedure that allows to determine the diagnostic value of variables by calculating diagnostic coefficients (DCs) [14]. DC is a decimal logarithm of ratios of smoothed variables multiplied by 10. DC is represented by positive or negative numbers. At the same time, the greater the value of the DC, the more differential diagnostic information it carries. DCs of each found genetic trait are summed up, and when the limit values (threshold) are reached, the probability of the presence or absence of one of the alternative diseases (conditions) is established.

When calculating the integral characteristics of genetic traits as diagnostic and prognostic criteria, in addition to calculating DC, the specificity of the biomarker (Sp) was calculated as the probability of a truly negative proportion [15].

The differences in the genotype frequencies were determined by the two-tailed Fisher's exact test with 2 x 2 contingency tables. Statistical processing of the obtained results was performed using IBM SPSS Statistics 23 software package (USA). The differences were considered statistically significant at $p < 0.01$. The critical significance level in multiple comparisons was assumed with account of the Bonferroni correction [16, 17].

RESULTS

The comparison of the distribution of single and complex genetic traits in groups of patients with POAG and cataract with similar data from the reference group of healthy individuals without signs of ophthalmic diseases was carried out in 11 polymorphic sites (Table 1).

Table 1

Polymorphic sites of the studied genes in groups of patients with POAG and cataract and individuals without ophthalmic pathology

	Parameters	Polymorphic site	Locus (chromosome)	Reference sequence number
Cytokine genes				
1	*TNFA*	-238 G/A	6q21.3	rs361525
2	*TNFA*	-308 G/A	6q21.3	rs1800629
3	*TNFA*	-863 C/A	6q21.3	rs1800630
4	*IL1 B*	-31 C/T	2q14.2	rs1143627
5	*IL4*	-590 C/T	5q31.1	rs2243250
6	*IL6*	-174 G/C	7p21.	rs1800795
7	*IL10*	-592 C/A	1q31–q32	rs1800872
8	*IL10*	-1082 A/G	1q31–32	rs1800896
Metalloproteinase genes				
9	*MMP2*	-1306 C/T	16q12.2	rs243865
10	*MMP3*	-1171 5A/6A	11q22.3	rs3025058
11	*MMP9*	-1562 C/T	20q13	rs3918242

The results of the study of the POAG patients are presented in Table 2. During the initial analysis of the significance of differences between healthy individuals and POAG patients using the two- tailed Fisher's exact test, we revealed variables whose frequency significantly differed towards both an increase and a decrease in the POAG group with the significance of differences $p < 0.01$.

To obtain more significant values, which can be transferred into clinical practice to develop additional laboratory early diagnostic and prognostic criteria, we used the Bonferroni correction. It was used as a way to eliminate the effect of multiple comparisons that occurs when it is necessary to build a family of statistical conclusions, which avoids false conclusions about the presence of differences between groups, whereas in fact

the null hypothesis about the absence of differences is true. The application of this approach made it possible to select traits whose frequency differed most significantly in the group of POAG patients from the distribution of similar genetic traits in a sufficiently significant healthy group while maintaining the significance level of differences $p < 0.01$.

As part of complex genetic traits, whose frequency is significantly changed among POAG patients, both variants of cytokine genes with proinflammatory and anti-inflammatory activity and variants of matrix metalloproteinase genes are identified. Among the cytokine genes for these traits, variants of the *TNFA* and *IL 10* genes are most often detected, and among the metalloproteinase genes, variants of the *MMP2* gene are commonly detected. Variants of the *IL1B* and *MMP3* genes were not included in any of the complexes that significantly differ in the frequency of occurrence in the groups. The traits themselves are complex and include from two to six loci, variously combined and associated with different gene expression levels.

Among the complex genetic traits most closely associated with the development of POAG, the following complexes are distinguished: IL6-174:IL4-590:IL10-592:MMP2-1306:MMP9-1562, TNF-238:IL6-174:IL4-590:IL10-592:MMP2-1306, TNF-308:IL6-174:IL4-590:IL10-592:MMP2-1306, and *TNF-308:IL4-590, TNF-308:TNF-238.* Their prognostic value, according to the DC, exceeds 11.0, which corresponds to the reliability of the prognostic conclusion of over 95%. The combined genetic traits TNF-238:IL4-590:IL10-1082, IL10-1082:MMP2-1306:MMP9-1562, and TNF-308:IL10-1082:MMP2-1306 were practically not detected among patients with POAG, which indicates their probable protective value.

The comparative analysis on the frequency of occurrence of genetic traits in POAG patients and healthy individuals revealed formally similar, but different in content results. Thus, the analysis of the significance of differences according to the two-tailed Fisher's exact test revealed a significant group of 844 traits. The application of the Bonferroni correction as a way to eliminate the effect of multiple comparisons made it possible to select the traits whose frequency most significantly differed in the group of POAG patients as opposed to healthy individuals ($p < 0.01$). Among the complex genetic traits associated with the development of cataract, traits containing various variants of the *TNF* gene in all three studied sites prevailed. However, the *A* or *AA* variant in the position -308, associated with an increased ability of cells to produce this proinflammatory cytokine, was predominant (Table 3).

Table 2

The distribution frequency of the analyzed genetic traits in patients with POAG and healthy individuals

Polymorphic site	Genotypes	POAG, %	Control, %	OR	95% CI	Sp	Dc	*p*_cor
TNF-308	AA	7.07	0.66	11.41	2.33–55.90	99.34	10.3	0.0033
TNF-308:TNF-238	AA-GG	6.06	0.34	18.84	2.24–158.50	99.66	12.5	0.0091
TNF-308:IL4-590	AA-CC	7.07	0,34	22.37	2.72–184.21	99.66	13.2	0.0024
IL10-592:MMP2-1306	CA-TC	26.26	7.89	4.16	2.15–8.02	92.11	5.2	0.0008
TNF-308:IL10-592:MMP2-1306	GG-CA-TC	19.19	5.70	3.93	1.85–8.32	94.30	5.3	0.0080
TNF-238:IL10-592:MMP2-1306	GG-CA-TC	24.24	7.05	4.22	2.13–8.37	92.95	5.4	0.0015
IL6-174:IL10-592:MMP2-1306	GC-CA-TC	17.35	3.07	6.63	2.65–16.57	96.93	7.5	0.0023
IL4-590:IL10-592:MMP2-1306	CC-CA-TC	17.17	3.51	5.70	2.37–13.71	96.49	6.9	0.0022
TNF-308:IL6-174:IL10-592:MMP2-1306	GG-GC-CA-TC	14.29	2.63	6.17	2.29–16.58	97.37	7.3	0.0092
TNF-238:IL6-174:IL10-592:MMP2-1306	GG-GC-CA-TC	16.33	3.08	6.13	2.43–1.44	96.92	7.2	0.0038
IL6-174:IL4-590:IL10-592:MMP2-1306	GC-CC-CA-TC	12.24	0.88	15.77	3.46–71.91	99.12	11.4	0.0053
IL6-174:IL10-592:MMP2-1306:MMP9-1562	GC-CA-TC-CC	13.27	2.22	6.73	2.33–19.45	97.78	7.8	0.0098
TNF-238:IL6-174:IL4-590:IL10-592:MMP2-1306	GG-GC-CC-CA-TC	11.22	0.88	14.22	3.09–65.48	99.12	11.1	0.0073
IL6-174:IL4-590:IL10-592:MMP2-1306:MMP9-1562	GC-CC-CA-TC-CC	11.22	0.44	28.32	3.60–222.67	99.56	14.0	0.0090

Note (in all tables): *p*_cor - adjusted *p* value in the two-tailed Fisher's exact test (Bonferroni correction).

Table 3

The distribution frequency of the analyzed genetic traits in patients with cataract and healthy individuals

Polymorphic site	Alleles/Genotypes	Cataract, %	Control, %	OR	95% CI	Sp	DC	*p*_cor
IL1B-31	T	50.50	64.46	0.56	0.41–0.78	49.50	–1.1	0.0012
IL1B-31	C	49.50	35.54	1.78	1.28–2.46	64.46	1.4	0.0012
TNF-308	AA	12.00	0.66	20.45	4.49–93.12	99.34	12.6	0.0003
IL1B-31	CC	28.00	13.59	2.47	1.42–4.29	86.41	3.1	0.0057
TNF-863:TNF-308	CC-AA	11.00	0.67	18.42	4.01–84.64	99.33	12.2	0.0007
TNF-863:MMP2-1306	CA-TT	9.00	0.87	11.32	2.40–53.42	99.13	10.2	0.0045
TNF-308:TNF-238	AA-GG	12.00	0.34	39.82	5.11–310.52	99.66	15.5	0.0007
TNF-308:IL1B-31	AA-TC	7.00	0.35	21.53	2.61–177.27	99.65	13.0	0.0036
TNF-308:IL6-174	AA-GC	6.00	0.00	21.81	2.65–179.55	100.00	15.8	0.0018
TNF-308:MMP9-1562	AA-CC	8.00	0.71	12.09	2.52–57.94	99.29	10.5	0.0040
IL6-174:IL10-1082	GC-GG	3.00	16.47	0.16	0.05–0.53	97.00	–7.4	0.0054
TNF-863:TNF-308:TNF-238	CC-AA-GG	11.00	0.34	36.09	4.60–283.40	99.66	15.1	0.0013
TNF-863:IL6-174:IL10-1082	CC-GC-GG	1.00	12.35	0.07	0.01–0.54	99.00	–10.9	0.0088
TNF-863:MMP2-1306:MMP9-1562	CA-TT-CC	8.00	0.44	19.65	2.42–159.36	99.56	12.6	0.0088
TNF-308:TNF-238:IL1B-31	AA-GG-TC	7.00	0.36	21.08	2.56–173.56	99.64	12.9	0.0085
TNF-308:TNF-238:IL6-174	AA-GG-GC	6.00	0.00	21.29	2.59–175.31	100.00	15.7	0.0054
TNF-308:TNF-238:MMP9-1562	AA-GG-CC	8.00	0.36	23.91	2.95–193.77	99.64	13.4	0.0013
IL6-174:IL4-590:IL10-1082	GC-CC-GG	0.00	10.06	0.08	0.01–0.64	100.00	–13.2	0.0100

In this group of patients, a significantly higher proportion of genetic traits closely associated with the development of the disease was found. Thus, of the traits whose frequency is significantly increased in cataract, 12 are characterized by a two-digit DC, 99–100% specificity, and a two-digit OR.

The following traits have the maximum prognostic value: TNF-308:TNF-238, TNF-863:TNF-308:TNF-238, TNF-308:TNF-238:MMP9-1562, TNF-308:IL6-174, TNF-308: IL1B-31, TNF-308:TNF-238:IL6-174, and TNF-308:TNF-238:IL1B-31. At the same time, their composition in all cases includes TNFA -308 AA. Complex genetic traits with high protective value were also identified: IL6-174:IL 4-590:IL 10-1082, and TNF-863:IL6-174:IL10-1082.

Obtaining data on the similarities and differences in the distribution of single and complex genetic traits among POAG and cataract patients prompted us to investigate these results in more detail. IL17A-197 A/G, TGFB-509 C/T, and IL8 -251 A/T genes were added to the comparative analysis of the distribution of genetic traits in both groups of patients due to data on the active involvement of their protein products in the regulation of inflammatory processes and remodeling of the ECM. Genes of tissue metalloproteinase inhibitors TIMP1-372 C/T, TIMP2-418 G/C, constituting a single regulatory complex with *MMP*, and TGFB receptor genes: *TGFBRI, TGFBR II* and *TGFBR III* were also added to the analysis (Table 4).

Table 4

Distribution frequency of the analyzed genetic traits in patients with POAG and cataract

Polymorphic site	Alleles/Genotypes	POAG, %	Cataract, %	OR	95% CI	Sp	DC	*p*_cor
IL1B-31	T	64.65	50.50	1.79	1.20–2.68	49.50	1.1	0.0092
IL1B-31	C	35.35	49.50	0.56	0.37–0.83	64.65	–1.5	0.0092
TGFBR2	C	98.48	91.50	6.04	1.74–20.95	8.50	0.3	0.0040
TGFBR2	G	1.52	8.50	0.17	0.05–0.57	98.48	–7.5	0.0040
TGFBR2	CC	96.97	84.00	6.10	1.72–21.65	16.00	0.6	0.0084
IL1B-31:MMP2-1306	TT-TC	22.22	5.00	5.43	1.96–15.00	95.00	6.5	0.0036
TNF-238:IL1B-31:MMP2-1306	GG-TT-TC	22.22	5.00	5.43	1.96–15.00	95.00	6.5	0.0072
IL8-251:IL17-197:MMP9-1562	TA-GG-CC	21.21	4.08	6.33	2.08–19.21	95.92	7.2	0.0088
TNF-863:TNF-238:IL1B-31:IL4-590	*CC-GG-CC-CC*	1.01	16.00	0.05	0.01–0.41	98.99	–12.0	0.0060

Table 4 (continued)

Polymorphic site	Alleles/Genotypes	POAG, %	Cataract, %	OR	95% CI	Sp	DC	*p*_cor
TNF-863:TNF-238:IL1B-31:TIMP2-418	CC-GG-CC-GG	4.04	21.00	0.16	0.05–0.48	95.96	–7.2	0.0092
TNF-308:IL6-174:IL17-197:MMP2-1306	GG-GC-GG-TC	15.31	1.00	17.89	2.31–138.31	99.00	11.8	0.0051
IL6-174:IL17-197:MMP2-1306:TGFBR2	GC-GG-TC-CC	15.31	1.00	17.89	2.31–138.31	99.00	11.8	0.0037
IL8-251:IL17-197:MMP9-1562:TGFBR2	TA-GG-CC-CC	20.20	3.06	8.02	2.30–27.97	96.94	8.2	0.0066
TNF-308:IL6-174:IL17-197:MMP2-1306:TIMP2-418	GG-GC-GG-TC-GG	15.31	1.00	17.89	2.31–138.31	99.00	11.8	0.0060
TNF-308:IL6-174:IL17-197:MMP2-1306:TGFBR2	GG-GC-GG-TC-CC	14.29	0.00	17.82	2.31–137.72	100.00	14.7	0.0000
IL6-174:IL17-197:MMP2-1306:TIMP2-418:TGFBR2	GC-GG-TC-GG-CC	15.31	1.00	17.89	2.31–138.31	99.00	11.8	0.0047
TNF-308:IL6-174:IL10-592:IL17-197:TIMP2-418:TGFBR2	GG-GC-CA-GG-GG-CC	13.27	0.00	16.44	2.12–127.60	100.00	14.4	0.0067
TNF-308:IL6-174:IL17-197:MMP2-1306:TIMP2-418:TGFBR2	GG-GC-GG-TC-GG-CC	14.29	0.00	17.82	2.31–137.72	100.00	14.7	0.0000

When analyzing these results, three main aspects are of interest. Firstly, with the Bonferroni correction, the number of significantly different genetic traits increases significantly compared to the group of healthy individuals with a given level of the significance $p < 0.01$. Secondly, the composition of complex genetic traits includes a large number of variants of newly included genes, which confirms the correctness of their inclusion in the study. Thirdly, the number of highly significantly different traits increases significantly, which increases their prognostic value. We increased the significance level of the differences by five times from 0.05 to 0.01 in order to select the analyzed genetic traits suitable for possible transfer into clinical practice.

The predominant participation of *TNFA* gene variants in the formation of complex genetic traits differentiating comparable diseases is again of great interest when analyzing the results obtained. The results of the study showed a significant increase in the frequency of the T allele in the *IL1B* gene at -31T in the group of POAG patients ($p = 0.0046$), whereas in the study of patients of the Balkans' population, the protective role of *IL1B* rs16944 in the development of this disease was shown [18]. More significant differences between the groups were found in the study of the distribution of the *TGBR 2* gene. D50683 C was detected in more than 98% of POAG patients, which is significantly more common than in patients with cataract (OR = 6.04; $p = 0.002$).

The same pattern was revealed for the homozygous *TGFBR2 CC* genotype. To date, there has been no data on the effect of *TGBR 2* gene polymorphism on the expression of its protein products, however, the available data on a significant increase in the content of the TGFß2RII protein in the trabecular network of POAG patients suggest the presence of such a link in the development of fibrotic processes in POAG [19]. Significant changes in the level of TGFß isoforms in the intraocular fluid of POAG patients were described by us earlier [20].

A comparison of the distribution of gene variants involved in the ECM remodeling revealed significant differences between the groups of patients. The number of genetic traits, which presence in the patient's genome is significantly associated with the development of POAG increases, which is characterized by two-digit values of OR in the range from 16.44 to 17.89, the DC of up to 14.7, and specificity of 99–100%. Twelve such complex genetic traits are presented in Table 4. There are also genetic traits, whose frequency is significantly increased in patients with cataract.

The data obtained in the digital format are clearly illustrated in Figure, which presents the patterns of frequency distribution of the studied genetic traits in both diseases in a diagram.

The figure clearly demonstrates pronounced differences in the frequency of occurrence of complex genetic traits, although a zone of repeated combinations is visible in the central part of the figure, which probably reflects the presence of common links in the pathogenesis of eye diseases associated with inflammation, fibrosis, and ECM remodeling.

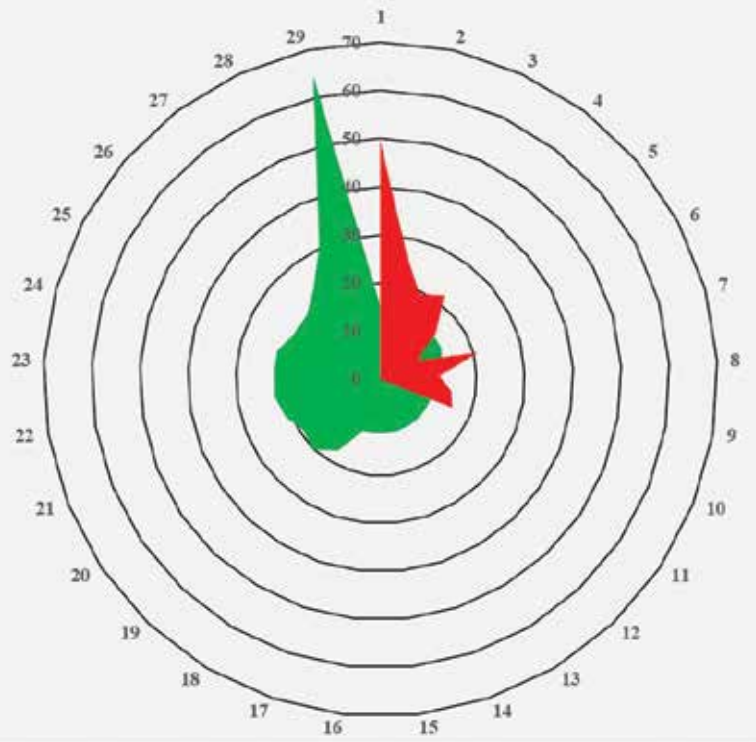

Figure. Graphical representation of differences in the frequencies of combinations of polymorphic variants in the studied genes in POAG and cataract: the numbers and scale of the radial axes correspond to the numerical values in Table 4.

DISCUSSION

We began the analysis of the distribution of polymorphic gene variants by comparing data of patients with POAG with similar data in the control group, which included 302 individuals of the Caucasian race born and permanently living in Russia. The results of the study showed significant deviations in the distribution of the studied traits both for a number of variants of individual genes and for their combinations. The analysis of single-nucleotide polymorphism (SNP) distribution revealed an increase in IL10-1082 A and IL10-592 A among patients with POAG, with a corresponding relative decrease in alternative *G* and *C* variants, which is confirmed by changes in the frequencies of the corresponding genotypes. The -1082 G/A polymorphism in the promoter region is associated with higher IL-10 production in the presence of the *G* allele relative to the *A* allele in the same position [21]. These data may indirectly indicate that among patients with POAG, alleles of the *IL-10* gene associated with low production of the corresponding cytokine with anti-inflammatory activity synthesized by Th2 cells and suppressing the production of cytokines by Th1 cells are more common.

It is worth noting that complex genetic traits closely associated with POAG, as a rule, include gene variants with both proinflammatory and anti-inflammatory activity.

The analysis of the obtained data showed that among patients with cataract, the frequency of occurrence of the *A* allele and *AA* genotype in TNF-308 was increased. It was previously shown that the *A* allele is associated with an increased level of production of this proinflammatory cytokine [22]. There are only isolated meta-analysis data on a weak association of the development of POAG with the *A* allele and the AA genotype of the *TNFA* – 308G/A (OR 1.6–1.7), more pronounced in Mongoloids. None of the other polymorphisms was significantly associated with the risk of POAG [23].

In our study, no isolated association of any of the three polymorphic variants of this gene with POAG or cataract was noted. However, in the composition of significantly associated complex genetic traits, various genotypes of this gene were repeatedly identified. In contrast to the group of patients with POAG, among patients with cataract we found an increase in the frequency of the *C* variant and the homozygous CC genotype in the *ILB1 -31* gene. At the same time,

in the group of patients with POAG, an increase in the frequency of *IL10-1082 A* was revealed with a corresponding relative decrease in alternative variants *G*, which indicates the spread of the variant associated with a low ability of cells to produce IL-10 among patients with cataract.

The addition of IL17A-197 A/G, TGFB C/T, and IL8 -251 A/T cytokine genes, TIMP1-372 C/T, TIMP2-418 G/C tissue metalloproteinase inhibitor genes, which form a single regulatory complex with *MMP*, and TGFB receptor genes *TGFBRI, TGFBR II,* and *TGFBR III* more fully characterizes the state of ECM in comparable diseases.

Products of these genes are involved in the processes of vital activity in cells producing structural elements of the ECM, in the regulation of elasticity, looseness or stiffness of the ECM, in angiogenesis and lymphangiogenesis, in tissue fluid exchange, in the homeostatic nature of its composition, in cell migration, apoptosis, etc. Practically, the analysis includes two ensembles of genes – the genes of cytokines and their receptors, as well as the genes of metalloproteinases and their tissue inhibitors. Along with the possible practical application of the results obtained for prognostic purposes, comparing the nature of the distribution of genes involved in the ECM remodeling is also of fundamental importance, allowing to identify general and specific traits underlying a genetic predisposition to the development of eye diseases of various genesis.

Although the genes analyzed in this study have only partially been included in the genes responsible for remodeling of human eye tissue structures in glaucoma so far [24], we believe that the high degree of association of their variant complexes with eye diseases of various genesis makes their analysis promising for further research. Previously, the association of POAG with TIMP polymorphisms in Mongoloids [25] and MMP-TIMP polymorphisms in Caucasians [26] was shown.

CONCLUSION

The comparative analysis of the results of the study on the frequency of the analyzed genetic traits, including cytokine and metalloproteinase genes, in patients with POAG and healthy individuals showed the presence of complex genetic traits, whose frequency is extremely high among POAG patients, which is characterized by two-digit OR, high specificity (99–100%), and high DC.

Alternative results were obtained by us in a comparative analysis of the frequency of genetic traits in groups of patients with cataract and healthy individuals. The application of the Bonferroni correction as a way to eliminate the effect of multiple comparisons made it possible to select the traits whose distribution frequency most significantly differed in the group of patients with cataract compared to the distribution of similar genetic traits in the control group of 302 healthy individuals while maintaining the given $p < 0.01$.

The direct comparison of the distribution of two gene ensembles whose products are involved in the processes of ECM remodeling – genes of cytokines and receptors to some of them (*TGFB1 – TGFBR2*), as well as genes of metalloproteinases and their tissue inhibitors (MMP – TIMP) revealed a significant number of genetic traits characteristic of both diseases, which indicates significant differences in the implementation of genetic predisposition to their development.

The received data indicate a fundamental possibility of developing reliable laboratory criteria (riskometers) for early diagnosis and prognosis of predisposition to the development of POAG and cataract before the development of clinical and laboratory signs of the disease at a young age.

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Authors' contribution

Konenkov V.I. – conception and design, critical revision of the manuscript for important intellectual content, drafting of the article. Shevchenko A.V. – carrying out of the laboratory studies, analysis and statistical processing of the data, drafting of the article. Prokofiev V.F. – analysis and statistical processing of the data, drafting of the article. Chernykh V.V. – conception and design, critical revision of the manuscript for important intellectual content. Trunov A.V. – justification of the manuscript, design, selection of patients for the study, drafting of the article.

Authors' information

Konenkov Vladimir I. – Dr. Sci. (Med.), Professor, Academician of the Russian Academy of Sciences, Head of the Laboratory for Clinical Immunogenetics, Research Supervisor, Research Institute of Clinical and Experimental Lymphology – a branch of the

Federal Research Center “Institute of Cytology and Genetics of the Siberian Branch of the Russian Academy of Sciences”, Novosibirsk, vikonenkov@gmail.com, https://orcid.org/0000-0001-7385-6270

Shevchenko Alla V. – Dr. Sci. (Biology), Leading Researcher, Laboratory for Clinical Immunogenetics, Research Institute of Clinical and Experimental Lymphology – a branch of the Federal Research Center “Institute of Cytology and Genetics of the Siberian Branch of the Russian Academy of Sciences”, Novosibirsk, shalla64@mail.ru, https://orcid.org/0000-0001-5898-950X

Prokofiev Viktor F. – Cand. Sci (Med.), Leading Researcher, Laboratory for Clinical Immunogenetics, Research Institute of Clinical and Experimental Lymphology – a branch of the Federal Research Center “Institute of Cytology and Genetics of the Siberian Branch of the Russian Academy of Sciences”, Novosibirsk, vprok@ngs.ru, https://orcid.org/0000-0001-7290-1631

Trunov Aleksandr N. – Dr. Sci. (Med.), Professor, Head of the Science Department, S. Fyodorov Eye Microsurgery Federal State Institution, Novosibirsk branch, Novosibirsk, trunov1963@yandex.ru, https://orcid.org/0000-0002-7592-8984

Chernykh Valeriy V. – Dr. Sci. (Med.), Professor, Director, S. Fyodorov Eye Microsurgery Federal State Institution, Novosibirsk branch, Novosibirsk, sci@mntk.nsk.ru, https://orcid.org/0000-0002-7623-3359

(✉) **Shevchenko Alla V.,** shalla64@mail.ru

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Application of a hydrogel derived from porcine dermis for experimental treatment of superficial wounds

Melkonyan K.I.[1], Kozmay Ya.A.[1], Rusinova T.V.[1], Chuprynin G.P.[1], Kartashevskaya M.I.[1], Kartashevsky I.I. [1], Storozhuk S.V. [1], Selezneva I.I.[2], Gurevich K.G.[3]

[1] Kuban State Medical University
4, Mitrofana Sedina Str., Krasnodar, 350063, Russian Federation

[2] Institute of Experimental and Theoretical Biophysics
3, Institutskaya Str., Pushchino, 142290, Russian Federation

[3] Yevdokimov Moscow State University of Medicine and Dentistry
20/1, Delegatskaya Str., Moscow, 127473, Russian Federation

ABSTRACT

Aim. To study the efficacy of dermal hydrogel application in the experimental treatment of superficial scarified wounds in rats.

Materials and methods. The hydrogel was obtained from porcine dermis by alkaline hydrolysis. The DNA concentration was determined using the Nano Drop ND-1000 spectrophotometer. The study included 30 male Sphinx rats. Scarified wounds were created on the rat skin, then the rats were divided into two groups: group 1 – rats without treatment, or control group (n = 15), group 2 – rats with wound treatment with the dermal hydrogel for 5 days, or experimental group (n = 15). On day 3, 7, and 14 of the experiment, we explanted skin samples from the wound area and performed routine H&E staining.

Results. On day 3 of the experiment, moderate inflammation, edema, and collagen fiber disorganization were revealed in the experimental group, and pronounced inflammation with purulent exudate was found in the control group. On day 7 of the experiment, inflammation and foci of stratified epithelium were detected in the control group. The histologic analysis of the skin samples from the experimental group showed pronounced plethora of the vessels, necrotic changes of the dermis, and edema. The total thickness of the epidermis and the thickness of its stratum corneum were greater than in the control group samples. On day 14, the differences between the groups were minimal and the epidermis was thickened in the experimental group animals.

Conclusion. The study examined the effects of the dermal hydrogel on scarified wounds in rats. We found faster skin regeneration (by 1.5–2 days) in the experimental group compared to the controls. Besides, the rats of the experimental group were characterized by an increase in the number of fibroblasts in the dermis and thickened epidermis in the affected area.

Keywords: dermal hydrogel, scarified wound, morphological analysis, extracellular matrix

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Conformity with the principles of ethics. The study was approved by the local Ethics Committee at Kuban State Medical University (Protocol No. 102 of 01.10.2021).

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✉ *Melkonyan Karina I.,* kimelkonian@gmail.com

Применение гидрогеля на основе дермы свиньи для экспериментального лечения поверхностных ран

Мелконян К.И.[1], Козмай Я.А.[1], Русинова Т.В.[1], Чупрынин Г.П.[1], Карташевская М.И.[1], Карташевский И.И.[1], Сторожук С.В.[1], Селезнева И.И.[2], Гуревич К.Г.[3]

[1] Кубанский государственный медицинский университет (КубГМУ)
Россия, 350063, г. Краснодар, ул. Митрофана Седина, 4

[2] Институт экспериментальной и теоретической биофизики Российской академии наук (ИТЭБ РАН)
Россия, 142290, г. Пущино, ул. Институтская, 3

[3] Московский государственный медико-стоматологический университет (МГМСУ) им. А.И. Евдокимова
Россия, 127473, г. Москва, ул. Делегатская, 20/1

РЕЗЮМЕ

Цель – изучение эффективности использования дермального гидрогеля при экспериментальном лечении поверхностных скарифицированных ран у крыс.

Материалы и методы. Гидрогель получали из свиной дермы химическим методом с применением щелочного гидролиза. В полученных образцах гидрогеля определяли содержание ДНК с помощью спектрофотометра Nano Drop ND-1000. Исследование проведено на 30 самцах крыс породы сфинкс. Крысам наносили скарифицированные раны, затем животные были разделены на две группы: группа 1 – без лечения, или контрольная группа (n = 15), группа 2 – лечение раны дермальным гидрогелем в течение 5 сут (n = 15). На 3-и, 7-е и 14-е сут эксплантировались образцы кожи из области раны, которые подвергались гистологическому исследованию.

Результаты. На 3-и сут эксперимента в образцах кожи животных группы 2 отмечалось умеренное воспаление с поверхностным отеком и дискомплексацией коллагеновых волокон, а контрольной группы – выраженное воспаление с гнойным экссудатом. На 7-е сут эксперимента у крыс контрольной группы наблюдали воспаление, однако отмечали очаги пролиферации многослойного эпителия. Гистологический анализ кожи животных группы 2 продемонстрировал более выраженное полнокровие сосудов, некротические изменения дермы и ее отек. Общая толщина эпидермиса и толщина его рогового слоя была больше, чем в образцах контрольной группы. На 14-е сут эксперимента различия между изучаемыми группами были минимальны, отмечали утолщение эпидермиса у животных группы 2 по сравнению с контрольной группой.

Заключение. В проведенном исследовании продемонстрировано, что при использовании гидрогеля на основе дермы свиньи для лечения скарифицированных ран крыс полное восстановление кожи в пораженной области наступало на 1,5–2 сут быстрее, чем в контрольной группе. Помимо этого было зарегистрировано увеличение количества фибробластов в дерме и утолщение эпидермиса относительно аналогичного показателя у крыс контрольной группы.

Ключевые слова: дермальный гидрогель, скарифицированная рана, морфологический анализ, внеклеточный матрикс

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

Hydrogel dressings are the most promising material for the treatment of superficial wounds, as they prevent the formation of adhesions in the adjacent tissues, exert an analgesic effect, and have a positive effect on wound healing due to the contained biologically active components. Hydrogel dressings are semi-permeable to promote wound hydration, eschar rehydration, and autolytic wound debridement [1].

Hydrogels based on natural biologically active polymers (collagen, hyaluronic acid, chitosan, alginate, etc.) are biocompatible and biodegradable, have low cytotoxicity, and regulate the proliferation and functioning of fibroblasts, keratinocytes, macrophages, and endothelial cells [2]. These biomaterials can acquire anti-inflammatory and antibacterial properties when growth factors and bioactive peptides are added.

A promising material for biopolymer-based hydrogels is the extracellular matrix (ECM), which consists of collagen, elastin, glycosaminoglycan, and other biologically active molecules [3]. The source of ECM in most cases are animal tissues, in particular, the dermis of pigs and cattle, however, the technologies for their processing are quite expensive and time-consuming [4]. Recently, there has been an increased interest in the development of an optimal method for obtaining hydrogels based on ECM of animal tissues. The aim of this study was to evaluate the developed hydrogel based on porcine dermal ECM as a wound dressing for the experimental treatment of superficial wounds in laboratory animals.

MATERIALS AND METHODS

The research was carried out in compliance with the principles of humanity set out in the directives of the European Community (86/609/EEC) and the requirements of the Declaration of Helsinki, revision 2013. All manipulations met the requirements of the Order No. 708n of the Ministry of Health of Russia of 23.08.2010 “On Approval of the Rules of Laboratory Practice”, the Bioethics Committee, and the Federal Law of the Russian Federation on the Protection of Animals (Article 4 of the Law of the Russian Federation “On the Protection of Animals from Cruelty” of 01.12. 1999).

The material for the creation of the dermal hydrogel was the skin of the Landrace pig (male, aged 2 months) weighing 13.4 kg. The animal was anesthetized with solutions of Zoletil (1 mg / kg; Zoletil 100, Virbac, France) and Xylazine (4 ml / kg; Rometar, Spofa, Czech Republic). Dermal samples with a thickness of 0.5 ± 0.05 mm were obtained from the lateral surface of the body after preliminary mechanical removal of the epithelial layer with an electrodermatome (disk knife diameter 100 mm) under sterile conditions. The collected dermis was stored for 1–6 months at –80 °C for preliminary cryodestruction of the cellular elements in the dermis.The porcine skin samples were chemically decellularized, in particular treated with 5% aqueous NaOH solution, at a sample weight per solution volume ratio of 1:5 for 22.5 hours. After that, the samples were washed with deionized water until a stable neutral pH was reached. DNA content was determined in the dermal hydrogel samples with the Nano Drop-1000 spectrophotometer (Thermo Fisher Scientific Inc., USA) using the reagent kit (General DNA Quantification Kit, Abcam, UK) according to the manufacturer’s protocol.The efficiency of the obtained dermal hydrogel was studied on 30 male Sphinx rats (weight 160–200 g, age 3–4 months), kept in a vivarium with a balanced diet and natural light. The rats were divided into two groups: group 1 – rats without dermal hydrogel treatment or control group (n = 15), group 2 – rats with dermal hydrogel treatment (n = 15). Under general gaseous anesthesia with Isoflurane (induction 2–5%, flow 0.25–4%; Laboratorios Karizoo, Spain), scarified wounds 30 x 20 x 2 mm in size were created in the rats in the area of the withers along the marked surface. The wounds in the rats of group 2 were treated daily for 5 days with 0.5 g dermal hydrogel. After the surgery, all animals were injected with the analgesic drug Ketoprofen 10% (5 mg / kg; Nita-Pharm, Russia) and the antibiotic Convenia (4 mg / kg; Convention, Zoetis, USA). On day 3, 7, and 14 of the experiment, the skin samples (8 mm in diameter) were explanted in adjacent native tissues using a skin biopsy device (Medax, Italy); then these samples were histologically stained with hematoxylin and eosin.

Statistical processing of the obtained results on the content of DNA and morphometric data was carried out using the Graph Pad Prism version 6.04 and Microsoft Excel 2016 software. The results were presented as $M \pm S$, where M is the arithmetic mean, and S is the standard deviation. The differences were considered significant at $p < 0.05$, the significance of differences was calculated according to the Mann – Whitney test. To quantify histologic changes in the porcine dermis, computer morphometry was used by the ImageJ program (National Institution of Health, USA) and the IHC metrics plugin. Epidermal changes in the samples were evaluated using the Freehand Selection Tool.

RESULTS

The porcine skin samples after chemical decellularization acquired a gel-like structure after 22.5 hours (Fig. 1). The dermal hydrogel was clear, dense, and homogeneous. The finished dermal hydrogel contained a 1% solution of an antimycotic antibiotic (Gibco, Thermo Fisher Scientific, USA) and was stored under sterile conditions at a temperature of +4 °C.

The hydrogel based on the porcine dermis was an oxyphilic structure, which was predominantly homogeneous due to pronounced swelling of the polymers (Fig. 2, *b*).

The comparative quantitative analysis of the DNA content in the dermal hydrogel and native dermal samples showed that the amount of DNA in the dermal hydrogel decreased to 33.19 ng / mg of dry matter, $p < 0.05$ (17.43%) relative to the DNA content in the native dermis (190 .45 (100%) ng / mg of dry matter). The data obtained corresponded to the quality criterion for decellularized tissues – no more than 50 ng of DNA per 1 mg of dry tissue mass [5].The results of the study of dermal hydrogel showed that it has a fairly pronounced reparative effect and accelerates the process of wound healing in comparison with the animals in the control group. Thus, on day 9 of the study, the rats of group 2 showed no visual signs of inflammation, tissue necrosis, or scarring (Fig. 3).

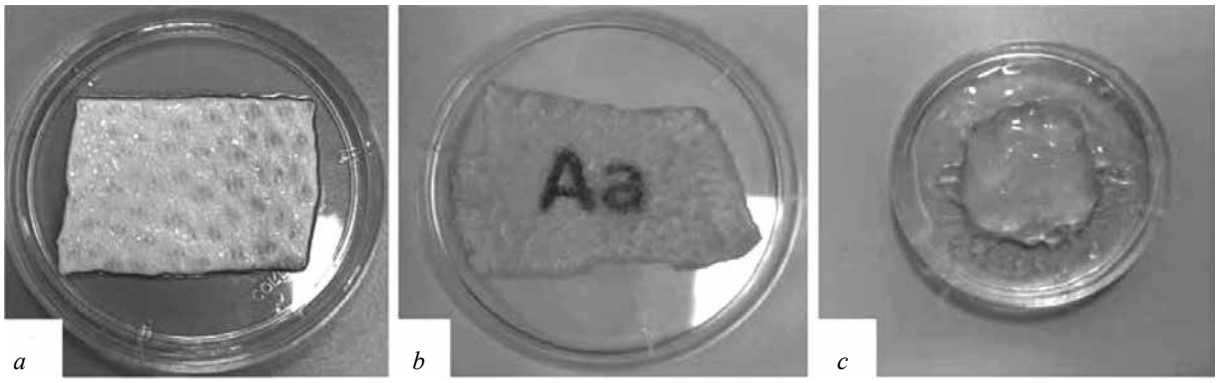

Fig. 1. The samples: *a* – native porcine dermis, *b* – dermis after 5 hours of treatment, *c* – sample of dermal hydrogel

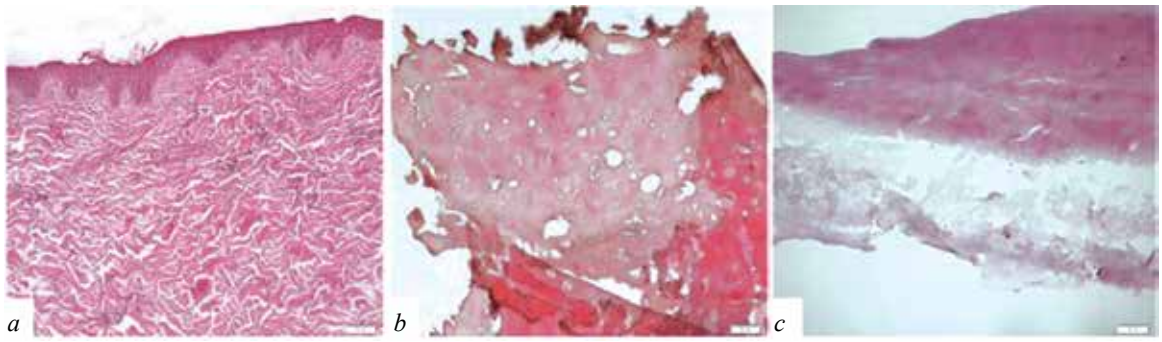

Fig. 2. Morphological analysis of the hydrogel: *a* – native porcine dermis, *b* – dermis after 5 hours of treatment, *c* – dermis after 22.5 hours of the treatment

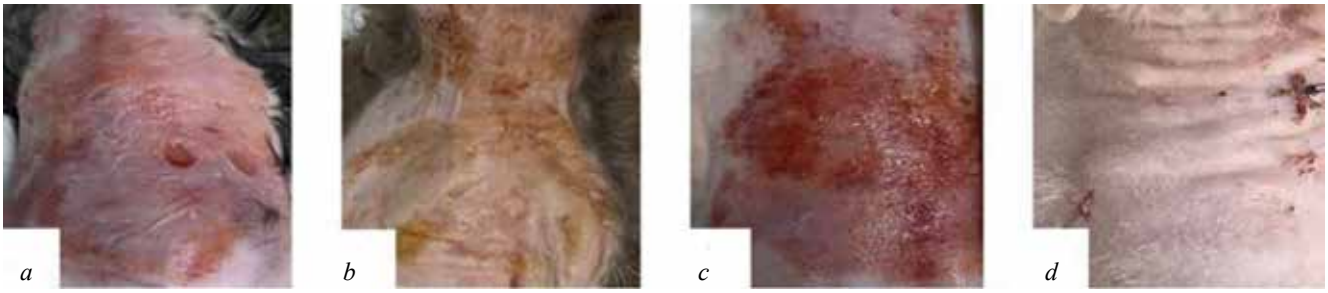

Fig. 3. The animals after the creation of a superficial scarified wound: *a, b* – the control group, no treatment; *c, d* – the experimental group, treatment with dermal hydrogel; *a, c* – right after the surgery; *b, d* – day 9 of the experiment

In the skin samples of untreated rats obtained on day 3 of the experiment, pronounced necrotic changes were observed, as well as obvious signs of inflammation and fibrinous purulent exudate (Fig. 4, *a*). On day 3 of the experimental treatment, the wounds treated with dermal hydrogel had moderately pronounced signs of inflammation. However, structural changes in this case were manifested through a pronounced edema of the wound surface with collagen fiber disorganization (Fig. 4, *d*).

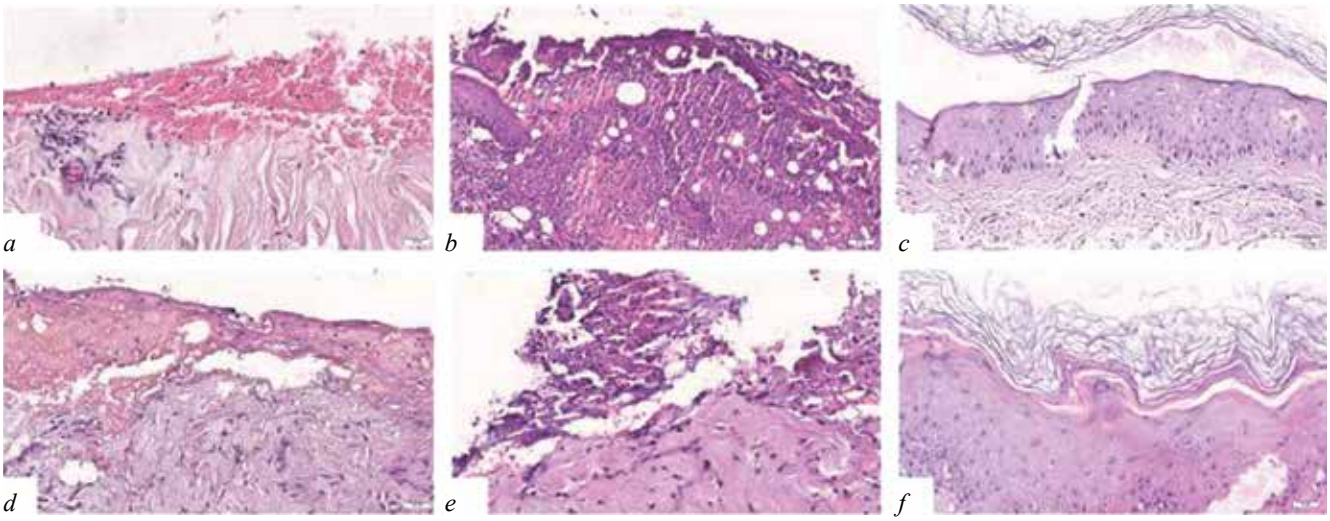

Fig. 4. Porcine skin from the wound area with adjacent tissues: *a, b, c* – the control group, no treatment; *d, e, f* – group 2, treatment with dermal hydrogel. *a, d* – day 3; *b, e* – day 7; *c, f* – day 14 of the experiment. Hematoxylin – eosin staining, x 100 magnification

On day 7, signs of inflammation were observed in the skin samples of the control rats, in particular, moderately pronounced infiltration. Against the background of resolving inflammation, signs of epidermal regeneration were noted in the form of foci of stratified epithelium proliferation (Fig. 4, *b*). The analysis of the skin samples of the animals of group 2 on day 7 showed more pronounced plethora of the microvasculature, necrotic changes in the dermis, and its edema (Fig. 4, *e*). Thickness of the epidermis in the rats of group 1 was 64.09 [52.82; 71.40] μm, which is less than that in the control animals (23.10 [16.56; 33.17] μm. The thickness of the stratum corneum in the epidermis of the control rats was also less ($p < 0.05$).

In the study of the porcine skin samples obtained 14 days after the gel was applied, the differences in the groups of animals were minimal: there was a slight difference in the thickness of the epidermis between the animals of group 2 (183.25 [155.07; 202.20] μm) and control rats (168.11 [144.26; 190.01] μm; $p < 0.05$; Fig. 4 *c, f*).

DISCUSSION

The most promising material for treatment of wounds are hydrogel dressings, whose high therapeutic efficacy as wound healing agents is proved by positive results of many studies [6]. It is known that hydrogels play a key role in the delivery of bioactive molecules and cellular products to the damaged area, unlike other types of modern wound dressings. Biological hydrogels promote autolysis of necrotic tissues, and their main property is a high degree of hydration to ensure a bactericidal effect and create optimal conditions for healing [7, 8].

Currently, an active search is underway for the most "perfect" collagen-containing hydrogel. There are numerous studies and developments for obtaining hydrogels from various tissues. The closest to our proposed method of processing the dermis to obtain a hydrogel is the method proposed by N.V. Kalmykova et al. [9]. The author obtained an ECM from the dermis of cattle in several ways – by treating with a 1M NaOH solution and a NaOH solution at a lower concentration with the addition of Na_2SO_4 and H_3BO_3 solutions and subsequent lyophilization of the obtained material. As a result of the processing of the dermis, they received a lyophilized ECM with a high content of collagen, but an additional processing step was necessary to obtain its hydrogel form.

In another study, F.T. Rodrigez et al. [10] developed a wound healing material based on the

porcine dermis treated with solutions of concentrated alkali and alkaline earth metal salts. The resulting material was further treated with a cross-linking agent, glutaraldehyde. However, the addition of crosslinkers can affect the toxicity and immunogenicity of the resulting material. Q.W. Tan et al. [11] obtained a hydrogel from porcine adipose tissue, which was decellularized using solutions of sodium dodecyl sulfate, pepsin, and hydrochloric acid, which are quite expensive. The dermal hydrogel proposed by us was obtained on the basis of porcine dermis, which is a less immunogenic biological material than synthetic materials or materials obtained using synthetic detergents. In addition, in our proposed method, there is no additional lyophilization step.

In the study, when using dermal hydrogel for the treatment of scarified wounds, on day 14, complete restoration of the skin in the affected area, a large number of fibroblasts, and thickening of the epidermis were noted compared to the animals in the control group. This is also confirmed by the data of other researchers, for example, H. Fujisaki et al. [12] noted that collagen hydrogels containing mainly type IV and I collagens support adhesion, proliferation, and growth of fibroblasts. It is known that collagen has a positive effect on early stages of wound healing, as it promotes platelet aggregation, stimulates the formation of granulation tissue, etc. Collagen lysis contributes to the enrichment of the wound with amino acids, which leads to the activation of protein biosynthesis in skin cells. Thus, in a study by T. M. Cherdantseva et al. [13], there was a slower increase in the area of granulation tissue, a slower decrease in the number of mast cells, and a decrease in their area and degranulation coefficient compared to animals in the control group. The author noted that in other studies on the effect of collagen-containing wound dressings, the ability of collagen to reversibly bind growth factors, protecting them from proteolysis, was revealed, which explains slower formation of granulation tissue in the group of rats whose burn wound was treated with a collagen matrix. Thus, due to its bioactive properties, the dermal hydrogel obtained by us promotes accelerated healing of scarified wounds, which correlates with the data of other researchers on the study of collagen-containing wound dressings. The simplicity and low cost of the technology for obtaining hydrogel from porcine dermis make it a potentially promising and competitive domestic biological material for wound healing.

CONCLUSION

The study demonstrated the effectiveness of using a dermal hydrogel based on the porcine dermal ECM in the experimental treatment of superficial scarified wounds. The use of the dermal hydrogel on scarified animal wounds led to earlier full recovery of the skin in the affected area, a greater number of fibroblasts, and more significant thickening of the epidermis compared to the control animals.The developed dermal hydrogel makes it possible to effectively protect the wound from bacterial microflora, accelerate wound healing, and create optimal conditions for active regeneration in the affected area. Further research on the use of dermal hydrogel as a therapeutic drug for wounds of various types will allow for the creation of a highly effective wound healing agent that has significant advantages among existing wound dressings.

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Authors' contribution

Melkonyan K.I., Rusinova T.V. – conception and design, justification of the manuscript, critical revision of the manuscript for important intellectual content. Kozmay Ya.A., Chuprynin G.P., Kartashevsky I.I. – carrying out of the experiment, analysis and interpretation of the data, drafting of the manuscript. Storozhuk S.V., Kartashevskaya M.I., Selezneva I.I. – review of literature on the research topic, analysis and interpretation of the data. Gurevich K.G. – final approval of the manuscript for publication.

Authors' information

Melkonyan Karina I. – Cand. Sci. (Med.), Associate Professor, Head of the Central Research Laboratory, Kuban State Medical University, Krasnodar, kimelkonian@gmail.com, http://orcid.org/0000-0003-2451-6813

Kozmay Yana A. – Junior Researcher, Central Research Laboratory, Kuban State Medical University, Krasnodar, yana.kozmay@gmail.com, http://orcid.org/0000-0001-5043-4315

Rusinova Tatyana V. – Cand. Sci. (Biology), Researcher, Central Research Laboratory, Kuban State Medical University, Krasnodar, rusinova.tv@mail.ru, http://orcid.org/0000-0003-2962-3212

Chuprynin Gleb P. – Junior Researcher, Central Research Laboratory, Kuban State Medical University, Krasnodar, chupryningp@ksma.ru, http://orcid.org/0000-0002-0120-2689

Kartashevskaya Marina I. – Cand. Sci. (Med.), Teaching Assistant, Department of Dermatovenereology, Kuban State Medical University, Krasnodar, marinaikar@mail.ru http://orcid.org/0000-0001-9060-2969

Kartashevsky Igor I. – 2nd-year Student, Department of Dentistry, Kuban State Medical University, Krasnodar, igor.igo.life@gmail.com, http://orcid.org/0000-0001-5725-6902

Storozhuk Sergey V. – Researcher, Central Research Laboratory, Kuban State Medical University, Krasnodar, sergejstorozuk232@gmail.com, https://orcid.org/0000-0002-9957-3567

Selezneva Irina I. – Cand. Sci. (Physics and Mathematics), Leading Researcher, Laboratory for Cell and Tissue Growth, Institute of Theoretical and Experimental Biophysics, Pushchino, selezneva_i@mail.ru, http://orcid.org/0000-0002-3444-5900

Gurevich Konstantin G. – Dr. Sci. (Med.), Professor, Head of the UNESCO Division "Healthy lifestyle – the key to successful development", Yevdokimov Moscow State University of Medicine and Dentistry, Moscow, kgurevich@mail.ru, https://orcid.org/0000-0002-7603-6064

(✉) **Melkonyan Karina I.,** kimelkonian@gmail.com

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Beta-adrenergic receptor reactivity of erythrocyte membranes in patients with left or right atrial dilatation against the background of atrial fibrillation

Muslimova E.F.[1], Popova V.O.[2], Rebrova T.Y.[1], Archakov E.A.[1], Batalov R.E.[1], Afanasiev S.A.[1]

[1] *Cardiology Research Institute, Tomsk National Research Medical Center (NRMC) of the Russian Academy of Sciences 111a, Kievskaya Str., Tomsk, 634012, Russian Federation*

[2] *Siberian State Medical University 2, Moscow Trakt, Tomsk, 634050, Russian Federation*

ABSTRACT

Hyperactivation of the sympathoadrenal system (SAS) leads to desensitization of β1-adrenergic receptors (β1-AR). This contributes to aggravation of myocardial contractile dysfunction and development of arrhythmias, including atrial fibrillation (AF). An indirect indicator of the viability of β1-AR is β-adrenergic receptor reactivity of erythrocyte membranes (β-ARM).

Aim. To evaluate β-ARM in patients with different forms of AF, including left (LAD) or right (RAD) atrial dilation.

Materials and methods. The sample included 38 patients, 65.8% of whom had paroxysmal AF, 21% had persistent AF, and 13.2% had long-standing persistent AF. All patients received surgical treatment for AF by radiofrequency ablation or cryoablation. LAD was detected in 39.4% of patients, RAD – in 34.2% of patients. Beta-ARM was determined before treatment, as well as at 3 days and at 12 months after ablation.

Results. The groups of patients with different forms of AF, as well as patients with LAD / RAD and without it showed comparable values of β-ARM at different measurement periods. In the group of patients without LAD / RAD, β-ARM increased 3 days after ablation compared to β-ARM before the treatment (p = 0.002 / p = 0.004) and returned to the pre-treatment level after 3 months. At the same time, in the group of patients with LAD / RAD, β-ARM did not significantly change before the ablation and in different periods after it.

Conclusion. In patients with AF without LAD / RAD, we detected an increase in β-ARM 3 days after the ablation compared to the level before the treatment and a decrease in the intensity of SAS 3 months after the surgery. In the presence of LAD / RAD, no changes in the β-ARM were revealed.

Keywords: beta-adrenergic receptor reactivity of erythrocyte membranes, atrial fibrillation, atrial dilatation

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

Source of financing. The study was carried out within the basic research topic No.122020300183-4.

Conformity with the principles of ethics. All study participants signed an informed consent. The study was approved by the local Ethics Committee at Cancer Research Institute of Tomsk NRMC (Protocol No. 208 of 20.01.2021).

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✉ *Muslimova Elvira F.,* muslimovef@yandex.ru

β-адренореактивность мембран эритроцитов у пациентов с дилатацией левого или правого предсердий на фоне фибрилляции предсердий

Муслимова Э.Ф.[1], Попова В.О.[2], Реброва Т.Ю.[1], Арчаков Е.А.[1], Баталов Р.Е.[1], Афанасьев С.А.[1]

[1] *Научно-исследовательский институт (НИИ) кардиологии, Томский национальный исследовательский медицинский центр (НИМЦ) Российской академии наук*
Россия, 634012, г. Томск, ул. Киевская, 111а

[2] *Сибирский государственный медицинский университет (СибГМУ)*
Россия, 634050, г. Томск, Московский тракт, 2

РЕЗЮМЕ

Гиперактивация симпатоадреналовой системы (САС) приводит к десенситизации β1-адренорецепторов (β1-АР). Это способствует усугублению сократительной дисфункции миокарда и развитию аритмий, в том числе фибрилляции предсердий (ФП). Косвенным показателем состоятельности β1-АР является β-адренореактивность мембран эритроцитов (β-АРМ).

Цель: оценка β-АРМ у пациентов с разными формами ФП, в том числе с дилатацией левого (ДЛП) или правого (ДПП) предсердия.

Материалы и методы. В выборку включены 38 пациентов, из них 65,8% с пароксизмальной, 21% с персистирующей, 13,2% с длительно персистирующей формами ФП. Всем пациентам проведено оперативное лечение ФП методом радиочастотной или криоаблации. ДЛП выявлена у 39,4% пациентов, ДПП – у 34,2% пациентов. В-АРМ определяли до лечения, через 3 сут, 3 и 12 мес после аблации.

Результаты. Группы пациентов с разными формами ФП, а также пациенты с ДЛП/ДПП и без нее показали сопоставимые значения β-АРМ на разных сроках измерения. В группе без ДЛП/ДПП β-АРМ повышалась через 3 сут после аблации по сравнению с β-АРМ до лечения (p = 0,002/p = 0,004) и через 3 мес вернулась к уровню до лечения. В то же время в группе пациентов с ДЛП/ДПП β-АРМ значимо не менялась до и в разные периоды после аблации.

Заключение. У пациентов с ФП без ДЛП/ДПП выявлено повышение β-АРМ через 3 сут после аблации по сравнению с уровнем до лечения и снижение напряженности САС через 3 мес. При наличии ДЛП/ДПП динамика в β-АРМ отсутствовала.

Ключевые слова: β-адренореактивность мембран эритроцитов, фибрилляция предсердий, дилатация предсердий

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

Источник финансирования. Работа выполнена в рамках темы ФНИ № 122020300183-4.

Соответствие принципам этики. Все участники исследования подписали информированное добровольное согласие. Исследование одобрено локальным этическим комитетом НИИ кардиологии Томского НИМЦ (протокол № 208 от 20.01.2021).

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INTRODUCTION

Cardiac rhythm disturbances are some of the most common problems in cardiology practice. Arrhythmias are associated with an increased risk of cardiovascular complications, lead to a decline in the quality of life, disability, and high mortality [1]. Currently, atrial fibrillation (AF) is one of the most common cardiac rhythm disturbances. Patients with AF experience an observable decline in the quality of life, exercise

tolerance; at the same time, left ventricular dysfunction appears and / or progresses with the development of heart failure in patients with AF [2].

It is known that the development of cardiovascular diseases, including cardiac rhythm disturbances, is accompanied by a persistent increase in the activity of the sympathoadrenal system (SAS). The level of catecholamines (adrenaline and norepinephrine) increases in the blood and myocardium, and it can affect the number and functional state of β1-adrenergic receptors (β1-AR) in cardiomyocytes [3].

Hyperactivation of the SAS leads to desensitization of β1-AR and a decrease in the number of receptors on the membrane of cardiomyocytes, which contributes to aggravation of myocardial contractile dysfunction [4]. This, in turn, can lead to a further increase in sympathetic activation, thereby forming a vicious cycle [5].

Evaluation of the functionality of β1-AR and the SAS activity is important for predicting the severity of the course of cardiovascular diseases, including cardiac rhythm disturbances. In particular, based on this approach, a method for predicting ventricular tachycardia in patients with coronary heart disease has been developed [6]. The β-adrenergic receptor reactivity of erythrocyte membranes (β-ARM) is an indirect indicator of the SAS activity. It is determined by the distribution density of β-AR on cell membranes, the degree of their affinity with plasma catecholamines, and the concentration of catecholamines. The method for evaluating β-ARM is based on studying the effect of various adrenergic agents on osmotic resistance of erythrocytes. A β-blocker, which binds to β-AR of the cell membrane and reduces the degree of hemolysis, is used [4].

Thus, the **aim** of this study was to evaluate β-ARM in patients with different types of AF, including those with left or right atrial dilation.

MATERIALS AND METHODS

The study sample included 38 patients (25 (65.8%) men and 13 (34.2%) women) admitted to the Department of Cardiac Pacing and Heart Arrhythmia Surgical Treatment of Cardiology Research Institute, Tomsk National Research Medical Center (NRMC) of the Russian Academy of Sciences. The age in the sample was 49 (26; 77) years. All clinical and laboratory studies were conducted in accordance with the ethical standards of the Biomedical Ethics Committee and the 1964 Declaration of Helsinki and its subsequent amendments.

All patients were diagnosed with atrial fibrillation (AF) based on the results of ECG and daily monitoring [7]. All patients underwent surgical treatment for AF using radiofrequency ablation (RFA) or cryoballoon ablation (CBA) according to the generally accepted method. The intervention included pulmonary vein antrum isolation with a circular electrode and a complete block of atrio – venous electrical conduction.

The clinical characteristics of the patients are presented in Table 1. Before surgical treatment of AF, the patients underwent an echocardiography during the examination. Echocardiography was performed using a Philips HD15 device (Netherlands) from standard positions with an evaluation of the size of the heart chambers and the left ventricular (LV) ejection fraction using the Simpson method. Some patients, in addition to AF, had other cardiac rhythm disturbances: 3 (7.9%) patients – ventricular extrasystole, 1 (2.6%) patient – supraventricular extrasystole, 2 (5.3%) patients – first-degree atrioventricular block.

Table 1

Clinical characteristics of patients, *n* (%)

Parameter	Value
Paroxysmal/persistent/long-standing persistent AF, *n* (%)	25 (65.8) / 8 (21) / 5 (13.2)
RFA / CBA, *n* (%)	31 (81.6) / 7 (18.4)
AF rhythm at admission, *n* (%)	18 (47.4)
Chronic heart failure, NYHA class I/II/III, *n* (%)	11 (28.9) / 3 (7.9) / 3 (7.9)
Coronary heart disease, *n* (%)	7 (18.4)
Essential hypertension, *n* (%)	22 (57.9)
Diabetes mellitus, *n* (%)	1 (2.6)
Obesity, *n* (%)	13 (34.2)
Left ventricular ejection fraction, %, *Me* (Q_1–Q_3)	65.5 (59.0; 69.0)
End-systolic volume, ml, *Me* (Q_1–Q_3)	34 (29; 46)
End-diastolic volume, ml, *Me* (Q_1–Q_3)	104 (89; 119)
Left ventricular sphericity index, *Me* (Q_1–Q_3)	0.54 (0.51; 0.56)
Left atrial dilation, *n* (%)	15 (39.4)
Right atrial dilation, *n* (%)	13 (34.2)
Left ventricular dilation, *n* (%)	3 (7.9)

Note: FC – functional class.

In order to determine β-ARM, venous blood samples were taken from patients in a vacutainer with EDTA before ablation and 3 days, 3 months, and 12 months after it. We used the commercial kit "B-ARM-Agat" (Agat-Med LLC, Russia) in accordance with the manufacturer's protocol. The method is based on the fact that the hemolysis of erythrocytes placed in a hypoosmotic medium is inhibited in the presence of a β-blocker. The value of β-ARM was calculated using the formula: (Eo1 + Eo2)/(Ek1 + Ek2) x 100%, where β-ARM is the value

of the adrenergic receptor reactivity; Eo1 and Eo2 are optical densities of experimental samples; Ek1 and Ek2 are optical densities of control samples.

Statistical analysis was carried out using the SPSS, version 13 software (IBM, USA). Quantitative data were previously checked for normality of distribution using the Shapiro – Wilk test. Further analysis of quantitative variables was performed using the Mann – Whitney test or the Kruskal – Wallis test. The analysis of dependent data was performed using the Wilcoxon test. The results were presented as the median and the interquartile range Me (Q_1–Q_3). The strength of the linear relationship between quantitative parameters was assessed using the Spearman's rank correlation coefficient r. The relationship between qualitative data was determined using the Pearson's $\chi 2$ test or the two-tailed Fisher's exact test. The differences were statistically significant at $p < 0.05$.

RESULTS

The changes in β-ARM were evaluated before ablation in 37 patients, 3 days after ablation – in 35 patients, 3 months after ablation – in 17 patients, and 12 months after ablation – in 4 patients. Beta-ARM was 19.7% (12.9; 27.5), 24.1% (15.1; 32.1), 20.3% (9.3; 29.3), and 32.5% (20.0; 43.2), respectively. There was a slight β-ARM increase 3 days after ablation compared to β-ARM before the treatment (Figure), but the significance level was $p = 0.060$. After 3 months, the β-ARM value was similar to the level before ablation ($p = 0.758$). After 12 months, the level of β-ARM increased by 1.5 times compared to the value before the treatment, however, due to the small sample of patients at this point, the differences did not show statistical significance ($p = 0.465$). We did not include the 12-month point in the further analysis due to a small sample size.

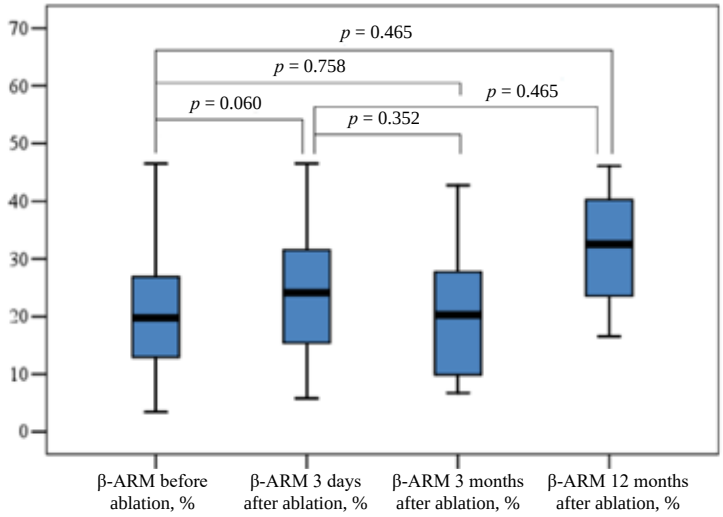

Figure. Changes in β-ARM before and after ablation

In the study sample, a direct linear correlation was found between β-ARM before ablation and end-systolic volume ($r = 0.331$, $p = 0.046$), as well as left ventricular sphericity index ($r = 0.436$, $p = 0.007$). However, there was no significant linear relationship between β-ARM before ablation and left ventricular ejection fraction (LVEF) in patients with AF ($r = –0.169$, $p = 0.316$).

Beta-ARM before ablation, 3 days and 3 months after ablation was comparable between the groups of patients with different types of AF. In addition, β-ARM did not change significantly after ablation compared to the point before therapy in groups with different types of AF. Patients with persistent and long-standing persistent AF compared to patients with paroxysmal AF showed an expected decrease in LVEF and an increase in end-systolic volume (ESV) and end-diastolic volume (EDV). The results are presented in Table 2.

In the studied sample, left atrium dilation (LAD) was detected in 5 (20%) of 25 patients with paroxysmal AF, in 6 (75%) of 8 patients with persistent AF, and in 4 (80%) of 5 patients with long-standing persistent AF ($p = 0.003$). Patients with LAD had higher ESV, EDV, and LVSI (Table 3).

There were no significant differences in β-ARM both before ablation and at different periods after the treatment between the groups of patients with and without LAD (Table 3). In the group without LAD, a significant increase in β-ARM was detected

3 days after ablation compared to β-ARM before the treatment (p = 0.002), and 3 months after ablation, β-ARM was similar to the level before the treatment (p = 0.678). At the same time, in the group of patients with LAD, β-ARM did not change significantly before and in different periods after ablation.

Table 2

Echocardiography parameters and β-adrenergic receptor reactivity of erythrocyte membranes in patients with different types of atrial fibrillation, *Me* (Q_1; Q_3)

Parameter	Paroxysmal AF	Persistent AF	Long-standing persistent AF	p
LVEF, %	67 (64; 70)	64 (47; 67)	62 (44; 63)	0.045
ESV, ml	32 (27; 38)	38 (31; 71)	48 (46; 76)	0.007
EDV, ml	96 (86; 108)	112 (96; 127)	126 (121; 131)	0.007
LV SI	0.54 (0.52; 0.56)	0.57 (0.52; 0.59)	0.56 (0.56; 0.58)	0.394
β-ARM before ablation, %	24.1 (14.2; 27.5)	15.1 (11.0; 26.3)	17.1 (16.2; 17.8)	0.476
β-ARM 3 days after ablation, %	24.6 (17.4; 36.6)	16.0 (11.3; 31.5)	21.6 (15.1; 23.3)	0.438
β-ARM 3 months after ablation, %	18.9 (8.5; 26.7)	21.3 (15.1; 40.9)	26.1 (11.9; 40.4)	0.664

Note: LVSI – left ventricular sphericity index.

Table 3

Echocardiography parameters and β-adrenergic receptor reactivity of erythrocyte membranes during left atrial dilation, *Me* (Q_1; Q_3)

Parameter	With LA dilation	Without LA dilation	p
LVEF, %	64 (47; 67)	67 (64; 70)	0.064
ESV, ml	39 (32; 77)	32 (28; 38)	0.024
EDV, ml	118 (97; 140)	98 (86; 112)	0.013
LVSI	0.57 (0.55; 0.60)	0.54 (0.52; 0.56)	0.048
β-ARM before ablation, %	18.7 (15.7; 33.0)	17.2 (12.0; 24.6)	0.171
β-ARM 3 days after ablation, %	20.8 (13.5; 32.1)	24.4 (16.7; 33.8)	0.474
β-ARM 3 months after ablation, %	21.3 (10.9; 34.0)	18.9 (8.8; 25.8)	0.606

Among all patients included in the study, right atrium dilation (RAD) was detected in 5 (20%) patients with paroxysmal AF, in 4 (50%) patients with persistent AF, and in 4 (80%) patients with long-standing persistent AF (p = 0.020). Patients with RAD had lower LVEF, higher ESV, EDV, and LVSI (Table 4). Also, β-ARM before ablation was 1.4 times higher in the patients with RAD than in those without dilation before ablation, but the difference did not reach statistical significance (p = 0.07). With RAD, β-ARM values remained at the same level both before ablation and after therapy at different times.

At the same time, in the group without RAD, a statistically significant increase in β-ARM at 3-days point was detected compared to β-ARM before ablation (p = 0.004), and then a return of β-ARM to the pre-ablation level after 3 months was noted (p = 0.959).

Table 4

Echocardiography parameters and β-adrenergic receptor reactivity of erythrocyte membranes during RA dilation

Parameter	With RA dilation	Without RA dilation	p
LVEF, %	62 (46; 66)	67 (64; 70)	0.030
ESV, ml	46 (31; 77)	32 (28; 38)	0.022
EDV, ml	121 (96; 145)	98 (86; 109)	0.018
LVSI	0.57 (0.56; 0.60)	0.54 (0.51; 0.56)	0.012
β-ARM before ablation, %	23.6 (15.9; 36.1)	17.2 (12.0; 24.6)	0.070
β-ARM 3 days after ablation, %	22.9 (14.3; 37.1)	23.8 (15.6; 30.9)	0.945
β-ARM 3 months after ablation, %	22.4 (10.9; 34.0)	19.6 (8.8; 25.8)	0.601

DISCUSSION

It is well known that SAS hyperactivation is a risk factor for cardiac rhythm disturbances. Elevated levels of catecholamines stimulate β1-AR of cardiomyocytes, which leads to activation of adenylate cyclase, an increase in the content of intracellular cAMP, and activation of protein kinase A. Hyperphosphorylation of ryanodine receptors promotes leakage of Ca^{2+} from

the sarcoplasmic reticulum, resulting in a decrease in the cardiac contractile function and an increased risk of arrhythmia [8].

Hyperactivation of the SAS also leads to pathological remodeling of β-AR. There is a dissociation of the receptor from G-proteins and a decrease in the density of receptors on the cell membrane up to cessation of receptor synthesis, which leads to progression of myocardial contractile dysfunction. An increased load on the left ventricle inevitably leads to an increase in pressure and overload of the atria, causing their subsequent dilation [3, 5].

Beta-ARM makes it possible to indirectly evaluate the functional state of β-AR. In most healthy individuals, this parameter is within the range of 2.0–20.0%, which indicates an increase in the osmotic resistance of erythrocytes as a result of blocking β-AR with an adrenergic blocker. An increase in β-ARM indicates receptor desensitization [4].

In our sample of patients with AF, β-ARM did not exceed the conditional norm before ablation. On day 3 after ablation, a slight increase in β-ARM values was observed compared to the level before ablation (although the significance level was not reached), which can be regarded as a response of the SAS to surgical intervention. After 3 months, β-ARM returned to the values before ablation. It can be assumed that, in general, there was no significant desensitization of β-AR in the study sample. However, in the studied sample, a direct linear correlation was found between β-ARM before ablation and ESV, as well as LVSI. In the group of patients with a long-standing persistent AF, the ESV values exceeded the limits of the reference values (> 43 ml / m^2) [9], which, together with an increase in β-ARM, indicates a deterioration in the LV systolic function.

It has been shown that the values of β-ARM significantly exceed the limits of the conditional norm in patients with myocardial infarction (46.8%), and the values turned out to be even higher in patients with advanced heart failure (58.8%) [10]. However, in the case of cardiac rhythm disturbances, lower levels of β-ARM also cannot be considered as an absolutely favorable indicator. Beta-ARM less than 51.26% and lower in patients with coronary heart disease is an independent predictor of ventricular tachycardia [6].

In our sample of patients with AF, the values of β-ARM in groups with different types of AF were comparable before ablation, as well as 3 days and 3 months after ablation. In addition, there were no significant changes in β-ARM in each group of patients with different types of AF.

At the same time, patients with persistent and long-standing persistent AF had reduced LVEF and higher ESV and EDV compared to these parameters in patients with paroxysmal AF, which indicates a deterioration in the LV systolic function. In addition, the group of patients with paroxysmal AF had the lowest prevalence of LAD and RAD.

It is well known that atrial dilation can create a substrate for electrophysiological myocardial remodeling, which, together with progressive structural remodeling, forms the basis for the onset of AF. The presence of this rhythm disturbance, in turn, contributes to further atrial remodeling [11].

In the studied sample, in groups of patients without atrial dilation, an increase in β-ARM above the conditional norm was detected 3 days after ablation. This can be explained by the normal response of the SAS to stress. After 3 months, the level of β-ARM again returned to the level before ablation, while not exceeding the limits of the conditional norm, which makes it possible to assess the restoration of the functional activity of β-AR.

However, in groups of patients with LAD / RAD, β-ARM values remained at the same level both before ablation and after the therapy at different times, while the values were at the upper limit or above the conditional norm. According to the feedback mechanism, which accounts for desensitization, the smaller the number of receptors on cell membranes, the higher the level of catecholamines in the blood [4]. The values of β-ARM in patients with atrial dilation may be due to increased activity of the SAS and indicate protective desensitization that maintains myocardial contractile reserve within the conditions of its structural changes.

Thus, the evaluation of the functioning of β1-AR and the SAS activity is of great importance for predicting the severity of the course of cardiovascular diseases.

CONCLUSION

Thus, higher values of β-ARM before ablation directly correlated with higher values of ESV and LVSI, which indicates a deterioration in LV systolic function. However, there was no correlation between β-ARM before ablation and LVEF. Patients with different types of AF had comparable levels of β-ARM. Patients with AF without left / right atrial dilation

showed an increase in β-ARM 3 days after ablation compared to the level of β-ARM before the treatment and a decrease in SAS tension after 3 months. In the presence of left / right atrial dilation in patients with AF, there were no changes in β-ARM.

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Authors' contribution

Muslimova E.F. – analysis and interpretation of the data, critical revision of the manuscript for important intellectual content. Popova V.O. – analysis and interpretation of the data, justification of the manuscript. Rebrova T.Yu. – analysis and interpretation of the data. Archakov E.A. – analysis and interpretation of the data, critical revision of the manuscript for important intellectual content. Batalov R.E., Afanasiev S.A. – conception and design, final approval of the manuscript for publication.

Authors' information

Muslimova Elvira F. – Cand. Sci. (Med.), Researcher, Laboratory for Molecular and Cellular Pathology and Genetic Testing, Cardiology Research Institute, Tomsk NRMC, Tomsk, muslimovef@yandex.ru, https://orcid.org/0000-0001-7361-2161

Popova Valeria O. – Student, the Department of Biomedicine, Siberian State Medical University, Tomsk, popovalerie@yandex.ru

Rebrova Tatiana Yu. – Cand. Sci. (Med.), Researcher, Laboratory for Molecular and Cellular Pathology and Genetic Testing, Cardiology Research Institute, Tomsk NRMC, Tomsk, rebrova@yandex.ru, https://orcid.org/0000-0003-3667-9599

Archakov Evgeny A. – Cand. Sci. (Med.), Researcher, Laboratory for High Technologies, Diagnostics and Treatment of Cardiac Arrhythmias, Cardiology Research Institute, Tomsk NRMC, Tomsk, aea_cardio@mail.ru, https://orcid.org/0000-0002-2530-361X

Batalov Roman E. – Dr. Sci. (Med.), Head of the Laboratory for High Technologies, Diagnostics and Treatment of Cardiac Arrhythmias, Cardiology Research Institute, Tomsk NRMC, Tomsk, romancer@cardio-tomsk.ru, https://orcid.org/0000-0003-1415-3932

Afanasiev Sergey A. – Dr. Sci. (Med.), Professor, Head of the Laboratory for Molecular and Cellular Pathology and Genetic Testing, Cardiology Research Institute, Tomsk NRMC, Tomsk, tursky@cardio-tomsk.ru, https://orcid.org/0000-0001-6066-3998

(✉) **Muslimova Elvira F.,** muslimovef@yandex.ru

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Prevalence of generalized anxiety disorder symptoms and their associations with behavioral attitudes and perception of the future in the Russian youth

Peshkovskaya A.G.[1, 2], Galkin S.A.[1, 2], Larionova A.V.[1], Kornetov A.N.[3]

[1] *National Research Tomsk State University*
36, Lenina Av., Tomsk, 634050, Russian Federation

[2] *Mental Health Research Institute, Tomsk National Research Medical Center (NRMC) of the Russian Academy of Sciences*
4, Aleutskaya Str., Tomsk, 634014, Russian Federation

[3] *Siberian State Medical University*
2, Moscow Trakt, Tomsk, 634050, Russian Federation

ABSTRACT

Aim. To investigate the prevalence of generalized anxiety disorder symptoms and their associations with behavioral attitudes and perception of the future among the Russian youth.

Materials and methods. The study involved 1,300 people aged 16–25 years and was conducted online in October 2022 using online questionnaires, which included the GAD-7 screening questionnaire on the severity of anxiety symptoms.

Results. According to the questionnaire, 25.5% of young people who participated in the study reported medium to high intensity of anxiety symptoms. These respondents were significantly more likely to perceive the image of the country' future negatively (p = 0.002). In addition, the largest proportion of people who reported a desire to leave the country (38.6%) was registered among young people who were at risk of generalized anxiety disorder. The authors emphasize that the study results should be interpreted in relation to the time period of data collection.

Conclusion. The study showed that generalized anxiety disorder symptoms negatively affected the subjective image of the future among young people and highlighted the need for primary psychological prevention in this age group.

Keywords: anxiety, youth, perceived future, screening, generalized anxiety disorder

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 respondents signed an informed consent to participation in the study and publication of the anonymized and generalized data. The study was approved by the Ethics Committee at Tomsk State University (Protocol No. 291 of 19.09.2022).

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✉ *Galkin Stanislav A.,* s01091994@yandex.ru

Распространенность симптомов генерализованного тревожного расстройства и их связь с поведенческими установками и восприятием будущего страны у российской молодежи

Пешковская А.Г.[1, 2], Галкин С.А.[1, 2], Ларионова А.В.[1], Корнетов А.Н.[3]

[1] Национальный исследовательский Томский государственный университет (НИ ТГУ)
Россия, 634050, Томск, пр. Ленина, 36

[2] Научно-исследовательский институт (НИИ) психического здоровья, Томский национальный исследовательский медицинский центр (НИМЦ) Российской академии наук
Россия, 634014, Томск, ул. Алеутская, 4

[3] Сибирский государственный медицинский университет (СибГМУ)
Россия, 634050, Томск, Московский тракт, 2

РЕЗЮМЕ

Цель: изучить распространенность симптомов генерализованного тревожного расстройства среди российской молодежи в современных реалиях и определить их связь с поведенческими установками и восприятием будущего.

Материалы и методы. Исследование, участие в котором приняли 1 300 человек в возрасте 16–25 лет, проводилось в октябре 2022 г. в онлайн-формате посредством заполнения электронных форм анкеты и скрининг-опросника уровня выраженности симптомов тревоги ГТР-7 (GAD-7).

Результаты. Согласно скрининг-опроснику, симптомы генерализованного тревожного расстройства были выявлены у 25,5% участников исследования. Молодые лица с риском генерализованного тревожного расстройства статистически значимо чаще воспринимали образ будущего страны резко негативно (p = 0,002). Кроме того, наибольшее число сообщивших о желании покинуть страну (38,6%) было зафиксировано среди молодых людей с риском генерализованного тревожного расстройства. Авторы подчеркивают, что результаты следует интерпретировать с привязкой к временному периоду сбора данных.

Заключение. Симптомы генерализованного тревожного расстройства ассоциированы с негативной оценкой будущего среди молодежи, что свидетельствует о необходимости первичной психологической профилактики в этой возрастной группе.

Ключевые слова: тревога, молодежь, восприятие будущего, скрининг, генерализованное тревожное расстройство

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

Источник финансирования. Результаты получены в рамках выполнения государственного задания Минобрнауки России, проект №FSWM-2022-0006.

Соответствие принципам этики. Все респонденты дали информированное согласие на участие в исследовании и публикацию данных в анонимном и обобщенном виде. Исследование одобрено этическим комитетом НИ ТГУ (протокол № 291 от 19.09.2022).

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INTRODUCTION

The health and psychological well-being of Russian young people, who are largely subjects of social change, are of key importance in the modern realities of the country's development. The COVID-19 pandemic, whose consequences for the mental health of the young working-age population will be observed for a long time [1, 2], the increase in international tension, which has an additional impact on the emotional state, social and psychological adaptation, and uncertainty about the future significantly increase the risk of developing

mental disorders, especially anxiety disorders [3]. Symptoms of anxiety and phobic disorders are among the most common mental health impairments in young people and are recorded more often in them than in the older population [4, 5]. Numerous studies have shown that symptoms of generalized anxiety disorder affect cognition by triggering emotion-driven decision-making, and also enhance avoidance behavior [6–10]. In particular, clinical samples show similar observations, for example, a study by E.M. Mueller et al., which included patients with generalized anxiety disorder [11]. The results of psychological studies on anxiety as a future-oriented mood state [12], which includes concern about what might happen and the expectation of a future threat [13, 14], emphasize the impact of anxiety on a negative evaluation of one's own future and a negative perception of the future in general [15].

Considering the above, the aim of the study was to investigate the prevalence of generalized anxiety disorder symptoms in Russian youth and to understand the association of anxiety symptoms with young people's behavioral attitudes and perception of the future.

MATERIALS AND METHODS

The study was conducted in compliance with the principles of the Declaration of Helsinki, approved by the Ethics Committee at Tomsk State University and was completely anonymous. Data collection was performed in the format of an online survey in October 2022 by sharing a link to an online form with questions for the respondent. The survey included three question blocks: (1) demographic (sex, age, academic major, educational institution); (2) the GAD-7 assessment questionnaire to diagnose anxiety symptoms and the risk of generalized anxiety disorder [16, 17]; (3) questions on perceived future, including perception of expected changes in the country over the next five years evaluated with the five-point Likert scale, and behavioral attitudes (motive and readiness to emigrate).

All respondents signed an informed consent to participate in the study and publish the data in an anonymous and generalized form. The study was carried out in compliance with all Russian and international regulations on scientific research involving human participants and in accordance with the Federal Law No. 152-FZ of 27.07.2006 "On Personal Data".

In total, the study involved 1,300 people aged 16 to 25 (18 [17; 19]) years, including 700 women (53.8% of the sample) and 600 men (46.2%). All the participants were students of higher educational institutions and vocational training colleges of Tomsk and the Tomsk region with majors in natural sciences (319 people, 24.5% of the total), social sciences (350, 27%), and engineering sciences (630, 48.5%).

The data were processed using the Statistica 12.0 software package for Windows (StatSoft). The data were presented as the median and the interquartile range Me [Q_1; Q_3] as well as in absolute and relative units (%). Normality of data distribution was verified using the Shapiro – Wilk test. The data obtained did not fit the normal distribution. The Pearson's test ($\chi 2$ test) and the Mann – Whitney U test were used to identify the significance of the differences in parameters between the groups. Correlations between the studied parameters were assessed using the Spearman's rank correlation coefficient (r_s). The differences were considered statistically significant at $p < 0.05$.

RESULTS

To investigate the prevalence and severity of anxiety and GAD symptoms in the young people, we analyzed data from the GAD-7 questionnaire of 1,300 participants. Based on the results, we divided all the respondents into two groups in accordance with the existing guidelines: group 1 with a minimal (0–4 points) or moderate (5–9 points) level of anxiety symptoms, n = 969 (74.5%), and group 2 with a medium (10–14 points) or high (15–21 points) level of anxiety symptoms, n = 331 (25.5%), which was considered as the risk of generalized anxiety disorder (Fig. 1). The resulting groups were comparable in terms of gender (p = 0.116) and age (p = 0.418).

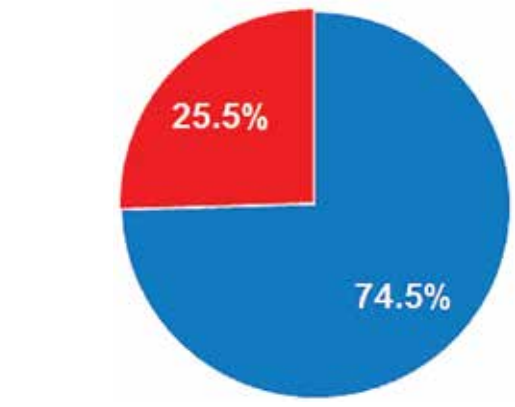

Fig. 1. Results of the assessment of generalized anxiety disorder symptoms (GAD-7) in Russian youth

Next, we analyzed the respondents' answers to questions clarifying the perception of Russia's future and migration behavior, particularly the motive and readiness to emigrate, taking into account the severity of reported anxiety symptoms. We found that the share of the respondents with a negative perception of the country's future was relatively low and ranged from 6.1 to 10.6% in groups of respondents with different levels of anxiety symptoms. However, the Pearson's test revealed significant differences in perception of the future between the groups ($p = 0.002$). Moreover, negative perception of the future showed a significant positive correlation with the severity of reported anxiety symptoms ($r_s = 0.372$; $p < 0.001$). Thus, young people who were at risk of generalized anxiety disorder based on the results of the GAD-7 assessment were significantly more likely to perceive the image of the country's future negatively (Fig. 2).

A similar analysis was carried out with respect to data on the intention of young people to emigrate if there is such an opportunity. The largest share of respondents who reported a motive to leave the country (38.6%) was among young people with medium to high level of anxiety symptoms who comprise the group at risk of generalized anxiety disorder (Fig. 3). Young people at risk of generalized anxiety disorder were significantly more likely to report a motive to leave the country ($p < 0.001$). The motive to emigrate also showed a positive correlation with the severity of anxiety symptoms in the surveyed sample ($r_s = 0.418$; $p < 0.001$).

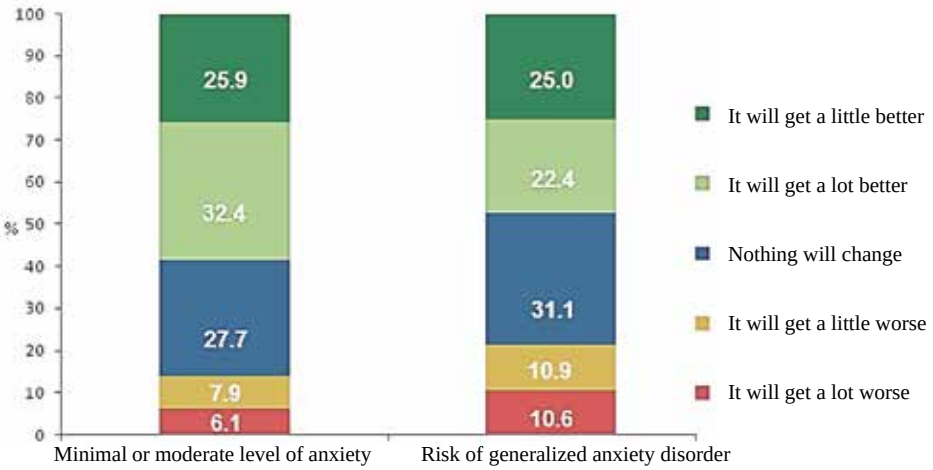

Fig. 2. Generalized anxiety disorder symptoms and perceived future of the country among Russian youth

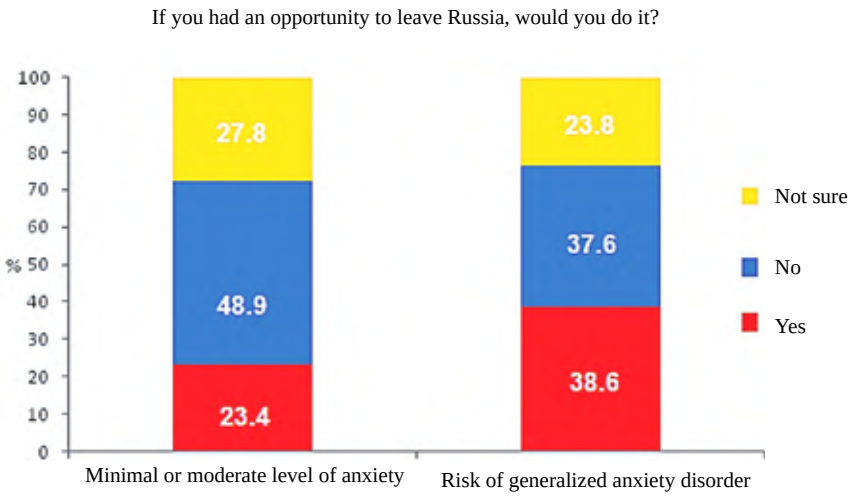

Fig. 3. Generalized anxiety disorder symptoms and migration intentions among Russian youth

DISCUSSION

The study results showed that a quarter (25.5%) of the surveyed young people had a high risk of generalized anxiety disorder. This parameter exceeds the average statistical data [6, 18, 19], which indicates the influence of current media and socio-political environment on the psychological well-being of young people. At the same time, the presence of generalized anxiety disorder symptoms among a relatively large number of young people requires attention from the psychological services of higher education institutions and vocational training colleges and psychological support services for the general population, since the identified anxiety parameters indicate vulnerability of this share of young people and a high risk of psychological distress, reduced level of social adaptation, and other mental health disorders. It is worth mentioning that, according to the majority of clinicians, unaddressed anxiety disorder in adolescents and young adults often progressed into depression in the future [18, 19].

We also found that in some young people the risk of generalized anxiety disorder was associated with a trend to perceive the future negatively. The data obtained were consistent with the results of previous studies [20, 21]. For example, in their study, I.A. Ralnikova [20] found that the level of anxiety affects perceived structure of the future: the more severe the level of anxiety, the lower the indicators of the future structure and the higher its uncertainty. Moreover, comparing our results with previous data obtained by the Levada Center in 2018 [22], 2019 [23], and 2020 [24], we observe a slight decrease in the positive expectations in youth and an increase in the share of those who reported a motive to emigrate. However, we emphasize that our results should be interpreted with reference to the time period of data collection.

Generally, anxiety symptoms including great severity associated with the risk of generalized anxiety disorder in youth are a significant socioeconomic and medical problem. Generalized anxiety disorder often leads to a disruption in social and professional activity and does not allow to maintain a fully-fledged lifestyle, which is especially important for working-age young people. Nevertheless, despite the importance of this disorder for public health, the vast majority of anxiety disorders remain undiagnosed, even in economically developed countries [25]. The prevention of anxiety disorders by other psychological tools obtained with the help of a psychologist and a psychotherapist is of particular importance. Taking into consideration the age group in which anxiety symptoms prevail, we should mention the work of an educational psychologist, which consists in creating a favorable psychological climate, supporting students' personal and professional growth, ensuring psychological security in students, teachers and employees, and supporting and strengthening their mental health and well-being.

CONCLUSION

The study showed that severity of anxiety symptoms associated with the risk of generalized anxiety disorder affects the subjective perception of the future in Russian youth. The obtained results can be used to provide psychological counseling to young people who are prone to experiencing anxiety in constructing a holistic view of the future, designing life prospects during crisis, as well as assistance in experiencing crisis periods.

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Authors' contribution

Peshkovskaya A.G. – conception and design. Peshkovskaya A.G., Larionova A.V. – methodology. Larionova A.V. – carrying out of the research. Galkin S.A. – analysis of the data. Galkin S.A., Peshkovskaya A.G. – drafting of the manuscript; Peshkovskaya A.G., Kornetov A.N. – editing of the manuscript. Larionova A.V., Peshkovskaya A.G. – research management.

Authors' information

Peshkovskaya Anastasia G. – Director of the Neuroscience Center, NR TSU; Junior Researcher, Mental Health Research Institute, Tomsk NRMC, Tomsk, peshkovskaya@data.tsu.ru, https://orcid.org / 0000-0002-3951-395X

Galkin Stanislav A. – Cand. Sci. (Med.), Junior Researcher, the Neuroscience Center, NR TSU; Junior Researcher, Mental Health Research Institute, Tomsk NRMC, Tomsk, s01091994@yandex.ru, https://orcid.org/0000-0002-7709-3917

Larionova Anastasia V. – Cand. Sci. (Psychology), Researcher, the Neuroscience Center, NR TSU, Tomsk, anpavlar@mail.ru, https://orcid.org / 0000-0002-8523-2913

Kornetov Alexander N. – Dr. Sci. (Med.), Professor, Head of the Fundamental Psychology and Behavioral Medicine Division, Siberian State Medical University, Tomsk, alkornetov@gmail.com, https://orcid.org/0000-0002-2342-7504

(✉) **Galkin Stanislav A.,** s01091994@yandex.ru

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The level of metabolic hormones in young people with arterial hypertension against the background of abdominal obesity

Polonskaya Ya.V., Kashtanova E.V., Stakhneva E.M., Shramko V.S., Sadovski E.V., Ledovskikh S.R., Shcherbakova L.V., Garbuzova E.V., Khudyakova A.D., Ragino Yu.I.

Research Institute of Internal and Preventive Medicine – Branch of the Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Sciences (IIPM – Branch of IC&G SB RAS)
175/1, B. Bogatkova Str., Novosibirsk, 630089, Russian Federation

ABSTRACT

Aim. To study the level of metabolic hormones in young people with arterial hypertension (AH) against the background of abdominal obesity (AO).

Materials and methods. The study included 498 people who were divided into two groups. The experimental group encompassed 250 people with AH, of which – 159 people had AO, the average systolic pressure – 141.9 ± 13.9 mm Hg, diastolic pressure – 95.6 ± 7.5 mm Hg. The control group included 248 people comparable to the experimental group by gender and age, of whom 104 people had AO, the average systolic pressure – 118.5 ± 9.8 mm Hg, diastolic pressure – 77.8 ± 7.4 mm Hg. The levels of amylin, C-peptide, ghrelin, glucose-dependent insulinotropic polypeptide (GIP), glucagon-like peptide-1 (GLP-1), glucagon, insulin, pancreatic polypeptide (PP), and peptide YY (PYY) were determined by the multiplex analysis. The level of glucose was determined by the enzymatic method. Statistical processing of the results was carried out using the SPSS 13.0 software.

Results. Patients with AH had higher levels of amylin, C-peptide, and glucose and lower levels of PYY. There was no significant difference between the experimental group and the control group for the rest of the studied parameters. In the experimental group, the C-peptide, GLP-1, glucagon, and insulin levels were associated with AO. In the control group, the association of AO with the levels of C-peptide, insulin, and glucose was shown. The odds of AH in people under the age of 45 years were associated with a decrease in the level of PYY, a rise in the amylin levels, and an increase in waist circumference.

Conclusion. Of the studied metabolic hormones, an increased level of amylin and reduced PYY can serve as potential biomarkers indicating high odds of developing AH in people under 45 years of age. AO is a factor that contributes to the development of AH at a young age.

Keywords: abdominal obesity, metabolic markers, arterial hypertension

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 study participants signed an informed consent. The study was approved by the Ethics Committee at the Research Institute of Internal and Preventive Medicine – Branch of the Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Sciences (Protocol No. 167 of 26.11.2019).

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✉ *Polonskaya Yana V.,* yana-polonskaya@yandex.ru

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Уровень метаболических гормонов у молодых людей с артериальной гипертензией на фоне абдоминального ожирения

Полонская Я.В., Каштанова Е.В., Стахнёва Е.М., Шрамко В.С., Садовский Е.В., Ледовских С.Р., Щербакова Л.В., Гарбузова Е.В., Худякова А.Д., Рагино Ю.И.

Научно-исследовательский институт терапии и профилактической медицины – филиал Федерального государственного бюджетного научного учреждения «Федеральный исследовательский центр Институт цитологии и генетики Сибирского отделения Российской академии наук» (НИИТПМ – филиал ИЦиГ СО РАН)
Россия, 630089, г. Новосибирск, ул. Б. Богаткова, 175/1

РЕЗЮМЕ

Цель: исследовать уровень гормонов метаболизма у молодых людей с артериальной гипертензией (АГ) на фоне абдоминального ожирения (АО).

Материалы и методы. В исследовании приняли участие 498 человек, которые вошли в две группы. Основная группа – 250 человек с АГ, из них 159 с АО, среднее систолическое давление (СД) – 141,9 ± 13,9 мм рт. ст., диастолическое давление (ДД) – 95,6 ± 7,5 мм рт. ст. Группа контроля – 248 человек, сопоставимых с основной группой по полу и возрасту, с АО – 104 человека, средний уровень СД и ДД – 118,5 ± 9,8 и 77,8 ± 7,4 мм рт. ст. соответственно. Методом мультиплексного анализа определяли уровень амилина, С-пептида, грелина, глюкозо-зависимого инсулинотропного полипептида (ГИП), глюкагоноподобного пептида-1 (ГПП-1), глюкагона, инсулина, панкреатического полипептида (ППП), пептида YY. Уровень глюкозы определяли энзиматическим методом. Статистическая обработка результатов проводилась в программе SPSS 13.0.

Результаты. У пациентов с АГ отмечены более высокий уровень амилина, С-пептида, глюкозы и более низкий уровень пептида YY. Достоверной разницы между основной и контрольной группой по остальным изучаемым показателям не выявлено. В основной группе с АО были связаны показатели С-пептида, ГПП-1, глюкагона, инсулина. В контрольной группе была показана связь АО с уровнем С-пептида, инсулина и глюкозы. Относительный шанс наличия АГ у людей в возрасте до 45 лет был связан со снижением уровня пептида YY, с повышением уровня амилина и увеличением окружности талии.

Заключение. Из изученных нами гормонов метаболизма повышенный уровень амилина и сниженный показатель пептида YY могут служить в качестве потенциальных биомаркеров, указывающих на высокую вероятность развития АГ у людей до 45 лет. Абдоминальное ожирение является фактором, который способствует развитию АГ в молодом возрасте.

Ключевые слова: абдоминальное ожирение, маркеры метаболизма, артериальная гипертензия

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

Источник финансировании. Работа выполнена в рамках государственного задания «Эпидемиологический мониторинг состояния здоровья населения и изучение молекулярно-генетических и молекулярно-биологических механизмов развития распространенных терапевтических заболеваний в Сибири для совершенствования подходов к их диагностике, профилактике и лечению» (рег. № 122031700094-5) и в рамках гранта РНФ № 21-15-00022, в работе использованы материалы биоколлекции НИИТПМ – филиал ИЦиГ СО РАН.

Соответствие принципам этики. Все участники исследования подписали информированное согласие. Исследование одобрено этическим комитетом НИИТПМ – филиал ИЦиГ СО РАН (протокол № 167 от 26.11.2019).

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INTRODUCTION

The prevalence of arterial hypertension (AH), which is the leading risk factor for cardiovascular diseases (CVDs), among the adult population reaches 45% [1, 2]. Obesity (primarily abdominal obesity (AO)) as a factor that causes an increase in AH is registered in the population, including young people, with increasing frequency [3, 4]. Both AO and AH contribute to CVDs, and their combination is associated with an even higher risk and a heavier course of AH. Some of the main causes of AH in obesity include hyperinsulinemia, insulin resistance, an imbalance of gastrointestinal hormones, such as ghrelin, amylin, YY peptide, glucagon-like peptide -1, and others, but the pathogenesis of AH in obese and non-obese people may differ. Despite improvements in the understanding of the pathogenetic mechanisms of AH and obesity, not all processes that underlie the comorbidity of obesity and AH have been studied, especially at a young age. Therefore, the aim of our research was to investigate the level of metabolic hormones in young people with AH and AO.

MATERIALS AND METHODS

The study was carried out on the basis of a sample from the population screening of young residents (aged 25–44 years) of Novosibirsk, which was conducted at the Research Institute of Internal and Preventive Medicine – Branch of the Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Sciences from 2013 to 2016. We selected 3,000 men and women aged 25–44 years from the database of the Territorial Compulsory Health Insurance Fund using a random number generator; 1,512 people responded to the invitation and took part in the screening. They filled out questionnaires, gave an informed consent to participate in the study, underwent anthropometric and instrumental examinations, and biological material was obtained from them.

The experimental group included all persons with newly diagnosed AH with systolic pressure greater than 140 mm Hg and / or diastolic pressure greater than 90 mm Hg, according to the clinical guidelines "Arterial hypertension in adults" approved by the Ministry of Health of Russia in 2020 [5]. The exclusion criterion was diabetes mellitus indicated in the questionnaire or fasting plasma glucose level greater than 7 mmol / l [6]. AO was recorded with a waist circumference of more than 80 cm in women and more than 94 cm in men [7]. Patients without AH, comparable in gender and age, were randomly recruited in the control group. The characteristics of the studied groups are shown in Figure.

In all patients, blood for the biochemistry test was taken in the morning on an empty stomach from the ulnar vein no earlier than 12 hours after the last meal. The levels of amylin, C-peptide, ghrelin, glucose-dependent insulinotropic polypeptide (GIP), glucagon–like peptide-1 (GLP-1), glucagon, insulin, pancreatic polypeptide (PP), and YY were determined by the multiplex analysis using the Human Metabolic Hormone V3 kit (EMD Millipore Corporation, Germany) on the Luminex 20 MAGPIX flow cytometer. Glucose was determined by the enzymatic method using the Thermo Fisher reagent kit (Finland) on the KONELAB Prime 30i biochemical analyzer (Finland).

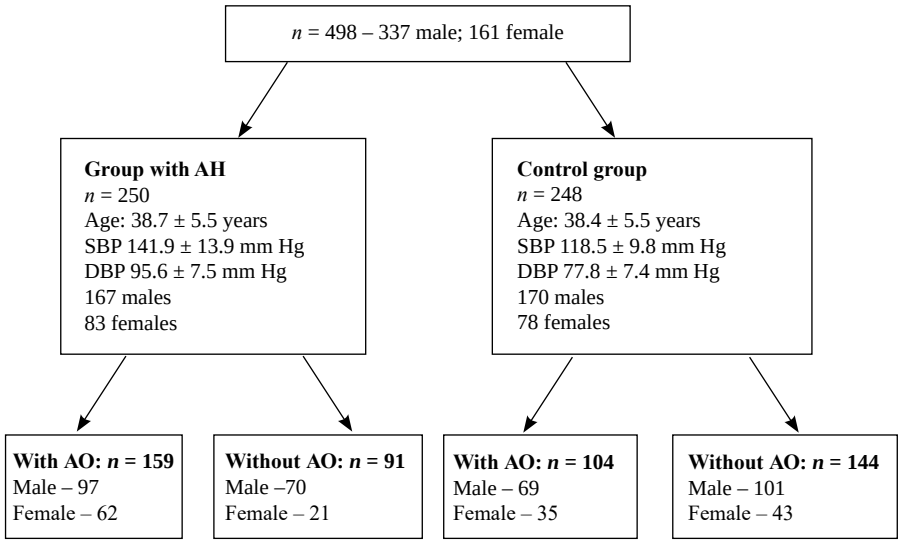

Figure.
Characteristics of the studied groups

Statistical processing of the results was carried out in the SPSS 13.0 software. The normality of data distribution was checked by the Kolmogorov – Smirnov test. Anamnestic data were presented as the mean and the standard deviation $M \pm SD$. Non-normally distributed quantitative variables were presented as the median and the interquartile range *Me* [*Q*25%; *Q*75%]. Normal distribution was seen only in two of the biochemical parameters studied by us; they were also presented as the median and the interquartile range *Me* [*Q*25%; *Q*75%]. The groups were compared using the nonparametric Mann – Whitney test. The correlation analysis was carried out using the Spearman's rank correlation coefficient. To identify the associations of the presence / absence of AH with the studied parameters, the multivariate logistic regression analysis was performed. The differences were statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

In young patients with AH, we found an imbalance of metabolic markers compared to healthy controls. Against the background of AO, these shifts are most pronounced (Table).

Table

The level of metabolic markers in young people with and without arterial hypertension, depending on the presence or absence of abdominal obesity

Parameter		Control group		Study group		*p*
		Me [$Q_{25\%}$; $Q_{75\%}$]	*p**	*Me* [$Q_{25\%}$; $Q_{75\%}$]	*p**	
Amylin, pg / ml	without AO	44.7 [39.4; 48.8]	0.551	71.9 [20.4; 73.2]	0.16	0.0001
	with AO	44.7 [38.5; 55.7]		72.2 [26.5; 75.8]		0.0001
C-Peptide, ng / ml	without AO	0.86 [0.55; 1.95]	0.003	0.86 [0.54; 2.01]	0.0001	0.903
	with AO	1.48 [0.70; 2.78]		1.99 [0.89; 3.58]		0.036
Ghrelin, pg / ml	without AO	156.3 [94.5; 256.2]	0.808	156.1 [107.8; 209.4]	0.124	0.707
	with AO	156.6 [91.9; 302.7]		170.7 [116.3; 297.1]		0.422
GIP, pg / ml	without AO	77.0 [43.5; 121.1]	0.456	78.6 [43.3; 117.9]	0.573	0.931
	with AO	83.9 [40.8; 124.7]		83.1 [44.0; 133.0]		0.96
GLP-1, pg / ml	without AO	583.6 [330.5; 788.7]	0.054	644.6 [299.1; 815.3]	0.017	0.981
	with AO	643.7 [381.2; 940.1]		720.3 [374.9; 935.7]		0.571
Glucagon, pg / ml	without AO	43.6 [28.1; 58.7]	0.695	45.2 [27.9; 57.9]	0.043	0.941
	with AO	43.3 [28.9; 72.4]		48.8 [36.1; 67.5]		0.196
Insulin, pg / ml	without AO	1,934.6[406.9; 2,889.7]	0.028	2,072.9[1,256.4; 2,776.3]	0.048	0.366
	with AO	2,343.3 [931.0; 3,262.1]		2,365.1 [1,256.4; 3,282.3]		0.512
PP, pg / ml	without AO	125.9 [59.9; 185.3]	0.468	90.0 [47.4; 185.1]	0.23	0.343
	with AO	135.4 [59.4; 201.7]		122.9 [62.0; 198.3]		0.743
YY, pg / ml	without AO	310.4 [79.5; 372.5]	0.936	208.5 [69.3; 303.4]	0.636	0.011
	with AO	310.4 [48.2; 420.6]		208.5 [82.6; 303.4]		0.273
Glucose, mmol / l	without AO	5.6 [5.2; 5.9]	0.002	5.9 [5.6; 6.1]	0.505	0.0001
	with AO	5.8 [5.4; 6.2]		5.9 [5.5; 6.4]		0.116

The level of amylin, a multifunctional peptide hormone, that is produced by the pancreas along with insulin and participates in carbohydrate metabolism, in our study was 1.6 times higher ($p = 0.0001$) in patients with AH compared to the control group. The effect of AO on the level of amylin was not revealed. M.T. Kailasam et al. [8] also found an increase in plasma amylin in AH, but, unlike our results, body mass index in their study was a strong predictor of an increase in circulating amylin.

Patients with AH also had higher levels of C-peptide, which is a fragment of endogenously produced proinsulin, and glucose (by 1.5 ($p = 0.003$) and 1.1 ($p = 0.0001$) times, respectively). In the study by S.I. Safronova et al. [9], the level of C-peptide was also higher in hypertensive patients compared to normotonic ones.

The level of the YY peptide in AH patients was 1.6 times lower (p = 0.006) compared to the control group, which is not consistent with the data obtained by E. Haj-Yehia et al. [10], where AH was more common in patients with high YY levels due to the direct vasoconstrictive effect of the peptide (in the authors' view). The difference can be explained by the fact that E. Haj-Yehia et al. studied patients with acute myocardial infarction.

There was no significant difference between the experimental and control groups in the rest of the studied parameters. Since the groups differed in waist circumference ($p = 0.0001$), further, in order to

consider the influence of AO, we analyzed the studied parameters depending on the presence / absence of AO. The results obtained in the study of serum metabolic markers are presented in Table.

In the control group, the level of statistical significance of differences $p < 0.05$ for C-peptide, insulin, and glucose was found between the subgroups with and without AO. The level of C-peptide was 1.7 times higher, and the level of insulin was 1.3 times higher in the subgroup with AO. The glucose concentration in this subgroup was 4% higher. Also, in the control group, there was a trend toward an increase ($p = 0.054$) in GPP-1 in patients with AO.

For the AH group, the differences between the subgroups were in the levels of C-peptide, insulin, GLP-1, and glucagon. C-peptide was 2.3 times higher in the subgroup with AO and AH, GLP-1 was 12% higher in patients, glucagon was 8% higher, and insulin was 15% higher compared to patients with AH but without AO. The remaining parameters in the AH group also did not depend on the presence of AO. The level of C-peptide was the highest in the subgroup with AO and AH.

When conducting the correlation analysis to assess the relationship of the studied biomarkers with the parameters characterizing AH, a weak correlation of systolic pressure parameters with amylin ($r = 0.224$; $p = 0.0001$), C-peptide ($r = 0.156$; $p = 0.001$), and glucose ($r = 0.23$; $p = 0.0001$) was revealed. Diastolic pressure was correlated with the same parameters: with amylin ($r = 0.260$; $p = 0.0001$), C-peptide ($r = 0.175$; $p = 0.0001$), and glucose ($r = 0.224$; $p = 0.0001$). There was no correlation with age for the studied markers.

To assess the odds of AH at a young age, the multivariate logistic regression analysis was performed, where the dichotomous parameter of the presence / absence of early AH was taken as a dependent variable, and the studied biomolecules and waist circumference were taken as independent variables. The relative chance of early AH was associated with a decrease in the level of YY (Exp(B) = 0.99; $p = 0.0001$; confidence interval (CI): 0.988–0.994), an increase in amylin levels (Exp(B) = 1.04; $p = 0.0001$; CI: 1.032–1.059), and an increase in waist circumference (Exp(B) = 1.05; $p = 0.0001$; CI: 1.019–1.062). Thus, with a decrease in the concentration of YY by 1 pg / ml and an increase in amylin by 1 pg / ml, the odds of having AH at a young age increase by 1 and 4%, respectively. With an increase in waist circumference by one centimeter, the odds of having AH in people under 45 years increase by 5%.

CONCLUSION

Of the studied metabolic markers, an increased level of amylin and a reduced level of YY can serve as potential biomarkers indicating high odds of AH in people under 45 years, and AO is a modifiable factor that contributes to the development of AH at a young age.

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Authors' contribution

Polonskaya Ya.V. – conception and design, statistical processing and interpretation of the results, drafting of the article. Kashtanova E.V. – conception and design, significant contribution to the interpretation of the data. Stakhneva E.M. – conception and design, editing of the manuscript. Shramko V.S. – carrying out of clinical and biochemical studies, editing of the manuscript to increase its scientific value. Sadovski E.V. – carrying out of clinical and biochemical studies, editing of the manuscript. Ledovskikh S.R. – compilation of the database, carrying out of the clinical and biochemical studies, editing of the manuscript. Shcherbakova L.V. – statistical processing and analysis of the data. Garbuzova E.V. – editing of the manuscript to increase its scientific value. Khudyakova A.D. – significant editing of the manuscript. Ragino Yu.I. – significant contribution to conception and design of the study, significant editing of the manuscript to increase its scientific value.

All authors approved the final version of the manuscript for publication and agreed to bear responsibility for all aspects of the work associated with proper study and solution of issues related to the accuracy and honesty of any section of the article.

Authors' information

Polonskaya Yana V. – Dr. Sci. (Biology), Senior Researcher, Laboratory for Clinical Biochemical and Hormonal Studies of Therapeutic Diseases, Research Institute of Internal and Preventive Medicine – Branch of the Institute of Cytology and Genetics, Siberian Branch of Russian Academy of Sciences, Novosibirsk, yana-polonskaya@yandex.ru, https://orcid.org/0000-0002-3538-0280

Kashtanova Elena V. – Dr. Sci. (Biology), Associate Professor, Head of the Laboratory for Clinical Biochemical and Hormonal Studies of Therapeutic Diseases, Research Institute of Internal and Preventive Medicine – Branch of the Institute of Cytology and Genetics, Siberian Branch of Russian Academy of Sciences (IIPM – Branch of IC&G SB RAS), Novosibirsk, elekastanova@yandex.ru, https://orcid.org/0000-0003-2268-4186

Stakhneva Ekaterina M. – Cand. Sci. (Biology), Senior Researcher, Laboratory for Clinical Biochemical and Hormonal Studies of Therapeutic Diseases, Research Institute of Internal and Preventive Medicine – Branch of the Institute of Cytology and Genetics, Siberian Branch of Russian Academy of Sciences (IIPM – Branch of IC&G SB RAS), Novosibirsk, stahneva@yandex.ru, https://orcid.org/0000-0003-0484-6540

Shramko Victoria S. – Cand. Sci. (Biology), Researcher, Laboratory for Clinical Biochemical and Hormonal Studies of Therapeutic Diseases, Research Institute of Internal and Preventive Medicine – Branch of the Institute of Cytology and Genetics, Siberian Branch of Russian Academy of Sciences (IIPM – Branch of IC&G SB RAS), Novosibirsk, nosova@211.ru, https://orcid.org/0000-0002-0436-2549

Sadovski Evgeny V. – Junior Researcher, Laboratory for Clinical Biochemical and Hormonal Studies of Therapeutic Diseases, Research Institute of Internal and Preventive Medicine – Branch of the Institute of Cytology and Genetics, Siberian Branch of Russian Academy of Sciences (IIPM – Branch of IC&G SB RAS), Novosibirsk, stinger000@mail.ru, https://orcid.org/0000-0001-7350-534X

Ledovskikh Sofya R. – Resident, Research Institute of Internal and Preventive Medicine– Branch of the Institute of Cytology and Genetics, Siberian Branch of Russian Academy of Sciences (IIPM – Branch of IC&G SB RAS), Novosibirsk, ledovskikh.sofiya@mail.ru, https://orcid.org/0000-0001-7345-0473

Shcherbakova Lilia V. – Senior Researcher, Laboratory for Clinical and Population Studies of Therapeutic and Endocrine Diseases, Research Institute of Internal and Preventive Medicine – Branch of the Institute of Cytology and Genetics, Siberian Branch of Russian Academy of Sciences (IIPM – Branch of IC&G SB RAS), Novosibirsk, 9584792@mail.ru, https://orcid.org/0000-0001-9270-9188

Garbuzova Evgeniya V. – Cand. Sci. (Med.), Researcher, Laboratory for Clinical Biochemical and Hormonal Studies of Therapeutic Diseases, Research Institute of Internal and Preventive Medicine – Branch of the Institute of Cytology and Genetics, Siberian Branch of Russian Academy of Sciences (IIPM – Branch of IC&G SB RAS), Novosibirsk, stryukova.j@mail.ru, https://orcid.org/0000-0001-5316-**4664**

Khudyakova Alyona D. – Cand. Sci. (Med.), Head of the Laboratory for Genetic and Environmental Determinants of the Human Life Cycle, Research Institute of Internal and Preventive Medicine – Branch of the Institute of Cytology and Genetics, Siberian Branch of Russian Academy of Sciences (IIPM – Branch of IC&G SB RAS), Novosibirsk, alene.elene@gmail.com, https://orcid.org/0000-0001-7875-1566

Ragino Yulia I. – Dr. Sci. (Biology), Corresponding Member of the RAS, Head of the Research Institute of Internal and Preventive Medicine – Branch of the Institute of Cytology and Genetics, Siberian Branch of Russian Academy of Sciences (IIPM – Branch of IC&G SB RAS), Novosibirsk, ragino@mail.ru, https://orcid.org/0000-0002-4936-8362

(✉) **Polonskaya Yana V.,** yana-polonskaya@yandex.ru

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Sarcoidosis as a disease associated with metabolic syndrome

Bespalova I.D.[1], Romanov D.S.[1], Denisova O.A.[1], Bragina E.Yu.[2], Koshchavtseva Yu.I.[1], Mitrichenko U.M.[1], Teteneva A.V.[1], Kalyuzhina E.V.[1], Porovskiy Ya.V.[1], Bukreeva E.B.[1]

[1] *Siberian State Medical University*
2, Moscow Trakt, Tomsk, 634050, Russian Federation

[2] *Research Institute of Medicical Genetics, Tomsk National Research Medical Center (NRMC) of the Russian Academy of Sciences*
10, Ushaika River Embankment, Tomsk, 634050, Russian Federation

ABSTRACT

The review summarizes and analyzes the results of domestic and major foreign studies of recent years concerning the prevalence of metabolic syndrome components and the explanation of their role in the mechanisms of sarcoidosis development. A deep understanding of the pathogenesis of metabolic syndrome (MS) in terms of the role in it of risk factors for a severe course and complications of most socially sensitive noncommunicable diseases clustered within MS can underly the development of effective pathogen-specific approaches to MS treatment.

Keywords: sarcoidosis, metabolic syndrome, obesity, atherosclerosis, diabetes mellitus, dyslipidemia

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Саркоидоз как ассоциированное с метаболическим синдромом заболевание

Беспалова И.Д.[1], Романов Д.С.[1], Денисова О.А.[1], Брагина Е.Ю.[2], Кощавцева Ю.И.[1], Митриченко У.М.[1], Тетенева А.В.[1], Калюжина Е.В.[1], Поровский Я.В.[1], Букреева Е.Б.[1]

[1] *Сибирский государственный медицинский университет (СибГМУ)*
Россия, 634050, г. Томск, Московский тракт, 2

[2] *Научно-исследовательский институт (НИИ) медицинской генетики, Томский национальный исследовательский медицинский центр (НИМЦ) Российской академии наук*
Россия, 634050, г. Томск, Набережная реки Ушайки, 10

РЕЗЮМЕ

В обзоре обобщены и проанализированы результаты отечественных и крупных зарубежных исследований последних лет, касающихся изучения распространенности компонентов метаболического синдрома (МС)

✉ *Bespalova Inna D.,* innadave@mail2000.ru

и объяснения их роли в механизмах развития саркоидоза. Глубокое понимание патогенеза данного заболевания с позиций участия в нем кластера факторов риска тяжелого течения и осложнений большинства социально значимых неинфекционных заболеваний, объединенных рамками МС, может лечь в основу разработки эффективных патогенетических подходов к лечению.

Ключевые слова: саркоидоз, метаболический синдром, ожирение, атеросклероз, сахарный диабет, дислипидемия

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 growth of interest of fundamental and applied sciences in the problem of metabolic syndrome (MS) is explained both by the drastically growing prevalence of its components (visceral obesity, hyperglycemia, dyslipidemia, arterial hypertension, hyperuricemia, etc.) and by their proven involvement in the pathogenesis of adverse outcomes in a range of noncommunicable diseases, determining high level of morbidity, disability, and mortality and a significant decrease in the quality of life of the population at the present time [1–3]. Currently, the list of diseases clustered within MS is not limited to coronary heart disease and type 2 diabetes mellitus. This group of pathologies includes all diseases caused by atherosclerosis, cancers of different localization, metabolic diseases (gout, urolithiasis and cholelithiasis, fatty liver disease), and connective tissue diseases, etc. [4–11]. In this regard, the need to consider sarcoidosis as a disease associated with MS seems to be quite reasonable.

Timely diagnosis and effective treatment of sarcoidosis continue to be among the most relevant issues for the medical community. The attention of clinicians and researchers to this pathology is due to a number of reasons. Sarcoidosis is a multisystem inflammatory disease of unknown etiology, manifested by the formation of specific granulomas in the affected tissues. The socio-medical significance of this pathology is explained both by a recent increase in its incidence, a trend toward a progressive course, high level of disability in the able-bodied population and by complicated diagnosis and monitoring of the disease course due to variability of its manifestations and the lack of etiotropic therapy. The variety of clinical manifestations of sarcoidosis is determined not only by the target organ affected by granulomas, but also by the influence of comorbid pathology, which also complicates the diagnosis of the disease and the strategy of managing patients [12–14].

The epidemiology of sarcoidosis is characterized by an increase in the incidence and disability of the population in different countries, including Russia and the CIS countries [15]. The prevalence, incidence rate, and clinical characteristics of sarcoidosis are determined by geographic, ethnic, social, and even professional affiliation, as well as by gender, age, and premorbid history [16, 17]. The most reasonable explanation for the recent increase in the incidence of sarcoidosis may be the widespread epidemic level of the prevalence of MS components [15, 18–20]. At the same time, it is important to take into account that MS and its individual components can be considered both as risk factors for the development of sarcoidosis and as a consequence of corticosteroid therapy, which also characterizes the relevance of research in this area.

A number of scientific articles provide data on the influence of MS components on the prevalence of sarcoidosis and vice versa. Thus, South Korean researchers conducted an epidemiological study that showed that in patients with metabolic disorders, the incidence of sarcoidosis, calculated per 100,000 population, was significantly higher than in patients without metabolic disorders: in patients with diabetes mellitus (DM) – 2.40 versus 0 .76, in patients with arterial hypertension – 1.81 versus 0.74, in patients with dyslipidemia – 2.60 versus 0.74. It was also

shown that these categories of patients not only have a higher risk of developing sarcoidosis but also a significantly higher risk of death [21].

Colleagues from the Istanbul University of Medical Sciences conducted a study of 47 patients with sarcoidosis and 45 apparently healthy individuals. MS was diagnosed according to the NCEP-ATP III criteria; the level of insulin resistance was also assessed by calculating the HOMA-IR index. The groups did not differ significantly in gender and age. The comparative analysis of body mass index (BMI), waist circumference (WC), triglycerides, and blood glucose levels showed significantly higher values of these parameters in patients with sarcoidosis than in the control group. 80% were diagnosed with stage 2 sarcoidosis, and almost half of the patients in the main group received steroids. The relative risk of developing MS in patients with sarcoidosis was 7.66, while the relative risk of developing insulin resistance was 5.48 [22].

Another group of Turkish scientists conducted a study that included 133 patients with newly diagnosed sarcoidosis, 133 age- and sex-matched controls, and 51 patients with rheumatoid arthritis (RA); all patients were investigated before receiving pathogen-specific therapy. A comparative analysis of the frequency of occurrence of MS and its individual components was carried out in accordance with the NCEP-ATP III criteria. It was found that MS was significantly more common in the sarcoidosis group than in the control group. These results were comparable with the data in the group of patients with RA. An important conclusion of this study, as the authors emphasize, is the evidence that MS components are associated with sarcoidosis and can be regarded as risk factors for its development, independent of the effects of corticosteroid therapy [23].

Sarcoidosis is a multisystem disease that is characterized by lesions of not only respiratory organs but other body systems. Extrapulmonary manifestations of sarcoidosis include damage to the eyes, nervous system, heart, kidneys, etc., which can aggravate the prognosis [24].

Some studies have shown an association between the course of sarcoidosis and a number of MS-associated diseases. In particular, a mutual aggravating effect of sarcoidosis and coronary heart disease has been established. In the presence of postinfarction cardiosclerosis and circulatory failure, a decrease in forced vital capacity (FVC) was explained by a decrease in myocardial contractility due to systolic and diastolic dysfunction of the left ventricle. At the same time, a decrease in functional parameters coincided with the timing of cardiac disease [20]. In this regard, it is difficult to differentiate the presence of cardiac sarcoidosis in patients, which also reduces left ventricular contractility and ultimately affects a decrease in the function of the respiratory system [25]. Clinically, symptoms of a heart disease are observed in only 5% of sarcoidosis cases, but in a series of autopsies, it was found that 27% of patients with sarcoidosis had granulomatous infiltration in the heart [26, 27].

Currently, there is no evidence that severe coronary artery stenosis is characteristic of sarcoidosis, but angina-like complaints have been described [28]. At the same time, sarcoidosis, like atherosclerosis, has a chronic inflammatory nature, which underlies microvascular damage and endothelial dysfunction and determines a high cardiovascular risk in patients of this category [29, 30]. Therefore, damage to the cardiovascular system in sarcoidosis can be caused not only by damage to the heart muscle by a specific process, but also by comorbid pathology [31]. In this regard, research can be aimed at establishing common links in the pathogenesis, namely, determining the role of MS components.

Thus, Russian scientists of the Moscow City Scientific and Practical Center for Combating Tuberculosis determined the association of dyslipidemia with the activity of the inflammatory process and insufficient antioxidant defense in sarcoidosis, which increases the cardiovascular risk in patients of this category [32]. These results are consistent with the data presented in a review by Italian authors, who confirmed that the pathogenesis of sarcoidosis is associated with increased oxidative stress (protein carbonylation and lipid peroxidation) and changes in the lipid profile of the circulating blood. Lipid metabolism disorders, including a decrease in high-density lipoprotein cholesterol levels and apolipoprotein A-I concentrations, cause damage to the plasma membrane and bronchial and capillary endothelial cells in patients with sarcoidosis. Foreign researchers also confirm that dyslipidemia is associated with oxidative stress, a decrease in overall antioxidant defense, and, consequently, an increased risk of atherosclerosis [33, 34].

To assess the prevalence of DM in patients with sarcoidosis and to establish the relationship between these diseases, Egyptian researchers conducted a meta-analysis that included 19 studies (n = 18,686,162). The

mean prevalence of DM in patients with sarcoidosis was 12.7% (95% confidence interval (CI) 10–16.1): the highest prevalence was in North America with 21.3% (13.5–31.8); in Europe, it was 10.4% (7.9–13.7), and in Asia, it was 10% (1.8–39.7). Patients with sarcoidosis had higher rates of DM compared to the control group in all areas (odds ratio (OR) 1.75; 95% CI 1.49–2.05) [35].

This meta-analysis did not look for reasons for high prevalence of DM in patients with sarcoidosis, and the possible impact of corticosteroid therapy was not taken into account. A team of authors from Thailand and Sweden believe that high prevalence of DM in patients with sarcoidosis in North America can be explained both by higher prevalence of obesity in this continent and by the use of higher doses of corticosteroids in the treatment of sarcoidosis, which may potentiate the development of type 2 DM [36]. This statement is supported by a large population-based Swedish cohort study in which patients with sarcoidosis treated with corticosteroids demonstrated a high risk of developing type 2 DM within 2 years after the diagnosis of sarcoidosis compared to patients with untreated sarcoidosis [37].

According to most experts, the main component of MS is abdominal obesity due to the proven role of metabolically and endocrine active visceral adipose tissue in the development of associated pathologies [38, 39]. Obesity exacerbates symptoms of sarcoidosis, and corticosteroid therapy increases BMI. Prospective epidemiological studies conducted to investigate the role of obesity as a potential risk factor for the development of sarcoidosis are worth noting. Three studies in the United States and one study in Denmark demonstrated a higher risk of developing sarcoidosis among obese patients compared to non-obese patients; risk estimates ranged from 1.42 (95% CI 1.07–1.89) to 3.59 (95% CI 2.31–5.57) [40]. A health study of 59,000 African American women [41] found that obesity (BMI $\geq$ 30 kg / m^2) at baseline was associated with a 40% increase in the prevalence of sarcoidosis. Given obesity at the age of eighteen and a subsequent increase in body weight by 30 kg or more, an increase in the incidence of sarcoidosis was noted.

In a prospective health study of 116,430 American women who had been followed up for 14 years, 270 patients developed sarcoidosis, and obesity was associated with a 70% increased risk of developing sarcoidosis [42]. A population study in Olmsted County, Minnesota (USA) [43], which included 345 patients with sarcoidosis and the same number of apparently healthy controls, found a positive correlation between BMI and the risk of developing sarcoidosis. The odds ratio for developing sarcoidosis in people with obesity compared to those with a normal or low BMI was 2.54 (95% CI 1.58–4.06).

Understanding the mechanism of the mutual effect of obesity and sarcoidosis is extremely important in terms of substantiating new areas of prevention and treatment, since excess body weight can be not only a modifiable risk factor for this pathology, but also aggravate its course. Russian researchers indicated obesity as one of the most informative criteria for predicting the recurrent course of respiratory sarcoidosis [44], and BMI was identified as a risk factor for the development of extra-thoracic forms of sarcoidosis [19]. In order to understand the mechanism of the mutual influence of obesity and sarcoidosis, it should be taken into account that obesity, even in the absence of respiratory diseases, can affect many physiological respiratory factors, including static and dynamic spirogram parameters, as well as bronchial hyperreactivity, mechanical function of the upper respiratory tract, neuromuscular strength, and gas exchange [45].

It is also known that the average respiratory rate in patients with obesity is 30–50% higher than in individuals with normal body weight [46, 47]. Dyspnea on exertion associated with overweight underlies the decline in physical functioning in patients [48]. Indicators characterizing the severity of obesity (WC, waist – hip ratio (WC / HR), and subscapular skinfold thickness) have an inverse correlation with tidal volume, which is explained by the high position of the diaphragmatic dome [45, 49, 50]. The EPIC-Norfolk study of British patients of both sexes found an inverse relationship between WC / HC and FEV_1 and FVC [51]. It is also known that obesity is associated with such respiratory diseases as chronic obstructive pulmonary disease (COPD), bronchial asthma, pneumonia, and obstructive sleep apnea (OSA) [52–54].

The impact of obesity on the development and severity of OSA in patients with sarcoidosis has not been fully studied. Two published studies in Turkey reported that 66 and 52% of patients with sarcoidosis, respectively, had OSA [55, 56]. An earlier study using polysomnography showed that OSA was observed only in patients with a BMI greater than 30 kg / m^2 [57]. The relationship between obesity and sarcoidosis was assessed in terms of the impact of high BMI on symptoms of fatigue, dyspnea, health

status, and spirometry in 184 Serbian patients with sarcoidosis [58]. Compared to healthy controls of the same age and gender, patients with sarcoidosis were more likely to be overweight or obese, have a lower baseline dyspnea index (BDI) and lower FEV_1 values, more pronounced fatigue scores, and reported worse well-being. When the authors examined the independent and combined effects of sarcoidosis and BMI, they found that sarcoidosis itself contributed to aggravation of dyspnea and decreased subjective health scores.

Obesity may be both a consequence of sarcoidosis treatment and a risk factor for the disease, probably due to the metabolic and proinflammatory state of visceral adipose tissue. That is why abdominal obesity is associated with a number of noncommunicable diseases in addition to type 2 DM and atherosclerosis: with obstructive lung diseases (bronchial asthma, COPD), connective tissue diseases (RA, psoriatic arthritis, systemic lupus erythematosus), metabolic diseases (urolithiasis and cholelithiasis, fatty liver disease, gout), etc. [59–63].

According to modern concepts, visceral adipose tissue is classified as an organ of the endocrine and immune systems, which is confirmed by characteristic structural and functional changes. Fundamental studies in recent years have shown that adipose tissue in obesity is infiltrated with mononuclear leukocytes [38, 64, 65] and characterized by a proinflammatory and prooxidant state. This is confirmed by the ability of adipocytes to produce not only adipokines, but also proinflammatory cytokines and reactive oxygen species [38, 39, 64–66]. Biologically active substances synthesized by adipocytes in large quantities have significant systemic effects. The decisive role of chronic low-grade inflammation in the mechanisms of development of MS and its individual components has been proven by a large number of studies that show statistically significant relationships between metabolic parameters (severity of obesity, hyperglycemia, dyslipidemia, hyperuricemia, etc.) not only with the level of acute-phase proteins in blood, but also with the level of proinflammatory cytokines (interleukin (IL)-1β, IL-6, IL-8, TNFα, INFγ, etc.) [38, 39, 64–66]. The proinflammatory background set by MS cannot but affect the course of other diseases, in the pathogenesis of which the inflammatory process plays a leading role. In this regard, it can be assumed that the mechanism of association between sarcoidosis and MS is implemented through the same proinflammatory factors.

CONCLUSION

Thus, the analysis of scientific publications showed that sarcoidosis can be characterized as an MS-associated disease. On the one hand, MS components are modifiable risk factors for the onset and severe course of sarcoidosis, and on the other hand, they are the result of corticosteroid therapy for this disease. A deep understanding of the pathogenesis of the association between these two pathological conditions can form the basis for the prevention of severe sarcoidosis, its control, and effective pathogenetically grounded approaches to treatment.

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Authors' information

Bespalova Inna D. – Dr. Sci. (Med.), Acting Head, Division of Propedeutics of Internal Diseases with a Course in Therapy, Siberian State Medical University, Tomsk, innadave@mail.2000.ru, http://orcid.org/0000-0002-4513-6329

Romanov Dmitriy S. – Graduate Student, Division of Propedeutics of Internal Diseases with a Course in Therapy of the Pediatric Department, Siberian State Medical University, Tomsk, romanovds92@yandex.ru

Denisova Ol'ga A. – Cand. Sci. (Med.), Assistant Lecturer, Division of Advanced-Level Therapy with a Course in Physical Rehabilitation and Sports Medicine, Siberian State Medical University, Tomsk, oadeni@yandex.ru, http://orcid.org/0000-0003-4968-1110

Bragina Elena Yu. – Cand. Sci. (Biology), Senior Researcher, Laboratory of Population Genetics, Research Institute of Medical Genetics, Tomsk NRMC, Tomsk, elena.bragina@medgenetics.ru, http://orcid.org/0000-0002-1103-3073

Koshchavtseva Yuliya I. – Assistant Lecturer, Division of Propedeutics of Internal Diseases with a Course in Therapy of the Pediatric Department, Siberian State Medical University, Tomsk, kossy09@mail.ru, http://orcid.org/0000-0001-5260-4832

Mitrichenko Ulyana M. – Graduate Student, Division of Propedeutics of Internal Diseases with a Course in Therapy of the Pediatric Department, Siberian State Medical University, Tomsk, strashkovaum@gmail.com, http://orcid.org/0000-0001-6091-4849

Teteneva Anna V. – Dr. Sci. (Med.), Professor, Division of Propedeutics of Internal Diseases with a Course in Therapy of the Pediatric Department, Siberian State Medical University, Tomsk, anna.dubodelova@mail.ru, http://orcid.org/0000-0002-4323-2798

Kalyuzhina Elena V. – Dr. Sci. (Med.), Professor, Division of Advanced-Level Therapy with a Course in Physical Rehabilitation and Sports Medicine, Siberian State Medical University, Tomsk, kalyuzhina@sibmail.com, http://orcid.org/0000-0002-7978-5327

Porovskiy Yaroslav V. – Dr. Sci. (Med.), Professor, Division of Propedeutics of Internal Diseases with a Course in Therapy of the Pediatric Department, Siberian State Medical University, Tomsk, porovskijaroslav@gmail.com, http://orcid.org/0000-0002-0471-5084

Bukreeva Ekaterina B. – Dr. Sci. (Med.), Professor, Division of Propedeutics of Internal Diseases with a Course in Therapy of the Pediatric Department, Siberian State Medical University, Tomsk, kbukreeva@mail.ru, http://orcid.org/0000-0002-7699-5492

(✉) **Bespalova Inna D.,** innadave@mail2000.ru

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All you need to know about sarcopenia: a short guide for an internal medicine physician in questions and answers

Bikbavova G.R., Livzan M.A., Tikhonravova D.V.

Omsk State Medical University
12, Lenina Str., 644099, Omsk, Russian Federation

ABSTRACT

Sarcopenia is associated with social, economic, and individual burdens, including loss of independence, poor quality of life, and disability. In a short period of time, ideas about sarcopenia transformed from geriatric syndrome to disease. Initially, sarcopenia was considered in the context of gradual age-related deterioration in the functioning of all physiological systems. Over the years, it became clear that it can develop a second time, as a consequence of various diseases and pathological conditions.

To date, there have been no generally accepted diagnostic criteria for sarcopenia. There are several tests and tools available for screening sarcopenia, the choice of which depends on physical capabilities of the patient, capabilities of the medical institution, and the purpose for which it is detected (research or clinical practice).

From the point of view of human health, sarcopenia increases the risk of falls and fractures; impairs the ability to perform daily activities; is associated with the progression of major diseases and cognitive impairments; leads to movement disorders; contributes to a decrease in the quality of life, loss of independence or a need for long-term care. The presence of sarcopenia increases both the risk of hospitalization and hospitalization costs.

The aim of the literature review is to provide an analysis of up-to-date information on the causes, pathogenesis, screening, diagnosis, treatment, and consequences of sarcopenia, myosteatosis, and sarcopenic obesity. The search for literature containing information on relevant studies was conducted in PubMed and Google Scholar by the following keywords: sarcopenia, dynapenia, myosteatosis, sarcopenic obesity, nutritional status, malnutrition.

Keywords: sarcopenia, dynapenia, sarcopenic obesity, malnutrition, myosteatosis

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✉ *Bikbavova Galiya R.,* galiya1976@mail.ru

Все, что нужно знать о саркопении: краткий гид для современного терапевта в вопросах и ответах

Бикбавова Г.Р., Ливзан М.А., Тихонравова Д.В.

Омский государственный медицинский университет (ОмГМУ)
Россия, 644099, г. Омск, ул. Ленина, 12

РЕЗЮМЕ

Саркопения связана с социальным, экономическим и индивидуальным бременем, включающим потерю независимости, низкое качество жизни и инвалидизацию. За короткий промежуток времени представления о саркопении преобразовались с гериатрического синдрома до заболевания. Изначально саркопения рассматривалась в контексте постепенного возрастного ухудшения работы всех физиологических систем. С годами стало понятным, что она может развиться вторично, как следствие различных заболеваний и патологических состояний.

На сегодняшний день общепринятых диагностических критериев саркопении не существует. Доступно несколько тестов и инструментов для скрининга саркопении, выбор которых зависит от физических возможностей пациента, возможности медицинского учреждения и цели, с которой проводится ее выявление (научно-исследовательская работа или практическая деятельность).

С точки зрения здоровья человека саркопения увеличивает риск падений и переломов; ухудшает способность выполнять повседневную деятельность; связана с прогрессированием основных заболеваний и когнитивными нарушениями; приводит к нарушениям подвижности; способствует снижению качества жизни, потере независимости или необходимости в длительном уходе. Наличие саркопении увеличивает риск госпитализации и стоимость лечения во время госпитализации.

Цель обзора – представить анализ актуальной информации о причинах, патогенезе, скрининге, диагностике, лечении и последствиях саркопении, а также миостеатозе и саркопеническом ожирении. Поиск литературы, содержащей информацию о соответствующих исследованиях, проводился в системах PubMed и Google Scholar по таким ключевым словам, как саркопения, динапения, миостеатоз, саркопеническое ожирение, нутритивный статус, мальнутриция.

Ключевые слова: саркопения, динапения, саркопеническое ожирение, мальнутриция, миостеатоз

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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WHAT IS SARCOPENIA? BACKGROUND, DEFINITION, AND TERMINOLOGY

Sarcopenia is generally regarded as an age-related progressive condition characterized by impaired skeletal muscle function and weight loss, associated with an increased risk of falls, fractures, hospitalization, and mortality. M. Critchley described the loss of muscle mass in elderly people in 1931. The term sarcopenia was proposed by the American professor I. Rosenberg in 1989 (Greek “sarx” – body, flesh and “penia” – deficiency) [1]. In 2010, the European consensus on definition and diagnosis proposed the first definition of sarcopenia. This term is understood as a condition characterized by progressive and generalized skeletal muscle disorder with increased risk of adverse outcomes, such as deterioration in the quality of life, physical disability, and mortality [2]. In 2016, sarcopenia was officially recognized as a disease in the International Classification of Diseases (ICD-10: M62.84).

So, in a short period of time, ideas about sarcopenia were transformed from a geriatric syndrome to a disease. The status of the disease gives “increased

awareness" of this problem [3] and stimulates research interest and commercial interest of pharmaceutical companies in the development of new drugs. According to experts, sarcopenia will become a global problem by 2045, which is associated with an increase in human life expectancy [4].

Primary sarcopenia is a consequence of age-related changes in the muscle tissue. In certain cases, it can develop a second time, as a consequence of other diseases and pathological conditions (cancer, chronic heart failure, liver cirrhosis, inflammatory bowel diseases, etc.) due to systemic inflammation, limited physical activity (bed rest), malabsorption syndrome, obesity, endocrine disorders, and nutrient deficiency [5].

Sarcopenia can be acute, lasting less than 6 months, and chronic, lasting at least 6 months [6]. There is a term presarcopenia, which is defined as an isolated decrease in muscle mass with normal muscle strength and function. Classifying sarcopenia into acute, chronic, and presarcopenia is necessary for early intervention and management of the disease using available methods to improve the quality of life and life expectancy of patients.

It is reasonable to separate the use of such sarcopenia characteristics as muscle mass (quantitative disorder) and muscle strength and function (qualitative disorder), since muscle strength depends not only on their mass [7]. To define muscle strength, it is proposed to use the term dynapenia [8], while the term sarcopenia is a broader concept [9]. Some scientists suggest_using the term dynapenia only in relation to elderly and senile people [10]. However, the Foundation for the National Institutes of Health, which is established by the US Congress, suggests using the term dynapenia in patients of any age [9].

The combination of sarcopenia and obesity is called sarcopenic obesity (SO). It is a loss of muscle mass and fat accumulation that synergistically increase life-threatening consequences. SO is associated with an increased risk of disability, cardiovascular diseases, metabolic syndrome, and mortality [11]. The ability of adipose tissue to produce signaling molecules and influence metabolism, that is, to work as an organ of the endocrine system, was discovered relatively recently, becoming one of the main achievements in this field [12]. Among the effector organs is muscle tissue, the paracrine regulation of which is implemented by adipocytes. Under physiological conditions, muscle tissue contains a minimal amount of fat which is used as an energy source during aerobic activity, while excessive pathological muscle fat infiltration is called myosteatosis [13]. Fat deposition in the muscles can occur between the muscles (intermuscularly), in the extracellular region, but within the same muscle (intramuscularly), and inside cells (intracellularly). Thus, there is a change in muscle architectonics and a significant decrease in the functional activity of muscles [14].

WHAT IS THE PREVALENCE OF SARCOPENIA?

The prevalence of sarcopenia varies from 5 to 13% among people over 60–70 years and from 11 to 50% among people over 80 years [15, 16]. According to other studies, the prevalence of sarcopenia among the elderly is 29% and among individuals living in long-term care institutions – 33% [17]. The International Clinical Guideline on Sarcopenia recommends annual screening in all persons over 65 years of age in hospitals and clinics using the SARC-F questionnaire (A Simple Questionnaire to Rapidly Diagnose Sarcopenia) [18].

WHAT ARE THE RISK FACTORS FOR SARCOPENIA?

Muscle mass starts to decrease from the third to the fourth decade and progresses at a rate of 0.5–1% per year with a drastic decline after the eighth decade [19]. Muscle strength also decreases in parallel, but not in direct proportion to the loss of muscle mass. Such factors as malnutrition, physical inactivity, and polymorbidity in the elderly are involved in the development of primary sarcopenia, while taking medication, malabsorption syndrome, systemic inflammations, endocrine disorders, obesity, and malnutrition are involved in the development of secondary sarcopenia (Figure).

The theory of the emergence of sarcopenia is discussed taking into account the impact of internal and external risk factors. Internal factors include the influence of proinflammatory cytokines, oxidative stress, mitochondrial dysfunction, and insulin resistance. In addition, double-blind studies have shown that the heritability of some parameters of muscle mass and strength reaches 80%. External risk factors for sarcopenia include exposure to radiation, dietary habits, smoking, alcohol and / or drug abuse, infectious agents, social environment, and physical activity. The interaction of internal and external factors is a complicated, parallel, and dynamic process that leads to an imbalance between protein synthesis and proteolysis in skeletal muscles [20].

Figure. Factors contributing to the development of sarcopenia

METHODS OF SARCOPENIA DIAGNOSIS / HOW TO DIAGNOSE SARCOPENIA?

Currently there are no generally accepted diagnostic criteria for sarcopenia. There are several tests and tools available for screening sarcopenia, the choice of which depends on the physical capabilities of the patient, the capabilities of the medical institution, and the purpose for which it is detected (research or clinical practice). Sarcopenia should be diagnosed from the moment when the patient reports about such symptoms as falling, slow walking speed, difficulty getting up from a chair, weight loss, exhaustion, and weakness [22].

To identify sarcopenia, the European Consensus recommends using the SARC-F questionnaire which includes five questions concerning the degree of difficulty in performing routine activities (strength, walking, chair stand test, climbing stairs, falling during the previous year). The SARC-F questionnaire has good sensitivity and high specificity [22]. Its advantages are reliability, simplicity of use, convenience for screening, fast results, and low cost [23].

The Ishii screening test is a method that evaluates the probability of sarcopenia using three variables: age, grip strength, and calf circumference [24]. The calculation includes two steps and is represented by the following equations. Calculation of probability points: for men $0.62 \times (\text{age} - 64) - 3.09 \times (\text{grip strength} - 50) - 4.64 \times (\text{calf circumference} - 42)$; for women $0.80 \times (\text{age} - 64) - 5.09 \times (\text{grip strength} - 34) - 3.28 \times (\text{calf circumference} - 42)$.

The red flag method [25] is used for screening sarcopenia during a standard medical consultation. Table 1 presents the main components of this technique. In addition to collecting complaints and examining a patient, eating habits (for example, whether the patient consumes enough protein-containing foods) and physical activity (playing sports, working in the country or in the garden in the spring – autumn period and / or walking) are analyzed. The authors of the method suggest proceeding to more complex procedures for assessing sarcopenia when red flags are identified.

Table 1

The red flag method for screening sarcopenia

Parameter	Red flags
Examination	Weakness
	Visual identification of muscle loss
	Reduced gait speed
Complaints and anamnestic data	Weight loss
	Decreased muscle strength in the arms and legs
	Fatigue
	Falls
	Impaired mobility
	Loss of energy
	Difficulties doing exercises and daily activities
Clinician's assessment	Nutrition
	Body mass
	Physical activity

Determination of skeletal muscle strength: hand-grip dynamometry (grip strength) is a simple, reliable, and inexpensive method that is recommended for practical use. Levels lower than 27 kg in men and 16 kg in women are diagnostically significant in sarcopenia. Low grip strength is a predictor of long hospital stays, functional limitations, poor quality of life, and mortality [26, 27]. In cases where the use of hand-grip dynamometry is not possible, it is recommended to use the chair stand test. The patient is offered to get up from the chair 5 times in a row and sit down without the help of hands, while measuring the time during which the patient will be able to complete the task. A time interval greater than 15 seconds is diagnostically significant. Another version of the test with a chair is when times the patient gets up and sits down on a chair within 30 seconds are counted [25].

Such criteria as the total mass of skeletal muscles, the mass of appendicular muscles (muscle mass of skeletal muscles of the upper and lower limbs), and the cross-sectional area of muscles in various zones are important in order *to determine the mass of skeletal muscles*. There are several imaging techniques that make it possible to evaluate the above criteria; these include magnetic resonance imaging (MRI), computed tomography (CT), dual-energy X-ray absorptiometry (DXA), bioimpedance analysis, and ultrasound.

The procedure for conducting and describing the results obtained with MRI and CT is time-consuming, therefore, a method for calculating the lumbar index at the third lumbar vertebra (L3) was developed and tested [25]. The advantage of this method is its accuracy, reproducibility of the results, as well as the fact that the lumbar index can be determined during CT or MRI performed for other purposes (for example, in cancer patients). The disadvantages of these methods are rare application in primary health care due to their high cost, requirements for highly qualified personnel, and ionizing radiation load, which makes it difficult to use them during follow-up in response to prescribed treatment. An important point is that the cut-off points of low muscle mass are not currently defined [28].

Dual Energy X-Ray Absorptiometry (DXA) is also used to quantify sarcopenia (muscle mass) and calculate the content of adipose tissue. Abroad, this method is considered as the method of choice due to low radiation doses, speed of implementation, and non-invasiveness. The disadvantages of this technique include the facts that the equipment is not mobile and that different manufacturers report different results [6].

The bioimpedance analysis is less accurate for measuring quantitative parameters of sarcopenia, since this technique evaluates the distribution of fluid in the body, while the rest of remaining values are calculated ones, so there can be an error in measurement. Another disadvantage of the method is that its accuracy is affected by the position of the electrodes, room temperature, and body temperature [29].

Currently, methods for ultrasound diagnosis of sarcopenia are being actively developed, however, despite the improvement of equipment and software, the technique is operator-dependent, which has a significant impact on the representativeness of the results [30]. At the same time, the European Working Group on Sarcopenia in Older People [6] recommends this examination due to its reliability, convenience, cost, and timing, indicating its future potential. During the study, the thickness and cross-sectional area of the pennate (feather-like) muscles, echogenicity, beam length, and angle of inclination, for example, of the quadriceps femoris, are evaluated [31].

The measurement of calf circumference in the elderly is also useful as a diagnostic indicator in conditions where other methods for determining the quantitative characteristics of muscle mass are not available [32]. With all the conventions of the interpretation, the size of the calf less than 31 cm in general suggests a decrease in muscle mass [33].

Screening tools such as the Timed Up and Go (TUG) test are used *to determine physical performance*. When measuring the gait speed, the patient walks 4 meters at a normal speed, the medical staff records the walking time and calculates the speed (in m / s) [34]. The recommended cut-off point for determining severe sarcopenia is 0.8 m / s or less. The TUG test involves getting up from the chair, walking 3 meters to the mark, turning around, returning, and sitting back on the chair [35]. An indicator of severe sarcopenia is the time ≥ 20 seconds. The Short Physical Performance Battery (SPPB) test is a comprehensive test, but it is more often used in research than in clinical assessment, as it takes at least 10 min to complete it. The SPPB test includes an assessment of gait speed, a balance test, and a chair stand test. The maximum score is 12, and a score of ≤ 8 indicates poor physical performance [25].

Several international groups are currently involved in the development of screening and diagnostic criteria for sarcopenia: the European Working Group on Sarcopenia in Older People (EWGSOP); the Asian Working Group on Sarcopenia (AWGS), the International Working Group on Sarcopenia (IWGS), the Foundation for the National Institutes of Health (FNIH) established by the US Congress. The diagnostic criteria for sarcopenia which were offered by these groups are presented in Table 2.

Table 2

Diagnostic criteria for sarcopenia proposed by various research teams studying this problem

Research team	Definition	Criteria		
		Muscle mass	Muscle strength	Physical performance
EWGSOP-2 [6]	Probable sarcopenia – criterion 1. The diagnosis is confirmed additionally by criterion 2. If the patient has criteria 1, 2, and 3, then sarcopenia is severe. 1. Low muscle strength 2. Low muscle quantity or quality 3. Low physical performance	Appendicular Skeletal Muscle Mass Index (AMMI): the ratio of the total skeletal muscle mass of the upper and lower extremities to the patient's height per square, kg / m²: M < 7.0 kg / m² W < 5.5 kg / m² (DXA)	M < 27 kg W < 16 kg (dynamometry)	Gait speed M and W ≤ 0.8 m / s SPPB – total score ≤ 8 TUG ≥ 20 seconds
FNIH [9]	Combination of low muscle mass and weakness	AMMI M < 0.789 or < 19.75 kg (DXA) W < 0.512 or < 15.02 kg (DXA)	M < 26 kg W < 16 kg (dynamometry)	Gait speed M and W < 0.8 m / s
IWGS [36]	Combination of low muscle mass and reduced physical performance	M < 7.23 kg / m² W < 5.67 kg / m² (DXA) M < 7.23 kg / m² W < 5.67 kg / m² (BIA)	–	Men and women < 1 m / s

Note: M – men, W – women.

The European Working Group on Sarcopenia in Older People (EWGSOP) conducted its second meeting in 2018 [6]. Since their first meeting in 2010, researchers and clinicians have accumulated information, studied in detail many aspects of this problem, and identified issues that required resolution. One of the issues is, for example, that many practitioners are aware of sarcopenia and may suspect it in a patient, however no unique diagnostic criteria and management methods have been presented, while the consequences of sarcopenia are quite alarming [37–39].

Another important achievement was the understanding that timely diagnosis of muscle strength deficiency is still of paramount importance when comparing the significance of such sarcopenia parameters as muscle strength and muscle mass [40]. Measuring muscle strength is more applicable from a practical point of view, while measuring muscle mass is technically difficult due to reasons described above. In addition, according to studies, muscle strength is a more significant marker in terms of predicting poor patient outcomes [26, 27]. Scientists pay attention to the fact that the practical significance of this characteristic will also increase with the improvement of tools and methods for assessing the quantitative characteristics of muscle mass.

HOW TO TREAT SARCOPENIA?

Primary and secondary sarcopenia are a consequence of many diseases both in elderly or younger patients with comorbidities of varying severity, so the focus should be placed on the

treatment of underlying diseases. The positive impact of treatment strategies aimed at controlling diabetes mellitus, reducing inflammatory status, decreasing weight in obesity, and enriching diet with foods rich in various nutrients is obvious [41].

There is no doubt that physical activity and moderate strength workouts are beneficial for the elderly, which has a positive effect on muscle strength, muscle mass, and performance [25]. Moreover, strength workout is the most effective and available method for preventing the progression of sarcopenia that improves many aspects of overall health [42]. There are no studies in the field of standardization of physical activity, which creates difficulties in assigning the amount of physical activity that a particular patient needs. Only EWGSOP pays attention to the fact that the duration of physical exercise should be at least 3 months and combined programs should be recommended for patients with a sedentary lifestyle [6].

Currently convincing evidence of the impact of various therapeutic diets on the course of sarcopenia has not been obtained yet. However, observational studies have shown that an increasing protein intake to 1.2 g / kg daily in the elderly has a positive effect on muscle mass and, to a lesser extent, on muscle strength. Weak old people or old people with acute or chronic diseases need more dietary protein (1.2–1.5 g / kg of body weight per day) [43]. It has been suggested that dietary supplements, such as β-hydroxy-β-methylbutyrate, creatine, and vitamin D, have an impact on physical performance. It has been proven that supplements of β-hydroxy-β-methylbutyrate apparently increase muscle mass, while their effect on muscle strength and physical performance is controversial [44]. The meta-analysis demonstrated that vitamin D supplements increase muscle strength but do not affect their mass [45]. Based on this information, it seems reasonable for clinicians and / or nutritionists to pay attention to the calorie content, the quality and quantity of incoming protein, as well as the level of vitamin D in the elderly and consider the possibility of personalized prescription of dietary supplements.

Precision medicine is defined as a new paradigm that focuses on personalized, predictive, and preventive approaches and represents a completely new way to treat sarcopenia. Modern innovative technologies including smartphone software, neuromuscular electrical stimulation, smart home technologies, and interactive games with virtual reality elements help to personalize sarcopenia treatment programs [46]. These methods will help the elderly to remain independent and at the same time receive adequate physical activity and control their diet depending on individual needs. For example, the software, including remote measurements and monitoring of the intensity of physical activity, helps doctors remotely obtain information about the activity of patients and monitor their compliance with a scheduled treatment plan and progress in exercise. Robotic devices can also become useful tools in passive and active patient education [47]. A smart home includes many connected devices that can help older people stay independent by providing them with better experiences than regular exercise. For example, smart refrigerators have the function of helping old people maintain adequate nutrition by tracking daily food intake, providing them with individual meal plans and purchasing groceries through online systems. Of course, further research is needed to determine the role of currently available technologies in the treatment of sarcopenia.

The Food and Drug Administration (FDA) has not approved any specific drugs for the treatment of sarcopenia. The possibility of using such drugs as growth hormone, anabolic or androgenic steroids, selective androgen receptor modulators, protein anabolic agents, appetite stimulants, myostatin inhibitors, β-receptor blockers, angiotensin-converting enzyme (ACE) inhibitors, and troponin activators is being considered.

These groups of drugs have different efficacy. For example, growth hormone increases muscle protein synthesis and muscle mass but does not improve muscle strength or function [48]. The effects of anabolic steroid supplements differed between genders: an increase in weight and muscle mass in men and weight gain mainly due to increased fat mass in women [49]. In both men and women, testosterone supplements increased muscle strength [50]. Herbal supplements, such as curcumin, alkaloids, catechins, proanthocyanidin, gingerols, and segaols, have shown a moderate effect on skeletal muscle function [51]. Ghrelin and megestrol acetate, which are used as appetite stimulants, can increase body weight and muscle mass [52]. Myostatin, produced by muscles, prevents muscle anabolism [53]. Bimagrumab, a human monoclonal antibody that modulates activin type IIB receptors, increases muscle volume, muscle mass, and physical performance [54]. ACE inhibitors and troponin activators have a positive effect on muscle mass [55].

CONCLUSION

Scientific and technological progress in medical technology has made it possible to find out the causes of many diseases, decipher their pathogenesis, create new drugs, and develop preventive strategies. This has led to an increase in the life expectancy of the world's population [56]. However, population aging has emerged and requires a comprehensive public health response. Muscle mass and strength tend to decrease with age after the peak in adolescence. Thus, the term sarcopenia was coined. In a short period of time, ideas about sarcopenia have transformed from a geriatric syndrome to a disease. Initially, sarcopenia was considered in the context of gradual age-related deterioration of all physiological systems, leading to reduction of individual vitality reserves, which causes increased vulnerability to stress factors and increases the risk of adverse health consequences. Over the years, it became clear that in certain cases it can develop a second time, as a consequence of other diseases and pathological conditions.

At the moment, there are still many gaps in our knowledge about sarcopenia, namely the mechanisms of its occurrence, universal screening methods, diagnostic tools, validated control points, cut-off points, and outcomes. The research results gradually provide answers to the questions, but at the same time new problems appear and require further research and study.

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Authors' information

Bikbavova Galiya R. – Cand. Sci. (Med.), Associate Professor, Department of Advanced-Level Therapy, Endocrinology, Omsk State Medical University, Omsk, galiya1976@mail.ru, https://orcid.org/0000-0001-9252-9152

Livzan Maria A. – Dr. Sci. (Med.), Corresponding Member of the Russian Academy of Sciences, Rector, Head of the Department of Internal Diseases and Gastroenterology, Omsk State Medical University, Omsk, mlivzan@yandex.ru, https://orcid.org/0000-0001-6581-7017

Tikhonravova Daria V. – Sixth-year Student, Faculty of General Medicine, Omsk State Medical University, Omsk, nobrainnogain@mail.ru, https://orcid.org/0000-0002-0396-7853;

(✉) **Bikbavova Galiya R.,** galiya1976@mail.ru

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The role of endosarcomeric cytoskeleton proteins in the mechanisms of left ventricular diastolic dysfunction: focus on titin

Kalyuzhin V.V.[1], Teplyakov A.T.[2], Bespalova I.D.[1], Kalyuzhina E.V.[1], Chernogoryuk G.E.[1], Terentyeva N.N.[3], Grakova E.V.[2], Kopeva K.V.[2], Usov V.Yu.[2], Garganeeva N.P.[1], Livshits I.K.[1], Petrova I.V.[1], Lasukova T.V.[1]

[1] *Siberian State Medical University*
2, Moscow Trakt, Tomsk, 634050, Russian Federation

[2] *Cardiology Research Institute, Tomsk National Research Medical Center (NRMC) of the Russian Academy of Sciences*
111a, Kievskaya Str., Tomsk, 634012, Russian Federation

[3] *Surgut State University*
1, Lenina Av., Surgut, 628412, Russian Federation

ABSTRACT

Recognizing the fact that isolated left ventricular (LV) diastolic dysfunction (DD) underlies approximately 50% of all heart failure cases requires a deep understanding of its principal mechanisms so that effective diagnostic and treatment strategies can be developed. Despite abundance of knowledge about the mechanisms underlying DD, many important questions regarding the pathophysiology of diastole remain unresolved. In particular, the role of endosarcomeric cytoskeleton pathology in the deterioration of the so-called active (relaxation of the LV myocardium and the atrioventricular pressure gradient at the beginning of diastole, closely related to it in a healthy heart) and passive (myocardial stiffness) characteristics of diastole needs to be clarified.

The lecture briefly discusses the complex hierarchy of DD mechanisms (from the sarcomere to the whole heart) and covers the role of the giant protein titin in the latter, which is the main determinant of intracellular stiffness. Impairment of myocardial relaxation and deterioration of its wall compliance under a wide range of pathological conditions (pressure overload, ischemia, inflammation, cardiotoxic effects, oxidative stress, etc.) underlying DD can be explained by a shift in titin expression toward its more rigid N2B isoform, hypophosphorylation by protein kinases A and G or dephosphorylation by serine / threonine phosphatase 5 of its molecule in the extensible protein segment containing a unique N2B sequence, hyperphosphorylation of PEVK regions of titin by protein kinase C, as well as inhibition of the Ca^{2}+-dependent titin – actin interaction.

The results of deciphering these mechanisms can become a tool for developing new approaches to targeted therapy for diastolic heart failure that currently does not have effective treatment, on the one hand, and the key to understanding the therapeutic effects of drugs already used to treat chronic heart failure with preserved LV ejection fraction, on the other hand.

Keywords: heart failure with preserved ejection fraction, diastolic heart failure, left ventricle, diastolic dysfunction, mechanisms, endosarcomeric cytoskeleton, titin, alternative splicing, post-translational modification

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✉ *Kalyuzhin Vadim V.,* kalyuzhinvv@mail.ru

Роль белков эндосаркомерного скелета в механизмах диастолической дисфункции левого желудочка: фокус на титин

Калюжин В.В.[1], Тепляков А.Т.[2], Беспалова И.Д.[1], Калюжина Е.В.[1], Черногорюк Г.Э.[1], Терентьева Н.Н.[3], Гракова Е.В.[2], Копьева К.В.[2], Усов В.Ю.[2], Гарганеева Н.П.[1], Лившиц И.К.[1], Петрова И.В.[1], Ласукова Т.В.[1]

[1] Сибирский государственный медицинский университет (СибГМУ)
Россия, 634050, Томск, Московский тракт, 2

[2] Научно-исследовательский институт (НИИ) кардиологии, Томский национальный исследовательский медицинский центр (НИМЦ) Российской академии наук
Россия, 634012, Томск, ул. Киевская, 111а

[3] Сургутский государственный университет (СурГУ)
Россия, 628412, Сургут, пр. Ленина, 1

РЕЗЮМЕ

Признание того, что изолированная диастолическая дисфункция (ДД) левого желудочка (ЛЖ) лежит в основе примерно 50% всех случаев сердечной недостаточности, требует глубокого понимания ее основных механизмов, чтобы можно было разработать эффективные диагностические и терапевтические стратегии. Несмотря на то, что в настоящее время достаточно много известно о механизмах, лежащих в основе ДД, немало важных вопросов, касающихся патофизиологии диастолы, еще ожидают своего решения. В частности, нуждается в уточнении роль патологии эндосаркомерного скелета в ухудшении так называемых активных (релаксация миокарда ЛЖ и тесно связанный с ней в здоровом сердце атриовентрикулярный градиент давления в начале диастолы) и пассивных (миокардиальная жесткость) характеристик диастолы.

В лекции кратко рассматривается сложная иерархия механизмов ДД (от саркомера до целого сердца) и обсуждается участие в последних гигантского белка титина, который является основной детерминантой внутриклеточной жесткости. Лежащие в основе ДД нарушение активного расслабления миокарда и ухудшение податливости его стенки при широком спектре патологических состояний (перегрузка давлением, ишемия, воспаление, кардиотоксические воздействия, окислительный стресс и др.) могут объясняться смещением экспрессии титина в сторону его более жесткой N2B-изоформы, гипофосфорилированием протеинкиназами A и G или дефосфорилированием серин/треонин фосфатазой 5 ее молекулы в сегменте растяжимой части белка, содержащим уникальную N2B последовательность, гиперфосфорилированием PEVK элементов титина протеинкиназой C, а также нарушением Ca^{2+}-зависимого титин-актинового взаимодействия.

Результаты расшифровки этих механизмов могут стать инструментом для разработки новых подходов к направленной коррекции не имеющей эффективного лечения диастолической сердечной недостаточности, с одной стороны, и ключем для понимания саногенных эффектов препаратов, уже применяемых для терапии хронической сердечной недостаточности с сохраненной фракцией выброса ЛЖ – с другой.

Ключевые слова: сердечная недостаточность с сохраненной фракцией выброса, диастолическая сердечная недостаточность, левый желудочек, диастолическая дисфункция, механизмы, эндосаркомерный скелет, титин, альтернативный сплайсинг, посттрансляционная модификация

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

Diastolic dysfunction (DD) of the left ventricle (LV) is often referred to as a key link in the cardiovascular continuum, developing into clinically significant chronic heart failure (CHF) not only in common cardiovascular diseases (ischemic heart disease, arterial hypertension) [1–6], but also in pathologies that are rare in many regions of the world, in particular, endemic parasitic diseases (for example, Chagas disease and opisthorchiasis) [7–11].

Intact ventricular diastolic function is crucial for maintaining a normal level of blood circulation, aimed at the fullest satisfaction of the metabolic needs of body tissues, by ensuring a balance between stroke volume and the amount of blood entering the ventricles in diastole, not only at macro-, but also at micro-time intervals, despite the fact that the conditions of both blood flow to the heart and its ejection constantly change. While DD naturally leads to an increase in the filling pressure of the heart, sooner or later it also adversely affects the efficiency of ventricular systole [12–14].

Recognizing that isolated LVDD underpins approximately 50% of all cases of heart failure, which has a poor evidence base for improving the prognosis, requires a deep understanding of its underlying mechanisms so that effective diagnostic and therapeutic strategies can be developed [13, 15–21].

Despite the fact that the mechanisms underlying DD are well studied, many important issues related to the pathophysiology of diastole are still to be resolved. In particular, the role of the endosarcomeric cytoskeleton pathology in the deterioration of the so-called active (relaxation of the LV myocardium and the atrioventricular pressure gradient at the start of diastole, closely related to the LV myocardial relaxation in a healthy heart) and passive (myocardial stiffness) components of diastole needs to be clarified [22–26].

The aim of this lecture was to discuss the role of pathology of endosarcomeric cytoskeleton proteins in the mechanisms of LVDD, which have a hierarchy that is difficult to understand.

HIERARCHY OF DIASTOLIC DYSFUNCTION MECHANISMS

Hierarchy of the main mechanisms of DD (from sarcomere to the whole heart) is presented in the most general form in the Table [27].

Table

Hierarchy of the main mechanisms of impaired ventricular filling [27]

Level of change	Note
Myofibrils (sarcomeres)	At the level of myofibrils, increased stiffness and impaired relaxation may be due to modification of proteins that make up thick and thin filaments, the endrosarcomeric cytoskeleton (in particular, titin, nebulin, αa-actinin-2, myomesin), as well as myosin binding protein-C [28–32].
Cardiomyocyte	At the cardiomyocyte level, the ionic (Ca2+) transport system and the interaction of myofibrils play an important role in the pathogenesis [33]. At the same time, the change in the state of the system of membrane intracellular channels (for example, ryanodine receptors type 2), calcium uptake proteins by the sarcoplasmic reticulum (phospholamban, Ca^{2+} ATPase of the sarcoplasmic reticulum), sarcolemmal ion exchanger (sodium-calcium exchanger) and ion pumps (Na + / K+ ATPase) is of great importance [34–37].
Extracellular matrix	The diastolic properties of the ventricle are directly related to the state of extracellular matrix proteins (collagens, proteoglycans / glycosaminoglycans, elastin, fibronectin, laminin, and some other glycoproteins). The predominant glycoprotein of the extracellular matrix is collagen, the fibers of which surround each myocyte and provide connections between muscle fibers (the endomysium surrounds and connects individual cardiomyocytes, perimysial fibers divide cardiomyocytes into groups, the epimysium surrounds and groups a large number of muscle fibers, for example, papillary muscles) [38]. Extracellular matrix rremodeling, which occurs in many cardiovascular diseases, naturally leads to depressed myocardial compliance and is characterized by an increase in the total content of collagen and a change in the ratio of its types (a decrease in type III collagen found most commonly in tissues exhibiting elastic properties and an increase in the content of type I collagen which confers strength to the tissue and the molecules of which are cross-linked) [39].
Heart	At the organ level, ventricular filling is affected by systemic and intracardiac hemodynamic parameters (e.g., changes in afterload, the presence and severity of septal defects, and valvular insufficiency), geometric factors (the type of ventricular remodeling largely determines chamber and myocardial stiffness), and external limitations (constrictive pericarditis, pericardial effusion).

Table (continued)

Level of change	Note
	DD refers to such a pathological condition when the ventricle cannot receive blood at low pressure and fill without a compensatory increase in atrial pressure due to impaired active myocardial relaxation and / or deterioration of its wall compliance [39, 40]. It is necessary to distinguish between heart failure that has developed as a result of a primary impairment of active relaxation of the ventricular myocardium and / or deterioration of its wall compliance from that when the underlying impaired heart filling was not caused by ventricular DD [41]. The definition of LVDD does not include patients with mitral stenosis, in whom a mechanical obstruction of blood flow at the level of the left atrioventricular valve causes impaired ventricular filling and an increase in left atrial pressure [42]. A similar statement can be made in relation to constrictive pericarditis or pericardial effusion [16, 43]. Since in this pathology there is no impairment of myocardial relaxation and / or an increase in myocardial stiffness, after timely treatment (for example, valvotomy or effective removal of pericardial effusion), the LV regains the ability to receive blood at low pressure and fill without a compensatory increase in pressure in the left atrium [16].

Since the mechanisms of development and progression of diastolic heart failure have a complex hierarchy, it can be reasonably assumed that primary and secondary prevention will be effective only with a balanced (multifaceted) effect on various aspects of the pathogenesis [27]. At the same time, it is necessary to take into account the etiological heterogenic causes of heart failure which may include absolutely any cardiovascular disease, the features of the pathogenesis in which undoubtedly leave an imprint on the mechanisms and, very importantly, the sequence in which impairments of active myocardial relaxation develop and its stiffness increases [12, 44–48]. However, in most cases, the pathology of diastolic relaxation usually precedes an increase in ventricular stiffness [14, 42, 49]. As a rule, a decrease in cardiac output occurs later and is actually inevitable in patients with severe DD, since impaired filling eventually leads to a decrease in the cardiac index value [42, 50, 51].

The high diastolic stiffness of the damaged myocardium is stereotypically associated with the structural rearrangement of the extracellular matrix, characterized by changes in the qualitative and quantitative characteristics of interstitial proteins (primarily collagen), but with the development of DD modifications of proteins that make up the endrosarcomeric cytoskeleton are apparently no less important [52–54].

TITIN AND ITS ROLE IN THE MECHANISMS OF THE LEFT VENTRICULAR DIASTOLIC DYSFUNCTION

A significant difference between the myocardium and skeletal muscles, the contraction of which can normally be prolonged, is that the contraction in the muscle fiber of the heart always breaks naturally, and the relaxation cannot be prevented even with artificial extension of the cell excitation time. This feature of the myocardium is due to the need for mandatory relaxation to reduce pressure in the ventricles, without which it is impossible to fill them with blood from the venous bed during diastole. Complete recovery of length is typical even for isolated cardiomyocytes that do not experience any load.

This elastic straightening of myofibrils (elastic recoil) can be explained by some elastic formations in them that contract when shortened and straighten when relaxed [51, 54]. Similarly, the elastic structures of the myocardium make it more elastic than skeletal muscles, protecting sarcomeres from overstretching, which is theoretically possible during an overload of blood volume. Even when the muscle fiber of the heart is stretched with great force that is not encountered under physiological conditions, the length of the sarcomere increases very moderately, which prevents the complete extension of actin filaments from the interaction with myosin [54]. Elastin and collagen fibers of the extracellular matrix can poorly fulfill this function, since elastin is stretched only under the action of a sufficiently large force, and collagen proteins are practically inextensible (the extracellular matrix takes on the load only when stretched to large degrees) [54].

The significantly lower extensibility of the isolated cardiac muscle compared to the skeletal one is largely due to the presence of a well-developed endosarcomeric cytoskeleton in the cardiomyocyte. The state of several proteins of the endosarcomeric cytoskeleton is known to determine the elasticity of the cardiac muscle

fiber: titin (also known as connectin), nebulin, α-actinin, myomesin, etc. [32, 55–57]. But titin is the main determinant of intracellular stiffness, in the physiological range of the sarcomere length (1.9–2.2 μm) it causes 90% of passive tension in the cardiomyocyte (the elasticity of titin in the myocardium is 20 times higher than in the skeletal muscle) [27, 53, 58–60].

Titin is the largest of the known single peptides, consisting of a sequence of about 30,000 amino acids, the listing of which would take a third of this issue of the journal. Taking into account the molecular weight approximately equal to 3 MDa (up to 18% of all myocardial proteins), it is no coincidence that titin is called giant [54, 61, 62].

Titin with its N-terminus is anchored in the Z-line and is located in the I-band (the light band of the sarcomere, which like an elastic spring undergoes elongation when the muscle is stretched). In the A-band, titin with its C-terminus is attached to myosin (each myosin thread binds 6 titin molecules) (Figure), actually covering half of the sarcomere [51, 63]. When the sarcomere contracts to a length that is less than the unloaded or "passive" one, the elastic spring in the I-band compresses (Figure, *a*). When the sarcomere is stretched, titin exerts resistance, which is expressed in the creation of a resting tension (Figure, *b*).

During systole, when titin is compressed, potential energy is accumulated. During diastole, titin acting like a spring (elastic recoil force) applies this energy and develops the so-called restoring force to restore the initial sarcomere length, as a result of which the myocardium rapidly "straightens out" and the blood is sucked into the LV cavity, since at the beginning of diastole, the LV volume changes faster than the blood flow that should flow into it. At the beginning of systole, which is another part of the compression / stretch cycle, a stretched giant protein that accumulated elastic potential energy by the end of diastole transforms it into kinetic energy. This energy transformation and the optimal sarcomere length provided by the elastic titin components in diastole, when in accordance with the Frank – Starling law, the maximum contraction force is achieved, are extremely important factors for maintaining the systolic function of the cardiomyocyte, which, however, is not the subject of this lecture [51, 54, 64–66].

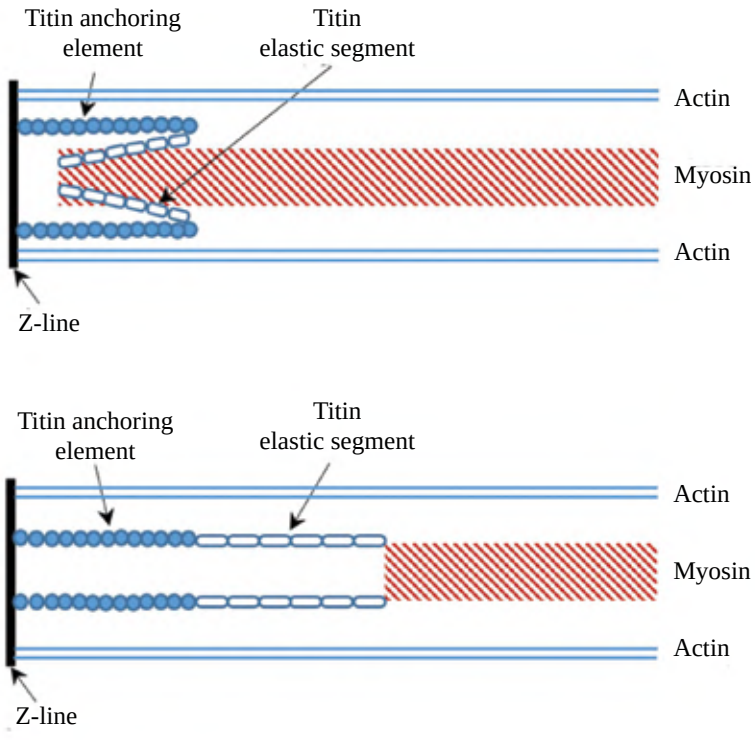

Figure. Titin in the sarcomere I-band (simplified scheme) (adapted from [63]): *a* – systole; *b* – diastole. Not adjusted to scale. To keep it simple, only two thin filaments (actin) and two titin molecules per thick filament (myosin) are shown, with titin A-bands and myosin heads omitted. A short inelastic titin I-band segment located at the A/I junction and regulatory proteins of thin filament are also omitted

In the structure of titin, there are: 1) sequentially connected immunoglobulin-like domains (Ig-segments), which are identified in the proximal (close to the Z-line), intermediate (alternatively spliced only in the N2BA-isoform), and distal part of the protein (close to the A-band); 2) a segment consisting of proline, glutamic acid, valine, and lysine residues (PEVK); 3) the unique N2B sequence (found in the myocardium, but not in skeletal muscles), to which the N2A element is added in the N2BA isoform, similar to that found in the skeletal muscle [54, 63, 67].

The elasticity of titin molecules, which determines both active (relaxation) and passive (stiffness) characteristics of the ventricular myocardium in diastole, is determined by the length and composition of the segments of the so-called extensible part of the protein in the I-band. Two main isoforms of titin have been described. The first one is a short (up to 26,926 amino acids in the human myocardium) and rigid N2B-isoform, which has a short extensible segment in the I-band. Due to its shorter length, higher resistance is created when the sarcomere is stretched. The second isoform is a more compliant N2BA-isoform with up to 34,350 amino acids, with a long elastic segment, characterized by a high content of immunoglobulin (the most extensible) and PEVK (more elastic) elements, as well as the presence of the N2A sequence [20, 25, 54, 63].

There is species-specific expression of the isoform profile: the N2B isoform dominates in rodents (the N2BA/N2B ratio is 20:80), while N2BA is dominant in most large mammals and in humans (the N2BA/N2B ratio ranges from 60: 40 to 80:20). In this case, two isoforms can coexist in one sarcomere, and each isoform functions independently. The co-expression of isoforms in different ratios leads to the modulation of the passive mechanical properties of the sarcomere and makes it possible to regulate diastolic parameters [54, 68].

A shift in expression toward the N2B isoform may determine an increase in diastolic ventricular stiffness. Moreover, the expression of various forms of titin (N2BA and N2B) largely determines the variant of myocardial remodeling that develops in various forms of myocardial damage and overload by the type of eccentric and concentric hypertrophy (concentric remodeling), which is characterized by the formation of classical systolic and diastolic CHF, respectively [53, 69–72].

In the experiments that focused on heart failure in dogs caused by accelerated pacing and spontaneous systemic arterial hypertension in rats, an increase in diastolic myocardial stiffness is associated with an increase in expression of the N2B-isoform of titin (more rigid), while in experimental infarction in rats and in patients with end-stage ischemic cardiomyopathy, the expression of the N2BA isoform that is sometimes called fetal is more pronounced [73–76]. Although the results of studies aimed at identifying the relationship between titin isoforms and myocardial stiffness generally correspond with each other well, the mechanisms that lead to isoform shift in heart failure remain unclear [27, 30, 77, 78].

Obviously, the structural rearrangement of the sarcomere, which underlies long-term adaptation, depends on the type of overload (volume or pressure) or damage to the myocardium (ischemic, metabolic, or cardiotoxic) and requires reconfiguration of alternative splicing [54, 76]. When there is a need for increased stroke volume (for example, aortic insufficiency or a loss of a significant proportion of viable cardiomyocytes due to myocardial infarction), the N2BA / N2B ratio shifts toward the long (N2BA) titin isoform. On the contrary, if the load is predominantly forceful (in particular, with systemic arterial hypertension, LV outflow tract obstruction, or aortic stenosis), the ratio shifts toward a shorter, more rigid N2B titin isoform [54]. The last scenario, in which the RNA-binding motif protein 20 (RBM20) is the repressor, is a classic one for the development of DD and heart failure with preserved LVEF [31, 39].

However, it is possible to exactly reproduce some scenario in the experiment, but, in clinical practice, a patient with comorbid pathology often has a combined type of damage and overload of the myocardium. For example, a patient with metabolic syndrome developed myocardial infarction which then led to papillary muscle dysfunction and relative mitral valve insufficiency. This all makes it difficult to understand the mechanisms of alternative splicing restructuring. However, it is clear that the final reorganization of alternative splicing will be determined by the characteristics of the dominant mechanical stress of the myocardium, an important sensor of which is titin together with other proteins of the endosarcomeric cytoskeleton. Titin interacts with these proteins with its N-terminus in the Z-line (α-actinin, telethonin and others) and its C-terminus in the A-band (for example, myomesin), as well as in the I-band and M-line (for example, obscurin), which are important points of mechanotransduction [32, 53, 54, 76, 79]. Altering the expression of numerous

titin-based mechanotransduction-associated proteins (for example, CRYAB, ANKRD1, muscle LIM protein, p42, CAMK2D, p62, NBR1, FHLs) makes it possible to regulate myocardial stiffness [54, 80, 81].

The functional state of the thyroid and pancreas also affects the sarcomere stiffness. A change in the isoform pattern toward a more rigid N2B-titin may be caused by hyperthyroidism and hyperinsulinemia, in which the phosphatidylinositol 3-kinase/Protein kinase B/mTOR axis is activated with an increase in RBM20 transcription. Conversely, low levels of triiodothyronine and insulin contribute to a shift in the isoform pattern toward a compliant N2BA titin [79, 82–85]. Another hormone angiotensin II whose maladaptive chronic activity surplus is observed in diastolic heart failure [19, 86, 87] increases myocardial stiffness by affecting the titin isoform profile, activating the mitogen-activated protein kinase/ELK1 signaling pathway, and increasing RBM20 transcription [88].

Changes in the expression of titin isoforms may take days or weeks, but the adjustment of the titin contribution to passive myocardial tension can also occur quickly (even within one cardiac cycle) [80]. As for the short-term modulation of cardiomyocyte elasticity, post-translational modification of titin is closely related to the mechanisms of rapid adaptation of the myocardium to varying hemodynamic requirements to the heart (for example, during exercise), when it is necessary to rapidly change the parameters of blood expulsion and its inflow to meet the metabolic needs of body tissues [67].

Catecholamines, nitric oxide, and natriuretic peptides can reduce the stiffness of titin springs, rapidly modulating diastolic function. Thus, β1-adrenergic agonists activate the signaling pathway through protein kinase A (cAMP-dependent), which phosphorylates the titin molecule in the segment of the I-band protein extensible part containing the unique N2B sequence (influence on PEVK elements can lead to the opposite effect), mainly in the N2B isoform (the elasticity of the N2BA isoform increases), which leads to a decline in its elasticity and an increase in myocardial extensibility, contributing to better ventricular filling [54, 79]. Nitric oxide and natriuretic peptides modulate titin elasticity in the same direction with the participation of another second messenger, cGMP-dependent protein kinase G, which along with proteinase A phosphorylates the same site in the N2B structure [54, 67, 89, 90]. In addition to the above protein kinases, protein kinase CaMKII and protein kinase D, which also phosphorylates the titin molecule in the segment containing the unique N2B sequence, have a similar effect on the extensibility of titin [54, 91].

Protein kinase C also phosphorylates the titin molecule in the segment of the I-band protein extensible part. Unlike the above-mentioned protein kinases A and G, phosphorylation of titin PEVK elements by protein kinase C (for example, during stimulation of α1-adrenergic receptors), the expression of which is increased in a wide range of pathological conditions (myocardial hypertrophy, heart failure with preserved LVEF) and cardiovascular diseases (coronary heart disease, essential hypertension), is accompanied by an increase in myocardial elasticity [54, 92–94].

Impaired phosphorylation of certain parts of the titin molecule or activation under the influence of certain factors of its dephosphorylation by serine / threonine phosphatase 5 (for example, HSP90 chaperone, oxidative stress inducers and products or proinflammatory cytokines) are considered titin-dependent mechanisms of developing increased myocardial stiffness and diastolic heart failure [12, 49, 53, 54, 95–98]. Since differentiated segments of individual titin isoforms in the I-band extensible part can be phosphorylated by different protein kinases, which leads to different effects, it is important to clarify the status of titin phosphorylation when a comprehensive understanding of the mechanisms of changes in myocardial stiffness during the development of heart failure with preserved LVEF is required [80, 99].

The diastolic stiffness of a cardiomyocyte does not only depend on the titin length (compliance), but is also determined by the titin – actin interaction, which is Ca^{2+}-dependent. Thus, the dependence of passive myocardial stress on Ca^{2+} concentration was described, which is associated with the ability of PEVK domains of titin I-band to bind to actin at an increased macroelement concentration, leading to a slowdown in sliding of titin and actin [54]. It is well known that the alteration of proteins regulating Ca^{2+} metabolism, such as the Ca^{2+} ATPase of the sarcoplasmic reticulum, its modulator phospholamban, other channels of the sarcoplasmic reticulum and their modulators (for example, FK506-binding protein 12.6) and the Na^{+}/Ca^{2+} exchanger is accompanied by impaired removal of Ca^{2+} from the cytosol and, as a result, a slowdown in relaxation [27, 100, 101]. Finally, titin-dependent diastolic elasticity may be determined by the work of stretch-activated potassium channels, the dysfunction (including a genetically determined one) of which can

increase the risk of developing CHF with preserved LVEF [102–104].

An acute and chronic increase in the passive stiffness of titin leads to an increase in its mechanical deformation and potentially nears the time of its degradation (half-life can vary from several hours to two or three days), the multi-stage process of which is very difficult to understand. The role of ubiquitin-proteasome and autophagosomal – lysosomal (autophagy) systems, proteases (such as calpains and matrix metalloproteinase-2, and heat shock proteins are discussed. However, to date, the exact mechanisms of degradation / protection of the titin molecule, given its impressive mass and length (about 1 μm), have not been fully studied [23, 78].

The impaired active myocardial relaxation and deterioration of its wall compliance in a wide range of pathological conditions (pressure overload, ischemia, inflammation, cardiotoxic effects, oxidative stress, etc.) underlying DD can be explained by a shift in titin expression toward its more rigid N2B isoform, hypophosphorylation by protein kinases A and G or dephosphorylation by serine / threonine phosphatase 5 in the segment of the extensible part of the protein containing the unique N2B sequence, hyperphosphorylation of titin PEVK elements by protein kinase C, as well as impaired Ca2+-dependent titin – actin interaction.

CONCLUSION

Maladaptive shifts in alternative splicing of titin and the pathology of its post-translational modification associated with impaired active relaxation of the myocardium and / or worsening compliance of its wall are the key mechanisms of the development of LVDD and diastolic heart failure. On the one hand, the results of deciphering these mechanisms can become a tool for developing new approaches to targeted therapy for patients with diastolic heart failure that does not have effective treatment. On the other hand, they can become the key to understanding the therapeutic effects of drugs already used to treat chronic heart failure with preserved LV ejection fraction.

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Authors' information

Kalyuzhin Vadim V. – Dr. Sci. (Med.), Professor, Head of the Advanced Therapy Division with Rehabilitation Training, Physiotherapy and Sports Medicine, Siberian State Medical University, Tomsk, kalyuzhinvv@mail.ru, http://orcid.org/0000-0001-9640-2028

Teplyakov Alexander T. – Dr. Sci. (Med.), Professor, Principal Researcher, Cardiology Research Institute, Tomsk NRMC, Tomsk, vgelen1970@gmail.com, http://orcid.org/0000-0003-0721-0038

Bespalova Inna D. – Dr. Sci. (Med.), Head of the Propaedeutics of Internal Diseases Division with Therapy Course of the Pediatrics Department, Siberian State Medical University, Tomsk, innadave@mail2000.ru, http://orcid.org/0000-0002-4513-6329

Kalyuzhina Elena V. – Dr. Sci. (Med.), Professor of the Advanced Therapy Division with Rehabilitation, Physiotherapy and Sports Medicine Course, Siberian State Medical University, Tomsk, kalyuzhina.e@mail.ru, http://orcid.org/0000-0002-7978-5327

Chernogoryuk Georgy E. – Dr. Sci. (Med.), Professor of the Advanced Therapy Division with Rehabilitation, Physiotherapy and Sports Medicine Course, Siberian State Medical University, Tomsk, chernogoryuk@yandex.ru, http://orcid.org/0000-0001-5780-6660

Terentyeva Nadezhda N. – Cand. Sci. (Med.), Associate Professor of the Department of Internal Diseases, Surgut State University, Surgut, nadiater@mail.ru, http://orcid.org/0000-0002-0462-3526

Grakova Elena V. – Dr. Sci. (Med.), Leading Researcher, Department of Myocardial Pathology, Cardiology Research Institute, Tomsk NRMC, Tomsk, gev@cardio-tomsk.ru, http://orcid.org/0000-0003-4019-3735

Kopeva Kristina V. – Cand. Sci. (Med.), Researcher, Department of Myocardial Pathology, Cardiology Research Institute, Tomsk NRMC, Tomsk, kristin-kop@inbox.ru, http://orcid.org/0000-0002-2285-6438

Usov Vladimir Yu. – Dr. Sci. (Med.), Professor, Leading Researcher, Department of Radiology and Tomography, Cardiology Research Institute, Tomsk NRMC, Tomsk, ussov1962@yandex.ru, http://orcid.org/0000-0001-7978-5514

Garganeeva Natalia P. – Dr. Sci. (Med.), Professor of the General Medical Practice and Outpatient Therapy Division, Siberian State Medical University, Tomsk, garganeeva@gmail.com, http://orcid.org/0000-0002-7353-7154

Livshits Inna K. – Cand. Sci. (Med.), Associate Professor of the Advanced Therapy Division with Rehabilitation, Physiotherapy and Sports Medicine Course, Siberian State Medical University, Tomsk, http://orcid.org/0000-0003-2357-6910

(✉) **Kalyuzhin Vadim V.,** kalyuzhinvv@mail.ru

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The role of PASS and STITCH in the verification of unknown properties of pyruvate and lactate. Literature review and fragments of authors' own research

Kolotyeva N.A.[1], Gilmiyarova F.N.[2], Gusyakova O.A.[2], Semashkova E.A.[2]

[1] *Brain Research Institute, Research Center of Neurology*
80, Volokolamskoye Highway, Moscow, 125367, Russian Federation

[2] *Samara State Medical University*
89, Chapayevskaya Str., Samara, 443099, Russian Federation

ABSTRACT

The aim of the study was to identify the predicted spectrum of biological activity of pyruvate and lactate using modern computer modeling methods and to determine potential protein partners in intermolecular interaction.

Materials and methods. The biological activity spectrum of pyruvate and lactate by the structural formula was determined using the PASS (Prediction of Activity Spectra for Substances) software. Potential protein interaction partners for small molecules were predicted using the Search Tool for Interactions Chemicals (STITCH).

Results. Analyzing the obtained results *in silico* reveals that pyruvate and lactate exhibit diverse biological activities, molecular mechanisms, and pharmacological effects. These include regulation of lipid, protein, and carbohydrate metabolism and effects on enzyme activity and gene expression. The data on the antihypoxic, antiischemic, antitoxic, immunomodulatory, antiinflammatory, antiviral, vasoprotective, and cytoprotective effects are presented. The neuroprotective and antineurotoxic effects of pyruvate and lactate are predicted.

Conclusion. The spectrum of biological activities of lactate and pyruvate were revealed by computer modeling methods, and protein interaction partners were characterized. The small molecules we studied have a coordinating role in the functioning and modulation of mediator, hormonal, receptor, immune, inflammatory, antibacterial, and antiviral responses and gene expression. The use of natural intermediates as therapeutic agents for the treatment of ischemic stroke, acute neurological disorders, and neurodegeneration is discussed, which is underlain by the stimulating effect of metabolites on neuroplasticity. These properties may be manifested through conformational rearrangement of receptors, active binding centers, expression of multiple genes, and changes in the functional manifestations of catalytic and other proteins. The obtained data will obviously expand our understanding of the role of small molecules in intermolecular metabolite – protein interactions.

Keywords: small molecules, pyruvate, lactate, biological activity, computer modeling, PASS, STITCH

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✉ *Kolotyeva Nataliya A.,* kolotyeva.n@yandex.ru

PASS и STITCH в верификации неизвестных свойств пирувата и лактата. Обзор литературы и фрагменты собственных исследований

Колотьева Н.А.[1], Гильмиярова Ф.Н.[2], Гусякова О.А.[2], Семашкова Е.А.[2]

[1] *Институт мозга, Научный центр неврологии*
Россия, 125367, г. Москва, Волоколамское шоссе, 80

[2] *Самарский государственный медицинский университет (СамГМУ)*
Россия, 443099, г. Самара, ул. Чапаевская, 89

РЕЗЮМЕ

Цель исследования заключается в выявлении прогнозируемого спектра биологической активности пирувата и лактата с применением современных методов моделирования, определение потенциальных белковых партнеров для межмолекулярного взаимодействия.

Материалы и методы. Определение спектра биологической активности пирувата и лактата по структурной формуле проводили в программном обеспечении Prediction of Activity Spectra for Substances (PASS). Прогнозирование потенциальных белковых партнеров взаимодействия для малых молекул выполняли в системе Search Tool for Interactions Chemicals (STITCH, инструмент поиска взаимодействующих химических веществ).

Результаты. Анализируя полученные результаты *in silico*, обращает на себя внимание проявление разнообразной биологической активности молекулярных механизмов, оказываемых фармакологических эффектов пирувата и лактата. Среди них регуляция липидного, белкового, углеводного обменов, влияние на активность ферментов, экспрессию генов. Приводятся данные антигипоксического, антиишемического, антитоксического, иммуномодулирующего, противовоспалительного, противовирусного, вазопротекторного и цитопротекторного действий. Спрогнозировано нейропротекторное, антинейротоксическое действие пирувата и лактата.

Заключение. Методами компьютерного моделирования раскрыт спектр биологической активности лактата и пирувата, а также охарактеризованы белки-партнеры по взаимодействию. Изучаемые нами малые молекулы выполняют координационную роль в функционировании и модуляции медиаторного, гормонального, рецепторного ответов, иммунологических, воспалительных, антибактериальных, противовирусных реакций, экспрессии генов. Обсуждается использование естественных интермедиатов в качестве терапевтических средств для лечения ишемического инсульта, острых неврологических расстройств, нейродегенерации, что имеет в своей основе стимулирующее действие метаболитов на процессы пластичности мозга. Проявление этих свойств, вероятно, реализуется через конформационную перестройку рецепторов, активных центров связывания, экспрессии множества генов, изменение функциональных проявлений каталитических и других белков. Полученные знания, очевидно, расширят наше понимание роли малых молекул в межмолекулярных взаимодействиях метаболит–белок.

Ключевые слова: малые молекулы, пируват, лактат, компьютерное моделирование, биологическая активность, PASS, STITCH

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 study of the role of metabolites in intercellular interaction systems is currently relevant. In particular, protein – small molecule interactions can regulate and control a variety of cellular processes, such as transport of substances and transmission of signals, playing a role in maintaining cellular homeostasis [1–3]. For economic and practical reasons, assessing intermolecular interaction of millions of chemical compounds with thousands of ligands in the experiment is difficult, so it is advisable to conduct

a preliminary assessment of the biological activity of specific chemicals *in silico* [4, 5].

Biological activity is the main characteristic of compounds with a known chemical formula, since its presence can be the basis for the use of the substance in medicine or it can limit its use due to the manifestation of undesirable side or toxic effects. The use of computer modeling can reduce the volume of necessary experiments by dozens of times in comparison with a blind search [6].

The focus of our attention is on small molecules pyruvate and lactate. One of the most important intermediate components of metabolism and a source of energy for mitochondria is pyruvate, which participates in the processes of cell matrix remodeling [7, 8]. Lactate is no longer considered as a dead-end product of anaerobic metabolism. Being an energy substrate, it plays an important role in regulating the function of many cells, participating in the processes of tissue proliferation and differentiation and angiogenesis [9, 10].

The article is devoted to *in silico* modeling and elucidation of biological activity and molecular mechanisms underlying the description of molecular structure, taking into account the search for structure – property relationships. Our understanding of cellular signal transduction may contribute to the elucidation of new interactions between lactate and pyruvate with proteins.

The aim of the study was to identify the predicted spectrum of biological activity of pyruvate and lactate using modern computer modeling methods and to determine potential protein partners in intermolecular interaction.

MATERIALS AND METHODS

PASS version 1.917 (Prediction of Activity Spectra for Substances) software is intended for prediction of the biological activity spectrum of a compound by its structural formula based on the analysis of structure – activity relationships using a training sample of compounds. The program has information on structures and known biological activities of over one million molecules. Biological activity for new compounds is predicted using Multilevel Neighborhoods of Atoms (MNA) descriptors, which are necessary to describe the structure of molecules in an organic compound, taking into account the search for structure – property relationships for heterogeneous samples [6].

The spectrum of biological activity predicted by the PASS computer system includes pharmacological effects, specific toxicity, side effects, effects of molecules on metabolism, molecular transport, gene expression, identification of undesirable targets, and molecular mechanisms of action. The prediction result is presented as *Pa* ("to be active") and *Pi (*"to be inactive") probabilities with values from 0 to 1 [11]. We took *Pa* over 0.5 as the optimal value of the probability of activity. The prediction of the biological activity spectrum was presented as an ordered list of *Pa* and *Pi* probability estimates.

Potential protein interaction partners for small molecules were identified using the Search Tool for Interactions of Chemicals (STITCH) version 5.0. The STITCH platform includes over 9,600,000 proteins from 2,031 eukaryotic and prokaryotic genomes and 430,000 chemical compounds. This program combines data on existing protein – small molecules interactions from DrugBank, GPCRligand (GLIDA), Matador, Therapeutic Target Database (TTD), Comparative Toxicogenomics Database (CTD), NCI – Nature Pathway Interactions, Reactome, BioCyc, and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases.

Since there can be overlaps between different manually generated datasets, STITCH considers repetitive interactions only once. Other major sources of intermolecular linkages are experimentally validated interaction data sets, which include data from ChEMBL, PDSP Ki Database, and Protein Data Bank. Sources of protein – chemical interactions are supplemented by automated text analysis and structure-based prediction methods. The text mining pipeline includes collaborative copying and processing of all MEDLINE abstracts and available full-text PubMed Central open access articles; NIH RePORTER grant abstracts were also taken into account [1].

To assess the effect and validity of protein – ligand binding, as well as the variability in affinity of known ligands, it is important to know the binding capacity between the compound and its target. Typically, this binding affinity is quantified as the inhibition constant Ki, and the IC50 (half-maximal inhibitory concentration) value is also taken into account [12].

The standard SMILES entry was used to search for identifiers and common chemical names that are stored in the small molecule information database. The program calculates the parameter *p* – the probability of protein – small molecule interaction. In STITCH, the interaction network can be mapped and tuned using different settings: by degree of evidence, confidence, molecular action or binding affinity. In our work, we

used the binding affinity index. The program predicts intermolecular interactions at a confidence threshold of 0 to 1 (low, medium, high, highest). We used a medium confidence threshold of $p > 0.4$ [1].

RESULTS AND DISCUSSION

PASS program for assessing the biological activity of small molecules. In the PASS computer environment, it is possible to analyze a total of 2,736 types of biological activities. Analyzing the data obtained, we can note the highest number of predicted bioactivities for pyruvate compared to lactate (1,926 and 1,792, respectively). It should be noted that pyruvate has a greater ability to exhibit molecular mechanisms of action and exert pharmacological effects, whereas lactate exhibits more adverse, toxic, and metabolic effects.

The studied predicted manifestations of small molecules and experimentally confirmed data are presented in Table 1. The findings indicate that pyruvate and lactate affect cellular processes: they act as cytoprotectors, stimulators of leuko- and erythropoiesis and platelet aggregation, and inhibitors of thrombopoiesis and platelet adhesion due to their ability to activate the hypoxia-inducible factor-1 alpha (HIF-1α) – erythropoietin (EPO) signaling pathway [13].

Table 1

Predicted effects of pyruvate and lactate in the PASS program

Effect	*Pa* lac	*Pi* lac	*Pa* pyr	*Pi* pyr	Experimentally proven data
Fibrinolytic	0.716	0.024	0.812	0.004	[14]
Lipid metabolism regulator	0.784	0.008	0.812	0.006	[15]
Hypercholesterolemic	0.727	0.019	0.757	0.003	
Leukopoiesis stimulator	0.803	0.003	0.727	0.005	[13]
Platelet aggregation stimulator	0.353	0.009	0.706	0.005	
Erythropoiesis stimulator	0.673	0.007	0.698	0.005	[13, 16]
Cytoprotector	0.554	0.017	0.670	0.009	[17]
Antiviral (rhinovirus)	0.623	0.009	0.663	0.005	[18]
Antihypoxic	0.743	0.004	0.650	0.002	[19]
Neuroprotective	0.646	0.066	0.648	0.05	[16, 20, 37–39, 41]
Antiischemic	0.414	0.154	0.611	0.005	[17, 19, 25, 40]
Restorer	0.480	0.019	0.610	0.011	[21]
Antiviral (picornavirus)	0.588	0.027	0.605	0.018	[18]
Antiinflammatory	0.614	0.026	0.600	0.004	[19, 22]
Platelet adhesion inhibitor	0.592	0.001	0.592	0.014	
Vasoprotector	0.681	0.024	0.564	0.025	[23]
Immunomodulator	0.811	0.023	0.554	0.04	[15]
Antineurotoxic	0.639	0.044	0.545	0.06	[17, 24, 39]
Inhibitor of thrombocytopoiesis	0.854	0.006	0.519	0.004	
Antitoxic	0.599	0.006	0.516	0.012	[21]

Note: *Pyr* – pyruvate, lac – lactate, *Pa* – probability of presence; *Pi* – probability of absence.

Pyruvate and lactate have anti-inflammatory and antiischemic effects, increase myocardial contractility and energy state, protect tissues from ischemic damage and oxidative stress by enhancing sarcoplasmic reticular Ca2+ transport, and increase NADPH production to maintain redox state of glutathione [19].

Pyruvate exerts neuroprotective effects, preventing death of postischemic astrocytes by inhibiting lactate dehydrogenase leakage, reducing redox ratio, and inhibiting activation of apoptotic events, such as cytochrome c release from mitochondria and fragmentation of caspase-3 and poly(ADP-ribose)polymerase. Pyruvate can accelerate its own metabolism by increasing pyruvate dehydrogenase activity and thus restore cellular levels of ATP in postischemic astrocytes [17, 20]. According to V.N. Yartsev, the vasoprotective effect of pyruvate and lactate is probably realized through a direct effect on the neurogenic tone of blood vessels [23]. Due to these predicted and experimentally confirmed properties,

pyruvate has been used as a therapeutic agent for the treatment of stroke and acute neurological disorders in studies by D. Frank [25].

Simulated antiviral effect of pyruvate and lactate against rhinovirus and picornavirus is worth noting. Lactate is highly likely to have an antiviral effect on arboviruses and papillomaviruses. There is an assumption that the virus loses its infectious activity due to conformational rearrangements in the structure and a loss of replication activity [18].

There is evidence of the involvement of these intermediates in various types of metabolism: lipid, protein, and carbohydrate (Table 2). We have identified the effect of pyruvate and lactate on the regulation of lipid metabolism, with a significant hypercholesterolemic effect. It is worth noting that these small molecules inhibit trans-2-enoyl-CoA reductase, which is involved in the biosynthesis of unsaturated fatty acids and elongation of mitochondrial fatty acids. Adipocytes have been experimentally shown to be the main source of lactate in white adipose tissue. Lactate levels range from 0.35–9.67 mM at a fat tissue density of 0.9 g / ml. It has been noted that adipocyte-derived lactate is a signaling metabolite that promotes activation of inflammatory macrophages by direct binding to the prolyl hydroxylase domain protein 2, highlighting the relationship between immunological processes and metabolism in obesity [15].

We revealed that pyruvate and lactate exert an inhibitory effect on enzymes of protein metabolism, such as alanine transaminase, serine-3-dehydrogenase, gamma-glutamyl transferase, and tryptophan transaminase. These intermediates are known to affect a number of carbohydrate metabolism enzymes: glycerol-3-phosphate dehydrogenase, L- and D- forms of lactate dehydrogenase, NAD-dependent malate dehydrogenase, NADP-dependent decarboxylating malate dehydrogenase, malate oxidase, pyruvate dehydrogenase complex [16].

Table 2

Probable molecular mechanisms of pyruvate and lactate action

Molecular mechanism of action	Enzyme code	*Pa* pyr	*Pi* pyr	*Pa* lac	*Pi* lac
Phosphoenolpyruvate phosphotransferase inhibitor	EC 2.7.3.9	0.91	0.001	0.922	0.001
Pyruvate decarboxylase inhibitor	EC 4.1.1.1	0.887	0.002	0.938	0.001
Aspartate – phenylpyruvate transaminase inhibitor	EC 2.6.1.70	0.854	0.003	0.816	0.004
Glutamine – phenylpyruvate transaminase inhibitor	EC 2.6.1.64	0.83	0.004	0.807	0.005
Pyruvate dehydrogenase inhibitor cytochrome	EC 1.2.2.2	0.812	0.002	0.59	0.005
Phenylpyruvate decarboxylase inhibitor	EC 4.1.1.43	0.808	0.002	0.614	0.004
Pyruvate dehydrogenase inhibitor	EC 1.2.4.1.	0.799	0.002	0.856	0.002
L-lactate dehydrogenase inhibitor	EC 1.1.2.3	0.778	0.001	–	–
Phosphoenolpyruvate carboxykinase inhibitor	EC 4.1.1.38	0.769	0.002	0.8	0.002
D-lactate dehydrogenase inhibitor	EC 1.1.2.4	0.765	0.001	0.783	0.002
Oxaloacetate tautomerase inhibitor	EC 5.3.2.2	0.723	0.001	0.881	0.001
Glycerol-3-phosphate oxidase inhibitor	EC 1.1.3.21	0.705	0.002	0.616	0.004
Malate dehydrogenase inhibitor	EC 1.1.1.37	0.674	0.005	0.705	0.004
Oxaloacetate decarboxylase inhibitor	EC 4.1.1.3.	0.674	0.002	0.742	0.003
Glycerol-3-phosphate dehydrogenase inhibitor	EC 1.1.1.8	0.669	0.003	0.698	0.003
Malate dehydrogenase acceptor inhibitor	EC 1.1.1.37	0.62	0.011	0.594	0.018
Malate oxidase inhibitor	EC 1.1.3.3	0.567	0.008	0.522	0.016
D-malate decarboxylating dehydrogenase inhibitor	EC 1.1.1.83	0.557	0.003	0.723	0.002
NADPH-dependent malate dehydrogenase inhibitor	EC 1.1.1.40	0.529	0.003	0.5	0.003
Lactate – malate transhydrogenase inhibitor	EC 1.1.99.7	0.524	0.001	0.808	0.001
Inhibitor of NADP-dependent decarboxylating malate dehydrogenase	EC 1.1.1.82	0.471	0.002	0.522	0.003

The influence of natural intermediates on gene expression is worth noting (Table 3). Pyruvate and lactate upregulate the expression of the *HMOX1* gene, which encodes the stress-induced protein heme oxygenase-1, regulating the viability, proliferation, and differentiation of many cell types. In the studies by R.A. Zager et al. (2014), the use of pyruvate resulted in an increase in cytoprotective messenger

RNA oxygenase-1 and IL-10, a selective decrease in proinflammatory MCP-1 and TNFα, and an increase in ATP levels [26]. Pyruvate attenuates the secretion of amphoterin in cells by inducing heme oxygenase-1 through activation of the phosphatidylinositol-3-kinase pathway, protein kinase, and nuclear factor 2 [27].

It is interesting that pyruvate and lactate increase the expression of the *TP53* gene, a transcription factor that regulates the cell cycle. It is known that the p53 protein acts as a suppressor of malignization, so the *TP53* gene is attributed the properties of an antioncogene [28]. An inhibitory effect of pyruvate on the expression of the gene encoding MMP-9, a protein of the matrix metalloproteinase family, was predicted. An increase in the level of the p53 protein contributes to the inhibition of the growth of non-small cell lung cancer cells, stops their invasion and migration, and induces apoptosis [29]. Pyruvate reduces the expression of the tumor necrosis factor (*TNF*) gene. It is known that this gene encodes a multifunctional proinflammatory cytokine, which is mainly secreted by macrophages and is involved in the regulation of cell proliferation, differentiation, and apoptosis. It has been shown to reduce lung tissue infiltration in radiation-induced lung injury by reducing the level of proinflammatory cytokines IL-1β, IL-6, TNFα, GM-CSF, M-CSF, TGF-β1, and HMGB1 [26, 30].

Table 3

Effect of pyruvate and lactate on gene expression

Effect on gene expression	*Pa* pyr	*Pi* pyr	*Pa* lac	*Pi* lac
BRAF gene expression inhibitor	0.724	0.003	–	–
TP53 gene expression enhancer	0.676	0.031	0.507	0.022
MMP-9 gene expression inhibitor	0.606	0.015	0.15	0.065
Inhibitor of *TNF* gene expression	0.589	0.014	0.163	0.084
HMOX1 gene expression enhancer	0.549	0.027	0.553	0.017
EIF4E gene expression inhibitor	0.544	0.005	0.015	0.005
TERT gene expression inhibitor	–	–	0.621	0.016

The inhibitory effect of pyruvate on the expression of the *EIF4E* gene was predicted by the PASS computer program, and its activation is associated with carcinogenesis. Pyruvate inhibits *BRAF* gene expression. The B-RAF protein, a proto-oncogene, is a part of the RAS / MAPK signaling pathway, which regulates cell growth, proliferation, differentiation, migration, and apoptosis [31]. Lactate has been predicted to inhibit the expression of the telomerase reverse transcriptase (*TERT)* gene, which is the catalytic subunit of the enzyme. The *TERT* gene is mainly active in progenitor and cancer cells, and to a lesser extent – in somatic cells, playing an important role in aging.

STITCH system for identifying intermolecular interaction partners. We searched for intermolecular interaction partners for pyruvate and lactate in the STITCH software. The number of interactions for pyruvate was 109 and for lactate – 384, with a mean significance level $p > 0.4$. Interactions of the studied small molecules with receptors, transfer proteins, hormones, and enzymes were noted, and molecular models were constructed from them. In Table 4, partner proteins typical of pyruvate and lactate are predicted with a high degree of certainty. The interaction of pyruvate and lactate as substrates with lactate dehydrogenase and its various isoforms is confirmed.

The probability of pyruvate and lactate binding to pyruvate kinase, which stimulates POU5F1-mediated transcription activation and plays a common role in the caspase-independent death of tumor cells, is predicted. The ratio between a highly active tetrameric form and an almost inactive dimeric form of pyruvate kinase determines whether glucose carbon will be directed to biosynthesis or used for glycolytic ATP production. The transition between the two forms contributes to the control of glycolysis and is important for the proliferation and survival of tumor cells [32].

Pyruvate and lactate are equally likely to interact with solute carrier family proteins (SLC16, SLC5) and mitochondrial pyruvate carrier (MPC) 1 and 2. Acting through lactate receptors HCAR1 / GPR81 or being transported by monocarboxylate transporters (MCT) across the cell membrane, metabolites influence the functional activity of cells in various tissues (endothelium, adipose tissue cells, neurons), which leads to changes in metabolism, proliferation, and differentiation [33, 34].

The studied natural intermediates pyruvate and lactate are highly likely to act as ligands for vascular endothelial growth factor (VEGF). In particular, the effects of lactate as a proangiogenic factor acting through an increase in VEGF expression are of interest. In some tissues, lactate is taken up and oxidized by macrophages, and lactate-mediated macrophage polarization promotes muscle revascularization and regeneration. The ability of

lactate to alter tumor vascular morphogenesis and perfusion has been established, thereby defining the relationship between metabolism in tumor tissues and angiogenesis [34, 35].

Table 4

Partner proteins in the interaction with pyruvate and lactate

Protein	Description	*p* pyr	*p* lac
PKM	Muscular pyruvate kinase	0.985	0.898
HAO1	Hydroxyacid oxidase	0.973	0.717
LDHA	L-lactate dehydrogenase A	0.969	0.998
LDHC	L-lactate dehydrogenase C	0.968	0.990
LDHB	L-lactate dehydrogenase B	0.968	0.986
LDHAL6A	Lactate dehydrogenase A type 6A	0.960	0.929
LDHAL6B	Lactate dehydrogenase A type 6B	0.957	0.915
LDHD	Lactate dehydrogenase D	0.910	0.911
SLC16A1	Solute carrier family 16, member 1	0.909	0.994
SLC16A3	Solute carrier family 16, member 3	0.909	0.969
SLC16A 7	Solute carrier family 16, member 7	0.900	0.969
SLC5A8	Solute carrier family 5, member 8	0.900	0.954
MPC1	Mitochondrial pyruvate carrier 1	0.900	0.908
MPC2	Mitochondrial pyruvate carrier 2	0.900	0.900
VEGFA	Vascular endothelial growth factor A	0.814	0.879

Note: p – the probability of a small molecule – protein interaction.

Table 4 shows the proteins that indicate interaction of pyruvate with such protein – enzymes as pyruvate carboxylase, mitochondrial phosphoenolpyruvate carboxykinase, dihydrolipoamide dehydrogenase, and dihydrolipoamide transacetylase – the components of pyruvate dehydrogenase complex. The interaction of pyruvate with various malate dehydrogenase isoforms has been predicted: cytosolic NADP-dependent isoform (ME1), mitochondrial NADP-dependent isoform (ME3), and mitochondrial NAD-dependent isoform (ME2). There is evidence that pyruvate is associated with glycerol-3-phosphate dehydrogenase and phospholipase A2, which catalyzes calcium-dependent hydrolysis of two acyl groups in 3-sn-phosphoglycerides. This releases glycerophospholipids and arachidonic acid, which are themselves precursors of signaling molecules.

Pyruvate has been predicted to be a ligand of cystathionine gamma-lyase, which implements methionine sulfidation; it activates sulfurtransferase, which carries out cyanide detoxification and thiosulfate biosynthesis. Both of these proteins generate an endogenous form of hydrogen sulfide, which is a synaptic modulator, a signaling molecule, a neuroprotector, and a regulator of blood pressure [36]. Pyruvate is able to interact with cytochrome b5, which is a membrane-bound hemoprotein that functions as an electron carrier for several membrane-bound oxygenases.

The data on the relationship of lactate with many receptors, including HCAR1, HCAR2, and HCAR3 hydroxycarboxylic acid receptors, which exhibit their effects using the G-protein-mediated pathway, are of interest. In addition, it has been shown that lactate can interact with the alpha-2 adrenergic receptors ADRA2A, ADRA2B, ADRA2C that mediate catecholamine-induced inhibition of adenylate cyclase by G-proteins. To a high degree, the intermediate under study interacts with opioid, dopamine, muscarinic cholinergic, bradykinin, glutamate, lysophosphatidic acid receptor, and G-protein-coupled receptor, which indicates the involvement of lactate in nerve impulse transmission [37]. Neuroprotective effect is probably related to the MTRNR2 protein, which reduces the production of the amyloid beta peptide in Alzheimer's disease [38]. The function of lactate as a ligand for

neurotransmitters, such as glutamate, dopamine, acetylcholine, histamine, and prostaglandin E2, is worth mentioning.

Thus, the metabolic role of lactate in reducing the development of neurodegenerative processes has been predicted and experimentally proven; some scientists consider lactate as a mediator or hormone involved in memory formation and neuroprotection [39]. The use of lactate in clinical studies in the treatment of ischemia and neurodegenerative diseases is discussed, which is determined by the stimulating effect of lactate on neuroplasticity. In particular, the role of lactate as a neuroprotective factor in Alzheimer's disease is of interest, which is determined by its ability to provide metabolic coupling between astrocytes and neurons in active brain regions and participate in the regulation of cerebral angiogenesis [40, 41].

We noted a high probability of interaction of lactate with adrenaline, somatostatin, melatonin, pancreatic polypeptide, and melanin-concentrating hormone. In addition, lactate can interact by a receptor-mediated mechanism with serotonin receptors and galanin and prostaglandin E hormone receptors.

The high ability of lactate and pyruvate to affect immune responses and inflammatory processes, as predicted by the PASS platform, is worth noting. Lactate binds to interleukin (IL)-8 and IL-6 – powerful acute-phase inducers involved in the final differentiation of B cells, lymphocytes, and monocytes; IL-10, which inhibits cytokine synthesis (γ-interferon, IL-2, IL-3, TNF, and GM-CSF); IL-1α, which is involved in the immune response and stimulates the release of prostaglandin and collagenase from synovial cells. Pyruvate, in turn, acts as a ligand for the IL-31 receptor.

These findings have been confirmed in a number of *ex vivo* and *in vivo* experiments. Intracellular pyruvate content has been shown to correlate positively with IL-1β and IL-10 levels in patients with community-acquired pneumonia. Increased levels of pyruvate partially support the ability of hyporeactive monocytes to produce cytokines [42]. Antiinflammatory and potential therapeutic effects of pyruvate in an experimental model of appendicitis in rats were noted [43]. The clinical application and therapeutic effect of pyruvate nasal spray in allergic rhinitis are discussed [44, 45].

The ability of lactate to be an interaction partner for different groups of chemokines has been noted. Thus, the chemokine CCL5 is an attractant for blood monocytes, T helpers, and eosinophils and causes histamine release from basophils. Binding to the chemokines CCR1, CCR3, CCR4, and CCR5, it is one of the major HIV-suppressive factors produced by CD8+ T cells. The chemokine CXCL1 activates neutrophils and may play a role in inflammation by exerting its effect on endothelial cells. D. Zhang et al. (2019) showed that lactylation of lysine residues serves as an epigenetic modification that directly stimulates macrophage chromatin gene transcription. Histone lactylation contributes to the understanding of lactate functions and its role in various pathophysiological manifestations of infectious and noninfectious nature, which may indicate a close functional relationship between the metabolic state and the expression profile of cells [46].

CONCLUSION

The spectrum of biological activities of lactate and pyruvate was revealed by computer modeling methods, and the interaction partner proteins were characterized. The small molecules we studied have a coordinating role in the functioning and modulation of mediator, hormonal, receptor, immune, inflammatory, antibacterial, and antiviral responses and gene expression. The use of natural intermediates as therapeutic agents for the treatment of ischemic stroke, acute neurological disorders, and neurodegeneration is discussed, which is underlain by the stimulating effect of metabolites on neuroplasticity.

These properties may be manifested through conformational rearrangement of receptors, active binding centers, expression of multiple genes, and changes in the functional manifestations of catalytic and other proteins. The obtained data will obviously expand our understanding of the role of small molecules in intermolecular metabolite – protein interactions. Publications in this area appeared only in 2009 and were summarized in a systematic review by X. Li et al. [47]. In 2022, an original viewpoint on the protein – small molecule interaction appeared. The term metabolic entanglement was coined by the authors. The presence of ligand-binding pockets in protein structures for small molecules is to be deciphered, which will allow for integration of metabolomics and protein interactomics [48].

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Authors' information

Kolotyeva Nataliya A. – Dr. Sci. (Med.), Associate Professor, Senior Researcher, Laboratory for Neurobiology and Tissue Engineering, Department of Molecular and Cellular Mechanisms of Neuroplasticity, Brain Research Institute, Research Center of Neurology, Moscow, kolotyeva.n@yandex.ru, https://orcid.org/0000-0002-7853-6222

Gilmiyarova Frida N. – Dr. Sci. (Med.), Professor, Honored Scientist of the Russian Federation, Department of Fundamental and Clinical Biochemistry with Laboratory Diagnostics, Samara State Medical University, Samara, kaf_biohim@samsmu.ru, http://orcid.org/0000-0001-5992-3609

Gusyakova Oksana A. – Dr. Sci. (Med.), Associate Professor, Head of the Department of Fundamental and Clinical Biochemistry with Laboratory Diagnostics, Samara State Medical University, Samara, o.a.gusyakova@samsmu.ru, https://orcid.org/0000-0002-5619-4583

Semashkova Ekaterina A. – Teaching Assistant, Department of Fundamental and Clinical Biochemistry with Laboratory Diagnostics, Samara State Medical University, Samara, e.a.semashkova@samsmu.ru, https://orcid.org/0000-0002-5023-9031

(✉) **Kolotyeva Nataliya A.,** kolotyeva.n@yandex.ru

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Possible role of features of the intestinal microbiome in patients with colorectal cancer as a cause of anastomotic leak

Kosareva P.V.[1, 3], Konev R.A.[2], Godovalov A.P.[1], Sivakova L.V.[1], Samodelkin E.I.[1]

[1] *Perm State Medical University named after Academician E.A. Wagner*
26, Petropavlovskaya Str., Perm, 614990, Russian Federation

[2] *City Clinical Hospital No. 9*
52, Industrial Str., Izhevsk, 426063, Russian Federation

[3] *Perm State University*
15, Bukchireva Str., Perm, 614990, Russian Federation

ABSTRACT

Aim. Following the analysis of literature data, to determine significant factors of intestinal obstruction in patients with colorectal cancer.

Materials and methods. We analyzed 84 literature sources from the Scopus, Web of Science, Google Scholar, and PubMed databases, as well as open access articles on Google.

Results. The predominant causes of anastomotic leaks after operations for colorectal cancer are discussed, the role of the microbiome in the development of postoperative complications is analyzed. The intestinal microbiome of patients with colorectal cancer contains bacteria that are not normally found under physiological conditions. These bacteria contribute to the development of disease, suture failure after surgery for intestinal obstruction, and progression of carcinogenesis. This effect is due to the production of bacterial metabolites, the effect on the human immunity, and competition with obligate intestinal microflora. On the other hand, the use of drug therapy, including antibiotics, leads to mass death of obligate bacteria. Therefore, it is important to search for drugs and treatment methods that, if possible, do not have a significant negative impact on the microbiome, but are capable of destroying pathogenic microorganisms. The concept of Russian authors was proposed, which consists in the intraluminal use of rifaximin-α for the prevention of purulent and septic complications and anastomotic leaks during reconstructive surgeries on the distal colon.

Conclusion. Anastomotic leaks after operations for colorectal cancer are largely facilitated by the imbalance of the intestinal microbiome typical of this group of patients, which can be eliminated by the use of antimicrobial drugs.

Keywords: intestinal microbiota, cancer, intestinal obstruction, anastomotic leak

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✉ *Konev Roman A.,* cancer500@mail.ru

Возможная роль особенностей кишечного микробиома у пациентов с колоректальным раком как причина несостоятельности анастомоза

Косарева П.В.[1, 3], Конев Р.А.[2], Годовалов А.П.[1], Сивакова Л.В.[1], Самоделкин Е.И.[1]

[1] *Пермский государственный медицинский университет (ПГМУ) им. акад. Е.А. Вагнера*
Россия, 614990, г. Пермь, ул. Петропавловская, 26

[2] *Городская клиническая больница (ГКБ) № 9*
Россия, 426063 г. Ижевск, Промышленная улица, 52

[3] *Пермский государственный национальный исследовательский университет (ПГНИУ)*
Россия, 614990, г. Пермь, ул. Букирева, 15

РЕЗЮМЕ

Цель исследования: на основе анализа литературных данных определить значимые факторы кишечной непроходимости у пациентов с колоректальным раком (КРР).

Материалы и методы. Проанализировано 84 литературных источника из баз данных Scopus, Web of Science, Google Scholar, PubMed, а также находящихся в свободном доступе в Google.

Результаты. Обсуждаются преобладающие причины несостоятельности анастомозов после операций по поводу КРР, анализируется роль микробиома в развитии послеоперационных осложнений. Микробиом кишечника больных КРР содержит бактерии, которые в норме не обнаруживаются в физиологических условиях, и сами эти бактерии способствуют развитию заболевания, а также несостоятельности кишечного шва после операции по поводу кишечной непроходимости, прогрессированию процесса канцерогенеза. Этот эффект обусловлен продукцией бактериальных метаболитов, влиянием на иммунную систему человека и конкуренцией с облигатной микрофлорой кишечника. Однако использование медикаментозного лечения, в том числе антибиотиков, приводит к массовой гибели облигатной микрофлоры. Поэтому важен поиск таких препаратов и методов лечения, которые по возможности не оказывают существенного негативного влияния на микробиом, но способны уничтожать патогенные микроорганизмы. Предложена концепция российских авторов, заключающаяся во внутрипросветном применении рифаксимина-α для профилактики гнойно-септических осложнений и несостоятельности анастомозов при реконструктивных операциях на дистальном отделе толстой кишки.

Заключение. Несостоятельности анастомозов после операций по поводу КРР в значительной мере способствуют специфические для этого контингента пациентов нарушения кишечного микробиоценоза, которые могут быть устранены использованием антибактериальных препаратов.

Ключевые слова: микробиота кишечника, рак, кишечная непроходимость, несостоятельность анастомоза

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

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INTRODUCTION

Currently colon cancer is one of the leading causes of morbidity and mortality in the Russian Federation [1], which reflects the global trend [2]. Colorectal cancer (CRC) affects more than 250,000 people each year and is the cause of about a third of cancer deaths [3].

Genetic predisposition to the disease plays a

certain role in the formation of colorectal cancer, in particular, mutation of the *K-Ras* gene can be accompanied by a constant level of its activity, which allows cells to evade apoptosis and proliferate rapidly and uncontrollably [3]. However, most cases of CRC are sporadic and are largely associated with a combination of manageable environmental risk factors [4].

According to the World Health Organization, risk factors may include race, age, poor family history and genetic predisposition (referring for less than 25% of CRC cases [5]), previous inflammatory diseases of the colon, including familial adenomatous polyposis, adenoma [6, 7].

Intestinal obstruction (IO) in CRC may occur due to the development of the disease (e.g., tumor overgrowth), anticancer therapy (e.g., scarring after radiation therapy), or due to other causes [8]. Usually, IO in such patients leads to severe consequences, and its treatment often presents great difficulties [9].

For many patients with malignant neoplasms of the gastrointestinal tract, dissemination of the abdominal cavity with tumor cells (peritoneal carcinomatosis) is a common route of metastasis and progression of the disease. Despite the encouraging results shown by cytoreductive surgery and intraperitoneal chemotherapy, most patients who develop peritoneal carcinomatosis associated with gastrointestinal cancer have a poor prognosis, and malignant ileus is a common terminal phase of the pathological process [10]. The prognosis in the case of intestinal perforation uncomplicated by carcinomatosis due to IO can also be fatal, especially in the case of fecal peritonitis [11]. There is no doubt that malignant obstruction of the colon is directly associated with cancer recurrence and lower overall survival, regardless of the disease stage and adjuvant chemotherapy [12].

The incidence of anastomotic leaks after surgery for CRC varies, averaging up to 15% of cases. At the same time, the factors leading to this complication are different and can be generalized as patient-related factors (the presence of chronic infections, internal and endocrine diseases) and as surgeon-related factors (the choice of the method of surgical intervention, etc.) [13–18]. According to other data, the incidence is 4–5% [19], 12% [20]. It is known that the risk of anastomotic leaks is higher in male patients than in female patients [21]. However, the factors that correlate with IO in patients with CRC are diverse and remain unclear; the dominant factor has not been determined yet [22].

MATERIALS AND METHODS

We analyzed 84 literature sources from Scopus, Web of Science, Google Scholar, and PubMed databases, as well as open access articles on Google. The analysis of the literature data was carried out taking into account ethical standards developed in accordance with the Declaration of Helsinki of the World Medical Association “Ethical Principles for Medical Research Involving Human Subjects” as amended in 2000 and the Rules of Clinical Practice in the Russian Federation approved by the Order of the Ministry of Healthcare of the Russian Federation No. 266 of 19.06.2003.

RESULTS

Basic approaches to the prevention of IO in patients with CRC. Since the development of anastomotic leaks after bowel surgery for CRC is a life-threatening complication [23], and a failure to properly heal anastomosis can lead to the development of peritonitis, these patients require additional care associated with longer hospital stays and increased costs. High morbidity and mortality rates and a less favorable cancer prognosis are noted in these patients, therefore, the search for optimal biomarkers of anastomotic failure, including microbiological parameters, is extremely important [24]. Especially since surgical trauma seems to cause such complex reactions as genotypic and phenotypic changes in the commensal microbiota, increasing their pathogenic potential, which causes tissue destruction and anastomotic leak [25].

Undoubtedly, adequate medical treatment, including appropriate fluid therapy, early initiation of antibiotics, and treatment of concomitant diseases in accordance with international guidelines, are important for patient recovery after surgery for IO associated with CRC [11].

Experimental and clinical studies have shown that combined perioperative systemic antibiotic prophylaxis and prolonged topical antibiotics against common enteric gram-negative and gram-positive pathogens in intestines after mechanical cleansing

are effective in preventing intestinal anastomotic leaks [26]. All of the above indicates that the search for optimal ways, approaches, and methodological concepts regarding the treatment of such a complex group of CRC patients who were diagnosed with IO is undoubtedly extremely relevant at the present time.

The role of the intestinal microbiota in the development of IO in patients with colorectal cancer. Normal microflora. Recent studies have demonstrated that the gut of patients with CRC contains microbiota that differs from that in the healthy colon, and that this microbiota may contribute to the onset of a malignant disease, intestinal suture failure after surgery for IO, and progression of carcinogenesis [26, 27].

The microbiota consists of various bacterial taxa that inhabit the epithelial barriers of various host organs. Microbiota (microbiome) is a metabolically active ecosystem that interacts with epithelial and stromal cells and plays an important role in human health, performing various functions, such as production of important metabolites, prevention of pathogen infections, and control of overgrowth of certain groups of bacteria to prevent changes in the local environment by toxic bacteria. In addition, microbiota is important for the activation of the host immunity. The number and diversity of microbial species in the intestine increase in the longitudinal direction from the stomach to the colon [28]. Short-chain fatty acids produced by obligate microflora are the main source of butyrate, propionate, and acetate, which are used as an energy source in the intestine and help proliferation and differentiation of intestinal epithelial cells [5].

In the last decade, numerous studies have established a clear relationship between changes in the composition of the gut microbiota and various human pathologies: obesity and associated metabolic disorders (for example, type 2 diabetes and non-alcoholic fatty liver disease), autoimmune diseases (for example, type 1 diabetes and inflammatory bowel disease), and some types of cancer that are characterized by changes in the microbiome and gut [28, 29].

In addition, the microbiota greatly contributes to the development of lymphoid tissue and can modulate the innate and adaptive host immunities. Gut microbiota interacts with elements of a full immune response through dendritic cells or through stimulation of epithelial receptors, even in the absence of bacterial translocation [30].

Based on localization, researchers distinguish between two types of intestinal microbiota: parietal (microbiota of mucous membranes) and luminal microbiota. Currently, luminal microflora is analyzed to a greater extent due to the ease of collecting fecal samples. On the contrary, parietal microbiota is usually examined using intestinal tissue biopsy obtained during endoscopy [31, 32]. Moreover, the composition of the microbiota varies between the epithelial cell layer, the mucus layer, and the lumen [33].

It is the parietal microbiota that is involved in stimulating mucus secretion and production of short-chain fatty acids, such as acetate, butyrate, and propionate, which are considered regulators of intestinal physiology and mediators of the host immunity. Butyrate is involved in colonocyte metabolism, enhances the barrier function of the intestine by increasing the production of mucus and the formation of tight junctions, stimulates the immunity of the mucous membranes, and also has antiinflammatory and antitumor effects, since it inhibits the proliferation of cancer cells [31, 32]. The antitumor effect of butyrate is due to its inhibitory effect on histone deacetylases (HDAC), which promote carcinogenesis. Due to the metabolic shift of cancer cells toward glycolysis, unused butyrate accumulates and inhibits procarcinogenic HDACs. In addition, recent studies show that butyrate can improve the healing of colonic tissue in surgical animal models, especially at the site of reconnection of colon ends, anastomosis, and after surgical resection [32].

Acetate produced by anaerobes, in particular *Bifidobacterium*, is involved in defense mechanisms against external agents, such as enterohemorrhagic Escherichia coli infection [31]. As it was shown in an experiment on rats, after colectomy, the composition of parietal microflora changes with a significant increase in the number of microorganisms of the genera *Enterococcus, Escherichia* and / or *Shigella* in the microbiome. However, it is still unclear to what extent the change in parietal microflora can be reflected in shifts in luminal microflora isolated during a bacteriological examination [31].

Pathological changes in the intestinal microflora in CRC. Dysbiosis is defined as the abnormal and predominant presence of pathogens in the

environment or as alterations in the considered normal proportion of different specimens composing the microbiota. This new ecosystem is also called the pathobiome [30].

A growing body of evidence indicates that disruption of the gut microbiota composition is strongly associated with CRC. Recent studies have identified *Streptococcus bovis*, enterotoxigenic *Bacteroides fragilis*, *Fusobacterium nucleatum*, *Enterococcus faecalis*, *Escherichia coli*, and *Peptostreptococcus anaerobius* as potential initiators of CRC [34, 26].

When the balance of normal microflora is disturbed, the number of intestinal probiotic species of microorganisms belonging to the genera *Bifidobacterium* and *Lactobacillus* decreases, and the number of bacteria producing enterotoxins *Bacteroides, Escherichia coli,* and *Clostridium difficile* increases. Bacteria secrete a variety of toxic factors that damage intestinal epithelial cells, causing a chronic inflammatory response and development of CRC, in particular by activating intestinal mucosal macrophages via M cells. In addition, chronic inflammation under conditions of high levels of oxidative stress leads to a loss of barrier functions of epithelial cells and disruption of humoral and T cell immunity [35].

Changes in the balance of gut bacteria can lead to changes in the levels of gut microorganism metabolites, such as short-chain fatty acids (SCFAs), polyphenols, vitamins, tryptophan catabolites, and polyamines; abnormal levels of SCFAs and molecules associated with amino acid metabolism like polyamines are involved in cancer progression and metastasis in various types of tumors [28]. These microbial metabolites interact with the host immunity and cause release of genotoxic virulence factors. Such microorganisms include *Bacteroides fragilis*, *Fusobacterium nucleatum*, *Enterococcaceae* or *Campylobacter*, *Peptostreptococus*, *Enterococcus faecalis*, *Escherichia coli*, *Shigella*, *Salmonella*, and *Streptococcus gallolyticus* [28, 29, 36]. To date, an excess of *Fusobacterium* in the intestine can be considered a potential biomarker for CRC [29].

Fusobacterium nucleatum is the most frequently observed species in the colorectal tumor microenvironment and affects the progression of the disease through multiple mechanisms [37]. Excess colonization of the intestine by microorganisms of the genus *Fusobacterium* is associated with the activation of macrophages after the activation of certain miRNAs, in particular mRNA-21; miRNA-21 activates interleukin-10 (IL-10) and prostaglandin E2 and causes a decrease in antitumor suppressor functions of T cells. A recent study showed that *Fusobacterium* promotes chemotherapy resistance in CRC by affecting innate immunity receptors TLR4 and MYD88, as well as specific mRNAs (mRNA18a and mRNA4082) responsible for autophagy activation. Thus, patients with high levels of *Fusobacterium* are more susceptible to chemotherapy failure and disease recurrence [29]. The so-called Western diet, characterized by a high intake of sugar and animal fat and low in fiber, is in particular associated with an increase in *Bacteroides* [31]. An increase in *Fusobacterium nucleatum* and *Bacteroides fragilis* is strongly associated with the occurrence of CRC due to inflammatory mechanisms, while *Faecalibacterium prausnitzii* is a protective factor, producing butyrate [38].

A hypothesis has been proposed regarding the relationship between *Fusobacterium nucleatum* and CRC. According to this hypothesis, the proposed pathogenic mechanism involves the activation of the β-catenin signaling pathway that causes cell proliferation (as a consequence of FadA binding with E-cadherin located on intestinal epithelial cells). The observation that *F. nucleatum* is more prevalent in patients with CRC than in healthy individuals is statistically significant. However, the number of *F. nucleatum* and *Bacteroides fragilis* (both in the stool sample and in the tumor tissue) appears to increase along with adenoma to adenocarcinoma progression [30].

Peptostreptococcus spp. is relevant in patients with CRC. A recent study showed that patients with bacteremia caused by *Peptostreptococcus spp.* have an increased risk of CRC. This microorganism produces many saccharolytic and fermented products, including acetic, isobutyric, isovaleric, and isocaproic acids, and may contribute to the acidic and hypoxic tumor microenvironment, which promotes bacterial colonization. However, no major research has been done in this direction to date. As for the procarcinogenic effect, it is known that this microorganism contributes to the accumulation of reactive oxygen species by affecting TLR2 and TLR4 [29, 36].

Peptostreptococcus anaerobius is an anaerobic bacterium that selectively lives in excess in the

colonic lumen and on the mucous membranes of patients with CRC, but its mechanisms of pathogenic and carcinogenic effects remain unidentified. To date, *P. anaerobius* is known to attach to the intestinal mucosa and accelerate the development of CRC in ApcMin /+ mice.

In vitro studies and transmission electron microscopy demonstrate that *P. anaerobius* attaches selectively to CRC cell lines (HT-29 and Caco-2) compared to normal colonic epithelial cells (NCM460) via the *P. anaerobius* cell wall protein, which binds directly to colonic cells via the integrin α2/β1 receptor, often overexpressed in human colorectal tumors and cell lines. The interaction between PCWBR2 and integrin α2/β1 induces active cell proliferation, which also involves the nuclear factor κB (NF-κB) activation pathway, which in turn induces a proinflammatory response as indicated by elevated levels of cytokines, such as IL-10 and interferon-γ, in tumors of ApcMin/+ mice treated with *P. anaerobius*. The identified relationship may be a promising therapeutic target in the management of CRC [39].

Streptococcus gallolyticus (*Streptococcus bovis*) is detected in approximately 20–50% of patients with CRC, while its prevalence in this biotope in the population is no more than 5% [5].

An increase in the enterotoxigenic variant of *Bacteroides fragilis* has been noted in stool samples of patients with CRC; *B. fragilis* degrades the E-cadherin protein and activates nuclear beta-catenin signaling and induces c-Myc expression and cell proliferation [5]. *B. fragilis* toxin activates the Wnt and NF-*k*B signaling pathways and enhances the release of proinflammatory molecules by the epithelium [28]. The presence of enterotoxigenic *B. fragilis* as well as *F. nucleatum* in the colonic mucosa is associated with more advanced CRC associated with elevated levels of inflammatory mediators, including MMP-9 [40].

Bacteroides, especially in combination with *Escherichia coli*, are crucial to the development of CRC (which was confirmed in experiments on mice), including its familial forms. Both the action through the activation of NF-κB and mucin degradation are mentioned among the mechanisms. However, they usually do not exhibit carcinogenic properties independently, outside of associations with other bacteria [29].

E. coli is characterized by the expression of genotoxins, such as cyclomodulins CIF (cycle inhibiting factor), cytotoxic necrotizing factor (CNF-1) or colibactin; in colonocytes, CNF-1 also affects the actin cytoskeleton, causing reversible cellular senescence, which is potentially associated with chromosomal aberrations and genomic instability [29].

Colibactin is another genotoxin of bacterial origin that can interfere with the cell cycle and promote epithelial cell proliferation through DNA damage, mutations, and genomic instability, which is followed by tumor growth [5]. Higher expression of *B. fragilis* toxin and colibactin genes was found in patients with familial adenomatous polyposis compared to healthy individuals. In addition, some microbial metabolites obtained from food can cause genotoxic and cytotoxic effects. *Clostrudium*, *Bacteroides*, and *E. coli* have been reported to have this capacity [30]. Some strains of *E. coli* and *B. fragilis* produce genotoxins [30].

In CRC patients, *Ruminococcus bromii*, *Clostridium clostridioforme*, and *Bifidobacterium longum* have low prevalence compared to normal population [5]. *S. bovis*/*gallolyticus* can colonize and grow in colon tissues through the binding of collagen and histone-like protein A to collagen I, IV, fibronectin, and fibrinogen in colonic tissues [5], and also acts through the activation of NF-κB and IL-8 [29]. *Clostridium difficile* is currently the most common cause of healthcare-associated infections, with an increase in the prevalence, severity, and mortality of nosocomial and community-acquired clostridial infections accounting for approximately one-third of all clostridial infections. There is also an increased incidence of asymptomatic colonization, especially in high-risk patients [41].

The role of Clostridia in this process is evidenced by the studies by individual authors. Pathogenic microflora is responsible for the excess of free radicals, especially *Enterococcus faecalis* [5]. *E. coli* toxin (colibactin toxin) causes cross-links and double-strand breaks in DNA [28]. The virulence of such an aggressive microorganism as *Pseudomonas aeruginosa* is regulated by the presence of specific fermentation products [33].

Possible role of viruses and fungi in the pathological process. The gut microbiome is not limited to bacteria only, but also includes viruses and microscopic fungi. A high viral DNA load is observed in tumors compared to normal benign tissue, which mainly concerns viral infections,

such as human papillomavirus, polyomavirus infections, human herpesviruses [28]. In addition, *Orthobunyavirus*, *Inovirus* and *Tunalikevirus*, *Bacteroides fragilis*, *Fusobacterium nucleatum* and genotoxic *Escherichia coli*, which are involved in the formation of CRC, are also relevant. Moreover, at early and late stages of cancer development, the mechanisms of the influence of microorganisms on the progression of the disease are different [28].

Metabolites of the microbiota. While some bacteria, such as *F. nucleatum*, *E. coli,* or *B. fragilis,* interact directly with the host by binding to receptors on tumor or immune cells, many effects caused by bacteria can be due to secreted metabolites. The gut microbiome is a vast source of secretory proteins and metabolites, constituting a common reservoir of metabolites in the tumor microenvironment [29].

During carcinogenesis, inflammatory cytokines (IL-6 and others) [42] and chemokines produced by cancer cells attract immature myeloid cells and helper T cells involved in inflammation. The pro-oncogenic microenvironment is characterized by the synthesis of growth factors, angiogenic factors, and tissue remodeling enzymes, as well as suppression of the antitumor T cell response, which contribute to tumor progression. In dysbiosis, the permeability of the intestinal wall increases, the cell wall lipopolysaccharides of some bacteria enter the host, which induces the immune system to secrete cytokines and trigger a cascade of reactions that ultimately lead to inflammation. Local inflammation promotes tumor progression through protumorigenic cytokines and chemokines, which act as growth factors and promote angiogenesis [28].

In general, the impact of the altered microbiota is ambiguous. Thus, *F. nucleatum* is associated with a lower level of $CD3^+$ T cells, increased production of TNFα, IL-6, IL-12, and IL-17 (all of which have a prooncogenic effect), which are involved in many immune responses. However, the Fap2 protein produced by this microorganism can prevent the antitumor effect of NK cells and other T cells that bind to inhibitory receptors [30].

In contrast, some microorganisms appear to have a direct protective effect against tumor growth, such as those that produce short-chain fatty acids (butyrate or acetate). According to previously published data, *Bifidobacterium* appears to be able to inhibit tumor progression by reducing infection by enteropathic microorganisms and reducing the production of bile products. Moreover, some microbes may exhibit antitumor activity through interactions with the immune system. This positive effect is associated with stimulation of phagocytes, increased NK cytotoxicity, and increased production of immunoglobulins, including IgA (which promotes mucosal barrier activity). Data from experimental studies show that *Bifidobacterium* can also contribute to the antitumor immune response by inhibiting the NF-κB signaling pathway. Similarly, *Faecalibacterium prausnitzii* may have a positive effect by inducing IL-10 secretion and modulating Treg response. IL-10 can control the proliferation of Th17 cells, stopping the progression of cancer. In addition, IL-10 suppresses TNFα production and iNOS expression [30].

CRC is usually treated with cytotoxic agents, such as 5-fluorouracil, capecitabine, and oxaliplatin, which interfere with DNA replication. Platinum-based anticancer drugs, such as oxaliplatin, cause severe toxicity to many organ systems, including the intestine. Its toxicity also affects the gut microbiome as it damages rapidly regenerating intestinal mucosal cells, disrupts immunological barriers, and alters environmental cytokines and inflammatory markers. High levels of *F. nucleatum* have been shown to promote chemoresistance in CRC, as *F. nucleatum* attaches to host epithelial E-cadherin, promoting colorectal carcinogenesis through Fusobacterium adhesion. *F. nucleatum* has also been found to mediate chemoresistance through targeting specific miRNAs and autophagy elements. Its direct association with CRC recurrence has even been proposed as a method for predicting patient outcomes or changing chemotherapy regimens [40].

Thus, CRC is characterized by altered production of bacterial metabolites directly involved in cancer metabolism. New evidence suggests that a high-fiber diet with polyunsaturated fatty acids, polyphenols, and probiotics, known to regulate the gut microbiota, may not only be a potential mechanism to reduce a CRC risk in primary prevention, but also contribute to an enhanced response to cancer therapy when used as an adjuvant to conventional treatment of the disease [28].

The gut microbiota composition altered in the postoperative period can lead to serious complications, including anastomotic failure and surgical site infections. In addition, intestinal microbiota can be used as a possible biomarker

in predicting long-term outcomes after surgical treatment for CRC [43].

Thus, the gut microbiota of patients after colorectal surgery changes due to surgical stress. The development of complications after colon surgery for CRC (including anastomotic failure and surgical site infections) may depend on bacterial shifts, which may also affect the prognosis and survival in patients with postoperative CRC [43].

Evidence has been accumulated for 60 years that anastomotic failure is caused by pathogens, classic examples of which are *E. faecalis* and *P. aeruginosa*, which have the ability to degrade collagen and / or host matrix metalloproteinase-9 (MMP-9) [26]. Specific bacterial infections increase the risk of anastomotic failure. In particular, *Pseudomonas aeruginosa* and *Enterococcus faecalis* (as bacteria that strongly affect collagen) have been shown to play a role in this process, while locally administered antibiotics turned out to be more effective [24].

Anastomotic leaks, which are a very serious problem [44], are currently associated with *Enterococcus faecalis*, since this pathogen has high collagenase activity and activates MMP-9, which are the main contributors to tissue destruction and intestinal inflammation. MMPs are a group of proteolytic enzymes that mediate the degradation of the extracellular matrix and regulate the release of growth factors, chemokines, and adhesion proteins. High levels of MMP-9 and MMP gelatinase with type IV collagen as the main substrate have been shown to be a marker of invasion and worsen cancer outcome in patients with CRC. The fact that strains of *E. faecalis* appear to play an important role in the pathogenesis of anastomotic leaks and remain in anastomotic tissues despite current bowel preparation before surgery suggests that microbiome suppression and the presence of a microbiome may be overlooked elements playing a role in local recurrence. These collagenase-producing *E. faecalis* strains can also interact with resident macrophages [40, 30]. Anastomotic leaks in CRC are associated not only with *Enterococcus* spp., producing beta-lactamase, but also with *Escherichia* spp. as the most common pathogens [45].

Collagenase-producing families Bacteroidaceae, Lachnospiraceae [46], and *Clostridium difficile* [41, 30] are also important for intestinal anastomotic failure. It was found that high abundance of *Bacteroides fragilis* is associated with a worse prognosis, while low abundance of *Prevotella*, *Bacteroides,* and *Faecalibacterium prausnitzii* seems to be a more favorable prognostic factor [47].

Impact of colorectal surgery on the gut microbiota. To date, the impact of colorectal surgery on the gut microbiota has not been fully clarified [43]. Undoubtedly, the use of isotonic laxatives (e.g., polyethylene glycol) as a preoperative preparation adversely affects the microbiota. At the same time, under favorable circumstances, in non-oncological patients who received such preoperative preparation, the parameters of the intestinal microbiota approach the normal range on average by day 14 after surgery [43]. It has been established that patients with CRC have an increased number of *E. coli* and *Staphylococcus* in the postoperative period [43].

Perioperative medications can also change the microbiome composition. Antacids neutralize gastric secretion, which can disturb the balance of acid-sensitive organisms in the intestine. Vasoactive drugs, which are often used in critically ill patients, can cause intestinal hypoxia affecting bacterial virulence. Opioids impair gastrointestinal peristalsis and motility, thereby reducing mechanical removal of excess bacteria from the lumen. This can lead to intestinal obstruction, dysbiosis, and / or bacterial overgrowth.

Perioperative interventions may cause increased multiplication of virulent bacterial strains (e.g., *Enterococcus*, *Pseudomonas*) capable of transforming into strains with a more aggressive tissue-destroying phenotype. These changes may contribute to the development of anastomotic leaks [33]. Instead of aggressive preoperative preparation with saline laxatives and broad-spectrum antibiotics, gentle bowel cleansing with nutritional supplements and non-microbicidal antivirulence agents is currently considered, which does not lead to mass destruction of the microbiome that is common nowadays. A successful practice is manifested by a decrease in the number of Enterobacteriaceae bacteria in this group of patients [40]. Carbohydrate food additives that suppress the virulence of *P. aeruginosa*, *E. faecalis,* and *Serratia marcescens* without affecting their growth are also considered [40].

However, one of the modern reviews conducted in accordance with the Oxford Center for Evidence-Based Medicine guidelines and principles (databases

used included PubMed, Cochrane Library, Embase, Scopus, and Google Scholar), summarizing published data on the prevention of anastomotic leaks after colorectal surgery, argued that mechanical bowel preparation does not reduce the risk of anastomotic failure, as well as the choice of surgical approach and strategy, excluding low ligation of the inferior mesenteric artery; while the use of an oral antibiotic reduces the incidence of anastomotic leaks [48].

Some authors recommend the use of postoperative antibiotics affecting *Escherichia coli* and *Enterococci* as the most common pathogens [49, 50]. At the same time, some modern authors suggest using antibiotics, such as gentamicin in combination with erythromycin, as preoperative preparation [20].

Recent studies by foreign authors provide data on the comparative efficiency of various oral antibiotics, including both selective and broad-spectrum ones. Selective antibiotics are known to target only certain (aerobic, Gram-negative) bacteria, while local anaerobic bacteria are mostly not affected. The disadvantage of broad-spectrum antibiotics is that they lead to more extensive destruction of bacteria, which can lead to microbial dysbiosis [51].

The following regimens are given: kanamycin and metronidazole orally with a short course of parenteral cefmetazole, kanamycin with erythromycin orally and parenteral cefotiam for 48 hours, kanamycin with erythromycin orally and cefmetazole administered parenterally, polymyxin B with tobramycin and amphotercin B orally and cefuroxime intravenously, etc. [51].

Many authors prefer topical (oral or intraluminal) use of antibiotics in this case [52, 53]. Russian authors report the successful intraluminal use of Alfa Normix® suspension (rifaximin-α) for the prevention of purulent – septic complications and anastomotic failure during reconstructive surgery in the distal colon [54].

CONCLUSION

Today the role of the pathobiome in the formation of IO and suture failure during anastomosis is undeniable, along with the fact that the use of antibiotics can disrupt the endogenous microbiome and cause resistance of pathogens to antibiotics. Therefore, it is important to search for such drugs and treatments that, if possible, do not have a significant negative effect on the microbiome, but are able to destroy pathogenic microorganisms [25] and, thereby, prevent intestinal suture failure and cancer progression.

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Authors' contribution

Kosareva P.V., Konev R.A. – conception and design, analysis and interpretation of the data. Godovalov A.P. – conception and design, justification of the manuscript. Sivakova L.V. – critical revision of the manuscript for important intellectual content. Samodelkin E.I. – final approval of the manuscript for publication.

Authors' information

Kosareva Polina V. – Dr. Sci. (Med.), Professor, Department of Inorganic Chemistry, Chemical Technology and Technosphere Safety, National Research Perm State University, Perm, perm-bagira@yandex.ru, https://orcid.org/0000-0002-0853-925X

Konev Roman A. – Chief Physician, City Clinical Hospital No. 9, Izhevsk, cancer500@mail.ru

Godovalov Anatoly P. – Cand. Sci. (Med.), Associate Professor, Department of Microbiology and Virology, Perm State Medical University named after Academician E.A. Wagner, Perm, agodovalov@gmail.com, https://orcid.org/0000-0002-5112-2003

Sivakova Lyudmila V. – Cand. Sci. (Med.), Associate Professor of the Department of Pathological Physiology, Perm State Medical University named after Academician E.A. Wagner, Perm, sivakova.lv@yandex.ru

Samodelkin Evgeny I. – Dr. Sci. (Med.), Professor, Department of Pathological Physiology, Perm State Medical University named after Academician E.A. Wagner, Perm, sei-p@mail.ru, https://orcid.org/0000-0002-0976-8863

(✉) **Konev Roman A.**, cancer500@mail.ru

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Diabetic ketoacidosis and cognitive impairment in children and adolescents

Magomedova K.Sh.[1], Bykov Yu.V.[2,3], Baturin V.A.[2]

[1] *Stepnovskaya District Hospital*
52a, Dodonova Str., Stepnoye village, Stavropol Region, 357930, Russian Federation

[2] *Stavropol State Medical University*
310, Mira Str., Stavropol, 355017, Russian Federation

[3] *Children's City Clinical Hospital named G. K. Filippsky*
5, Ponomareva Str., Stavropol, 355002, Russian Federation

ABSTRACT

The aim of the literature review was to highlight modern scientific sources on the formation and clinical manifestations of cognitive impairment in children and adolescents with type 1 diabetes mellitus (DM) after diabetic ketoacidosis (DKA). Type 1 DM is one of the most prevalent endocrine disorders in childhood and adolescence. DKA is the most common acute complication of type 1 DM that may cause cognitive impairment. Cerebral edema is the main cause of cerebral vascular insufficiency in patients with DKA. However, the mechanisms underlying the development of cognitive dysfunction in DKA have not been fully elucidated.

The leading hypotheses include development of neuroinflammation, oxidative stress, disruption of neurogenesis, and neurodegeneration. Hypoxic – ischemic injury and changes in the brain neuroanatomy may also cause cognitive dysfunction. Disruption of some brain structures has been reported after DKA episodes, primarily affecting the white matter. Clinical studies in the pediatric population support the presence of a correlation between the severity and frequency of DKA and the severity of cognitive impairment. Cognitive dysfunction in children and adolescents after a DKA episode can manifest through decreased attention, impaired memory and executive function, and reduced IQ. The earliest possible diagnosis of cognitive impairment in pediatric patients with symptoms of DKA in the context of type 1 DM can improve the treatment prognosis for this endocrinopathy.

Keywords: type 1 diabetes mellitus, diabetic ketoacidosis, cognitive impairment, children and adolescents

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✉ *Bykov Yuri V.,* yubykov@gmail.com

Диабетический кетоацидоз и когнитивные нарушения у детей и подростков

Магомедова К.Ш.[1], Быков Ю.В.[2, 3], Батурин В.А.[2]

[1] *Степновская районная больница*
Россия, 357930, Ставропольский край, Степновский район, с. Степное, ул. Додонова, 52а

[2] *Ставропольский государственный медицинский университет (СтГМУ)*
Россия, 355017, г. Ставрополь, ул. Мира, 310

[3] *Детская городская клиническая больница им. Г.К. Филиппского*
Россия, 355002, г. Ставрополь, ул. Пономарева, 5

РЕЗЮМЕ

Цель исследования – освещение современных научных источников по вопросам формирования и клиническим проявлениям когнитивных нарушений у детей и подростков с сахарным диабетом (СД) 1-го типа после перенесенного диабетического кетоацидоза (ДКА). СД 1-го типа является одним из распространенных эндокринных заболеваний в детском и подростковом возрасте. ДКА – наиболее частое острое осложнение СД 1-го типа, который может вызывать когнитивные нарушения. Отек головного мозга при ДКА является основной причиной, приводящей к церебральной недостаточности. Механизмы формирования когнитивной дисфункции при ДКА полностью не выяснены.

Ведущими гипотезами являются: возникновение нейровоспаления, оксидативный стресс, нарушение процессов нейрогенеза и нейродегенерация. Гипоксически-ишемические нарушения и изменения в нейроанатомии головного мозга также могут являться причинами когнитивной дисфункции. Отмечено нарушение некоторых структур головного мозга после ДКА, в первую очередь белого вещества. Клинические исследования, проведенные в педиатрической популяции, подтверждают корреляцию между тяжестью и частотой ДКА и выраженностью когнитивных нарушений. Когнитивная дисфункция у детей и подростков после ДКА может проявляться в снижении внимания, нарушении памяти и исполнительной функции, а также в низком уровне IQ. Максимально ранняя диагностика когнитивных нарушений в педиатрической практике при СД 1-го типа с проявлениями ДКА может улучшить терапевтический прогноз при лечении данной эндокринопатии.

Ключевые слова: сахарный диабет 1-го типа, диабетический кетоацидоз, когнитивные нарушения, дети и подростки

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

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INTRODUCTION

Type 1 diabetes mellitus (DM), one of the most prevalent metabolic disorders in children, presents an important public healthcare problem due to its rapidly increasing incidence [1–3]. According to available data, almost 15 million children across the globe have type 1 DM, with its prevalence growing steadily (by 2–5% each year), especially in the developing countries [3, 4]. The steadily growing prevalence of this endocrinopathy, especially among young children, leads to an increase in its acute complications [4].

According to the International Society for Pediatric and Adolescent Diabetes (ISPAD), diabetic ketoacidosis (DKA) is the most prevalent acute complication of type 1 DM [5–9]. The prevalence of DKA at the time of diagnosis in pediatric practice ranges from 12.8 to 80%, with the mean of 38.8% [4,

10–12]. DKA is the most frequent cause of death in children with type 1 DM, while the mortality rate for DKA is 0.3–0.5% in developed countries and much higher (about 10%) in developing countries [13–16].

DKA occurs due to the interaction between insulin (deficiency) and counterregulatory hormones (excessive concentrations) [13, 17]. While insulin deficiency leads to hyperglycemia and ketosis, an excess of counterregulatory hormones (epinephrine, cortisol, and growth hormone), which are produced in large amounts during stress, exacerbates hyperglycemia by suppressing the effect of insulin and increasing glycogenolysis in the liver [7, 17]. The characteristic manifestations of DKA are hyperglycemia, ketosis, and metabolic acidosis [7, 18]. DKA may be the primary manifestation of type 1 DM, but it may also develop further along the course of the disease and presents a serious relapsing issue in children and adolescents [5]. It has been demonstrated that 25–40% of children with newly diagnosed type 1 DM are also diagnosed with DKA, and in patients with a chronic disease course, DKA may develop due to poor compliance with treatment guidelines or malfunction of equipment used for DM treatment (for instance, failure of an insulin pump) [19]. According to the published data, the severity of DKA at the time of diagnosis affects the long-term clinical course of type 1 DM: children with DKA and newly diagnosed type 1 DM have poorer glycemic control and lower residual β-cell function for 2 years after the diagnosis, as well as a lower remission rate [20–22].

Cerebral circulation insufficiency is the most prevalent complication of DKA in children and adolescents with type 1 DM [12, 23]. Cerebral edema (CE) associated with serious neurological impairments has long been acknowledged as a rare but severe complication of DKA in children [24]. Severe, clinically evident manifestations of CE occur in about 1% of DKA episodes and often lead to death or persistent, chronic neurological symptoms [19, 24–26]. Insignificant (subclinical) manifestations of CE can be observed in the majority of children with DKA, even in cases when the clinical changes in the neurological status are minimal or absent [19, 27, 28]. It has been shown that changes in brain MRI in children and adolescents persist for 3 months after CE is diagnosed in patients with a DKA episode in the medical history [9].

This review describes cognitive impairments accompanying DKA in patients with type 1 DM as a pathological condition, with statistically significant differences in cognitive function compared to healthy children and adolescents.

PATHOPHYSIOLOGICAL MECHANISMS OF THE DEVELOPMENT OF COGNITIVE IMPAIRMENTS IN DK

The mechanisms leading to brain damage in DKA with subsequent development of cognitive impairments are not yet fully understood and currently remain a field of active research [19, 29]. Animal research experiments and clinical studies in the pediatric population have demonstrated that DKA may result in damage to neurons and astrocytes in patients with neuroinflammation [30, 31], apoptosis [32, 33], and impaired processes that suppress proliferation of neuronal cells (neurogenesis) [34, 35] and neurodegeneration [36]. Acute hyperglycemia which accompanies DKA may exacerbate oxidative stress, which could also trigger the development of cognitive deficits in children and adolescents with type 1 DM [37–39]. Data suggest that pathophysiological changes accompanying DKA adversely affect the brain, initiating an inflammatory response and development of vasogenic cerebral edema, which may trigger the development of cognitive dysfunction [29]. Animal studies have shown that DKA causes reactive astrogliosis and microglia activation in the brain, and these changes were most evident within the first 24 hours after the onset of DKA, although some inflammatory changes remained even 72 hours after the onset of DKA [29]. These persistent inflammatory disorders suggest ongoing brain damage even after DKA resolution [29].

Some authors reported the identification of specific biomarkers that indicate brain damage in children and adolescents with DKA [40]. S.T. Nett et al. [41] showed elevated plasma levels of interleukin (IL)-6 and tumor necrosis factor alpha (TNFα), a key indicator of astrocyte reactivity and neurodegeneration, in 45% of children with DKA, which had a positive correlation with the impairment in consciousness, indicating that systemic inflammation accompanies brain dysfunction in decompensated type 1 DM. Calcium-binding protein (S100β) secreted by astrocytes was elevated in DKA and was considered as an indicator of neuronal death, including one occurring during the inflammatory response [42].

S. Hamed et al. [43] observed elevated levels of neuron-specific enolase (NSE) in children with DKA at baseline and 12 and 24 hours after the initiation of DKA treatment in type 1 DM. The authors concluded

that the serum level of NSE was elevated on day 1 after the onset of DKA and correlated with the severity of hyperglycemia, ketosis, and acidosis [43]. S.L. Wootton-Gorges et al. [44] reported neuronal damage in DKA evidenced by a reduced N-acetylaspartate-to-creatine ratio (NAA / Cr), one of the markers of viability and normal functioning of neurons. Another inflammation marker in DKA is the kynurenine pathway with its kynurenine / tryptophan ratio, which may be elevated before DKA treatment in children and adolescents [45]. The elevated kynurenine / tryptophan ratio may result in excessive production of neurotoxins, which exacerbate cerebral circulation insufficiency [45].

It has been shown that ketone bodies can have a differential effect on brain capillary endothelial cells and increase the release of vasoactive peptides, for instance, endothelin-1 (ET-1) and vascular endothelial growth factor (VEGF), which adversely affect the cognitive function [46]. Development of hyperlipoproteinemia and emergence of toxic products of tryptophan catabolism are additional side effects stemming from dysregulation of metabolism in DKA, and they can have an adverse effect on cognitive functions [47].

Diabetic vasculopathy or angiopathy has long been considered as the cause of brain damage in DKA patients with subsequent development of cognitive dysfunction [48, 49]. DKA is known to cause CE and a decrease in cerebral blood flow with a potential long-term adverse effect on brain development in children and adolescents [23]. MRI-based studies in children and animal models demonstrate the impaired blood supply to the brain and metabolism alteration patterns accompanying DKA that are similar to the changes often observed in hypoxic – ischemic brain injury [19, 50]. It has been shown that cerebral hypoxia and / or ischemia associated with other conditions (for instance, altitude sickness, cardiac arrest or pediatric sleep-disordered breathing) may be linked to the development of cognitive impairments, which provides additional evidence for the involvement of hypoxic – ischemic disorders in the pathophysiology of cerebral circulation insufficiency in children and adolescents with DKA [51].

Acute hyperglycemia accompanying DKA may damage developing neurons and myelin in children with type 1 DM, which is consistent with data obtained in experimental models of DM that demonstrate degenerative changes in neurons and glial cells *in vivo*, along with disruption of myelin sheaths and a decrease in myelin in hyperglycemia [39]. Changes in the composition of brain sphingolipids (ceramides and sphingomyelin) caused by DKA may also trigger membrane remodeling in some cell populations, which may disrupt cell – cell interaction and result in brain tissue damage [52].

DKA also leads to changes in the neuroanatomy of the brain [19, 53]. Published MRI data reveal abnormalities in the gray and white matter of the brain in children and adolescents who previously had an episode of DKA [19]. The most pronounced changes were observed in the white matter of the brain, especially in the frontal lobes and are most noticeable in younger children who had the most severe acidemia that accompanied DKA [54]. Other authors report on persisting brain abnormalities in patients, detectable even 3 months after an acute DKA episode [9, 55]. Significant correlations have been reported between the decline in the overall volume of the gray and white matter of the brain and delayed memory at initial presentation, as well as subsequent impairment of sustained attention 6 months after the diagnosis of DKA [19].

F.J. Cameron et al. [54] investigated brain morphology and cognitive functions in children aged 6–18 years with and without DKA at presentation and at four time points: 48 hours, 5 days, 28 days, and 6 months after it. They demonstrated a significant correlation between changes in the brain morphology and the cognitive deficits observed at different time points. Another study [55] assessed whether the severity of clinical symptoms (presence of DKA at the time of diagnosis) corresponded to the differences in patients' cognitive functions and brain structure. The results showed a lower volume of the left temporo – parieto – occipital cortex in children with type 1 DM compared to the control group, which correlated with the severity of cognitive impairments. M.J. Marzelli et al. [56] discovered that young children with type 1 DM and frequent episodes of DKA in their medical history had decreased volumes of brain matter in key regions of the brain associated with cognitive functioning, compared to healthy individuals in the control group.

Thus, it may be said that the duration of post-DKA morphological and functional disorders of the CNS in children and adolescents may vary from 48 hours to 6 months. However, the time frames during which peak damage occurs and during which changes may be reversed remain unknown, which requires additional research [57]. Therefore, cognitive functions should be studied starting at 48 hours after a DKA episode with an almost six-month follow-up period [57].

CLINICAL MANIFESTATIONS OF COGNITIVE IMPAIRMENTS IN DKA

An assessment of cognitive impairments has been the subject of numerous studies, some of which reported a significant correlation between type 1 DM and a decline in cognitive function in children and adolescents, including those having DKA [40, 41, 53, 58, 59]. In a meta-analysis aimed at assessing the association between type 1 DM and cognitive function, P.A. Gaudieri et al. [60] concluded that this endocrine disease adversely affects various cognitive spheres in childhood and adolescence. The authors also reported that this correlation was more noticeable in children with an early onset of type 1 DM (an onset in early childhood) [60]. A different group of authors showed that children with type 1 DM have a lower level of intelligence compared to healthy children without DM [58, 59].

DKA often leads to morphological and functional changes in the brain which are associated with adverse neurocognitive outcomes [40, 53]. It has been shown that 40–70% of children with type 1 DM complicated by DKA exhibit diverse types of cognitive deficits, such as decreased attention, poor memory, impaired executive function, and low IQ [41]. According to several clinical, neuroimaging, and experimental studies, DKA may cause both light and severe cognitive impairments over the course of the disease, and these impairments develop even in the absence of subclinical manifestations of CE during decompensation [18, 40]. According to some studies, there is a trend in pediatric patients with newly diagnosed type 1 DM and DKA toward a decline in cognitive functions along the course of the disease compared to patients of the same age with type 1 DM and no symptoms of DKA [9]. For instance, children and adolescents with type 1 DM complicated by DKA at presentation coped worse with mathematical tasks than their siblings without DM in the control group [61]. S. Ghetti et al. [57] estimated whether an episode of DKA which occurred when type 1 DM was diagnosed or later, along the course of the disease, affects cognitive function in children and adolescents. The study involved 758 children with DKA and 376 children in the control group (with type 1 DM without DKA) aged 6–18 years. The authors demonstrated a correlation between the severe course of DKA and a lower coefficient of mental development [57].

The presence of DKA episodes in the medical history also correlated with a lower verbal intelligence quotient in children with type 1 DM and a decline in cognitive function [9, 61–63]. This study revealed memory deficits in children with type 1 DM and a history of DKA compared to children with a similar duration of DM and similar glycemic control, but without a history of DKA [64]. M.A. Cato et al. [65] reported a correlation between learning and memory impairment and a history of DKA in patients who had their first DKA episode 2 years prior to the assessment.

At the moment, it is not clear whether a single episode of DKA causes a long-term decline in cognitive functions in children and adolescents with type 1 DM [57]. However, it has been proven that the clinical severity of a DKA episode correlates with the severity of cognitive dysfunction 6 months after the diagnosis; therefore, DKA severity may be associated with the degree of CNS damage [54]. Still, not all clinical studies reveal an association between a history of DKA and cognitive dysfunction. For instance, a study revealed that children with type 1 DM and DKA were not cognitively impaired compared to children with type 1 DM and no DKA [66]. Therefore, based on the described clinical studies, it is possible to assume a relationship between DKA and the severity of cognitive impairments in children and adolescents with type 1 DM.

ASPECTS OF DIAGNOSIS AND MANAGEMENT OF COGNITIVE IMPAIRMENTS IN DKA

Cognitive dysfunction after an episode of DKA can be diagnosed using specialized neurophysiological methods adapted for children and adolescents and used in practical management of type 1 DM [67, 68]. For instance, the Wechsler Intelligence Scale for Children (WISC) is used to detect impairments of general intellectual ability and its components including verbal and non-verbal intelligence [69]. The Benton Visual Retention Test is used to measure visual perception and visual memory in children aged 8 years and older [70]. The Wisconsin Card Sorting Test is used to assess clinically important aspects of attention deficits [71]. The Stroop Color and Word Test is used to assess cognitive dysfunction in children and adolescents [72].

At present, despite the discovery of numerous pathophysiological mechanisms which may underpin the development of cognitive deficits after an episode of DKA, no specific (etiotropic) treatment has been developed for this cerebral dysfunction [73]. The following pharmacological agents can be potentially used to treat this condition: polypeptide

drug cortexin [74], hopantenic acid preparations [75], and memantine, a NMDA receptor antagonist [76], but the efficacy of these drugs in treating DKA in patients with type 1 DM has yet to be demonstrated in controlled studies. The possible non-pharmacological interventions include regular physical exercise and athletic activities, which have shown their efficacy in ameliorating mild cognitive impairment in adolescents with type 1 DM [77]. Much emphasis is placed on preventive measures aimed at maintaining a normal glycemic profile in order to reduce the risk of a DKA episode, and, as a consequence, minimize cognitive impairment [78].

CONCLUSION

DKA in children with type 1 DM is the most prevalent acute complication that can be an important trigger of cerebral circulation insufficiency further along the course of the disease. The pathophysiology of cognitive impairments in DKA remains understudied, even though children and adolescents with DKA are precisely the group of patients requiring special attention due to the severity of their disease, with a complicated and often negative prognosis in terms of brain dysfunction. Therefore, the search for new possible mechanisms underlying the development of cognitive deficits in this complication of type 1 DM in children and adolescents is a promising area of research in modern endocrinology.

Another major issue is that the theories about mechanisms of cerebral circulation insufficiency in children with DKA involve a wide variety of factors and the presumed mechanisms are of different nature and include neuroinflammation, apoptosis, disruption of neurogenesis, and neurodegeneration. Many research questions exist in relation to the duration of the formation of functional and morphological CNS impairments after a DKA episode in children and adolescents. The time periods during which the manifestations of cognitive dysfunction are at their peak and the aspects of potential reversibility of these impairments are still understudied. Furthermore, not all clinical studies reveal an association between a history of DKA and cognitive dysfunction.

Thus, the pathophysiological mechanisms that cause cerebral circulation insufficiency, along with the clinical manifestations of this pathology, are far from being fully understood, and further studies are needed in this direction within evidence-based medicine. At the same time, it can already be clearly assumed that timely and the earliest possible diagnosis of cognitive dysfunction during treatment of type 1 DM complicated by DKA may improve therapeutic approaches to this disease.

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Authors' information

Magomedova Kamila Sh. – General Practitioner, Stepnovskaya District Hospital, Stavropol Region, Stepnovsky District, Stepnoye Village, kamilla.2017@bk.ru, http://orcid.org/0000-0003-1167-907 - 4

Bykov Yuri V. – Cand. Sci. (Med.), Teaching Assistant, Department of Anesthesiology and Resuscitation with the Course of Further Vocational Education, Stavropol State Medical University, Stavropol; Children's City Clinical Hospital named G. K. Filippsky, Stavropol, yubykov@gmail.com, http://orcid.org/0000-0003-4705-3823

Baturin Vladimir A. – Dr. Sci. (Med.), Professor, Department of Clinical Pharmacology with the Course of Further Vocational Education, Stavropol State Medical University, Stavropol, prof.baturin@gmail.com, http://orcid.org / 0000-0002-6892-3552

(✉) **Bykov Yuri V.,** yubykov@gmail.com

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Monitoring of the intrauterine state of the fetus. Question history. New possibilities of phonocardiography

Repina E.S.[1], Kosteley Y.V.[2, 3], Bureev A.Sh.[3], Yuriev S.Yu.[1, 4], Petrov I.A., Tikhonovskaya O.A., Mikheenko G.A.

[1] *Siberian State Medical University*
2, Moscow Trakt, Tomsk, 634050, Russian Federation

[2] *Tomsk State University of Control Systems and Radioelectronics*
40, Lenina Av., Tomsk, 634050, Russian Federation

[3] *Diagnostika +, LLC*
11b, Frunze Ave., Tomsk, 634029, Russian Federation

[4] *Perinatal Health Center, LLC*
9/1, Sibirskaya Str., Tomsk, 634029, Russian Federation

ABSTRACT

The problem of decreasing perinatal mortality is one of the pressing problems in modern obstetrics. Unfortunately, current methods of monitoring the intrauterine state of the fetus that are at the disposal of an obstetrician – gynecologist (cardiotocography, Doppler velocimetry) do not guarantee fetal wellbeing in the near-term outlook, and the number of tests is limited due to safety concerns. Consequently, there is ongoing search for alternative methods of obtaining information about the intrauterine state of the fetus (phonocardiography, electrocardiography). Using IT and mathematical data analysis has considerably enlarged the phonocardiography potential, including implementation of remote monitoring of the fetal health state.

A Tomsk-based company Diagnostika + LCC developed software and hardware appliance FetalCare aimed at 24-hour monitoring of the intrauterine state of the fetus based on audio data on the fetal cardiovascular system. Cardiointervalograms (CIG) obtained by phonocardiography allow to estimate the state of the fetus based on standard assessment criteria: basal heart rate, heart rate variability, presence of accelerations and decelerations, short-term variation (STV), and long-term variation (LTV). The developed appliance is non-invasive, relatively cheap, portable, and safe both for the mother and the fetus.

Keywords: perinatal mortality, phonocardiography, cardiotocography, remote monitoring of the intrauterine state of the fetus

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✉ *Repina Ekaterina S.,* repinaekaterina.ssmu@gmail.com

Мониторирование внутриутробного состояния плода. История вопроса. Новые возможности фонокардиографии

Репина Е.С.[1], Костелей Я.В.[2, 3], Буреев А.Ш.[3], Юрьев С.Ю.[1, 4], Петров И.А, Тихоновская О.А., Михеенко Г.А.

[1] Сибирский государственный медицинский университет (СибГМУ)
Россия, 634050, г. Томск, Московский тракт, 2

[2] Томский государственный университет систем управления и радиоэлектроники (ТУСУР)
Россия, 634050, г. Томск, пр. Ленина, 40

[3] Общество с ограниченной ответственностью «Диагностика +»
Россия, 634029, г. Томск, пр. Фрунзе, 11б, офис 407

[4] Общество с ограниченной ответственностью «Центр перинатального здоровья»
Россия, 634029, г. Томск, ул. Сибирская, 9/1

РЕЗЮМЕ

Снижение перинатальной смертности – одна из ключевых проблем современного акушерства. К сожалению, имеющиеся в арсенале у акушеров-гинекологов методы наблюдения за состоянием плода (кардиотокография, допплерометрия) не дают гарантии его благополучного состояния в ближайшей перспективе, а количество проводимых исследований ограничено из соображений безопасности, так как до сих пор не определено влияние ультразвука на развивающийся плод. В связи с этим продолжается изучение альтернативных (не связанных с ультразвуковым излучением) методов получения информации о состоянии плода – фонокардиографии, электрокардиографии. Использование цифровых технологий и математических методов анализа данных существенно расширило возможности фонокардиографии, в том числе реализацию идеи дистанционного мониторирования состояния плода.

В компании ООО «Диагностика +» (г. Томск) разработан программно-аппаратный комплекс FetalCare, предназначенный для круглосуточного мониторирования состояния плода на основе аудиоданных деятельности его сердечно-сосудистой системы. Полученные путем фонокардиографии кардиоинтервалограммы позволяют судить о состоянии плода на основании стандартных критериев оценки: базального ритма, вариабельности, наличия акцелераций и децелераций, STV (short-term variation), LTV (long-termvariation). Созданный программно-аппаратный комплекс неинвазивный, сравнительно недорогой, портативный, безопасный в применении для матери и плода.

Ключевые слова: перинатальная смертность, фонокардиография, кардиотокография, дистанционное мониторирование состояния плода

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

Search for methods for decreasing perinatal mortality and morbidity remains one of the pressing problems in modern obstetrics. Improvement of perinatal care has led to a decrease in perinatal mortality, but fetal mortality and neonatal morbidity are still high.

The highest neonatal mortality rates (2019) are registered in the countries of Africa (up to 40.2‰), West and South Asia (up to 40.39‰), the lowest ones are in Japan (0.86‰), Europe (from 1.81‰

in Slovenia), Australia (2.36‰) and New Zealand (2.64‰), Canada (3.18‰), and the USA (3.53‰). As of 2019, leaders in the infant mortality ranking are Iceland with 1.6 (per 1,000 births), Slovenia – 1.7, and Finland and Japan – 1.9; the Russian Federation ranks 53rd (6.5) [1].

In 2018 in the Russian Federation, 11,659 (7.23‰) cases of perinatal mortality (per 1,000 live births and stillbirths) were registered, 8,894 of them are stillbirths (5.51‰). In 2019, the rate decreased to 7.10‰ (including 5.44‰ of stillbirths). In 2020, perinatal mortality increased insignificantly to 7.25‰ (including 5.67‰ of stillbirths). In 2021, perinatal mortality was 7.32 ‰ (including 5.77 ‰ of stillbirths). In the Tomsk Region in 2018, perinatal mortality was 5.66‰ (5.07‰ of stillbirths), in 2019 – 7.4 ‰ (6.22 ‰ of stillbirths), in 2020 – 7.5 ‰ (6.8 ‰ of stillbirths), in 2021 – 5.76‰ (5.24 ‰ of stillbirths). Over 4 months of 2022, given a decile in the birth rate (2,981 children in 2021, 2,752 – in 2022), perinatal mortality and stillbirth rates aggravated in contrast to 2021: perinatal mortality was 6.1‰, stillbirth – 5.4 ‰ [2–4].

The most common causes of antepartum fetal death are intrauterine hypoxia (43%), intrauterine infection (20%), diabetic fetopathy (17%), multifetal pregnancy (15%), and fetal congenital anomalies (5%). With an increase in gestation, the risk of stillbirth decreases: 60.3% at 22–37 weeks, 34.8% in 37–40 weeks, and 4.9% at 40 weeks and more [5].

Many Russian and foreign studies are devoted to sudden intrauterine fetal death of unspecified genesis. Attempts to explain it with thrombophilia, placental insufficiency, and developmental defects fail in 50% of cases [6–8]. Moreover, in this case even the risk-based approach is ineffective – 50% of sudden fetal deaths are registered within the group of multiparous women with a low perinatal risk [9, 10]. Given that, it is necessary to develop methods and tools for continuous monitoring of the intrauterine state of the fetus and search for causes and pathogenetic mechanisms of antepartum fetal death to provide emergency care in the presence of decompensation markers [9, 11–13].

Currently applied cardiotocography (CTG) and Doppler velocimetry are well-studied, informative, and evidence-based methods, but they are performed only in a medical institution, which is problematic during annual seasonal epidemics or occasional pandemics [14], when it is necessary to minimize contacts of pregnant women. Implementation of telemedicine technologies may resolve this issue. Daily remote monitoring of the intrauterine state of the fetus, given risk factors for intrauterine growth restriction (IUGR) or a present IUGR, starting from 32 weeks of pregnancy, is a desirable algorithm allowing to minimize severe fetal distress or a risk of intrauterine death [13,16,17].

Review of the literature shows an ongoing interest in remote fetal monitoring. Abroad, the first tests with the use of remote fetal electrocardiography (ECG) date back to 1980, fetal phonocardiography and vector electrocardiography – to 2008, accelerometry – to 2011. In current Russian healthcare practice, there is no one common method of remote fetal monitoring, which is explained by high price of imported equipment, lack of Russian alternatives, and underdeveloped legal framework for telemedicine. Therefore, reconsidering the fetal monitoring strategy and developing new effective methods for intrauterine fetal hypoxia prediction remain relevant.

The aim of the study was to perform review of the literature on methods for monitoring the intrauterine state of the fetus in modern obstetrics.

CURRENT METHODS OF DIAGNOSIS AND STRATEGY FOR MONITORING THE INTRAUTERINE STATE OF THE FETUS

Currently there are several methods for the diagnosis of the fetal state available: heart rate auscultation with fetoscope, cardiography, Doppler ultrasound of the fetomaternal circulation, actography. Apart from actography, all other methods require visits to the hospital and assistance of medical specialists.

According to the clinical guidelines “Physiological Pregnancy”, established by the Ministry of Health of the Russian Federation in 2020, the scope of fetal monitoring includes an interview concerning fetal movement, gravidogram and fetal heart rate (HR) auscultation every visit after 20 weeks, and CTG starting from 33 weeks every 2 weeks. In case of abnormalities in the gravidogram (fundal height of less than 10 or more than 90‰), changes in fetal movement, tachycardia or bradycardia, additional ultrasound and CTG are required.

With pronounced fetal growth restriction with anticipated weight of less than 3‰ given no Doppler echocardiography anomalies or oligohydramnios, delivery is recommended at 36–38 weeks of pregnancy [15]. Prior to this term, indications for hospitalization to level 3 inpatient services are only inversion of Doppler echocardiography parameters,

oligohydramnios or fetal anomalies registered with CTG.

The most significant markers of intrauterine hypoxia include fetal movement anomalies and pathological changes in CTG. Here a rather long period of outpatient monitoring starts, within which a CTG is recommended 1–2 times a week. The higher monitoring frequency, the more effective the algorithm, but this intensity might be excessive for many healthcare institutions. Monitoring becomes impossible during a seasonal increase in acute rhinovirus infections and flu, epidemics, pandemics, or if a woman lives far from a healthcare institution.

ACTOGRAPHY

Functional fetal development is reflected through changes in fetal movement patterns [17, 21–23]. Movements represent early neural activity, spontaneously generated by the central nervous system. The nature of movements is defined by fetal metabolic state and its neurological development. A decrease or changes in the nature of movement may be a sign of intrauterine fetal anomalies, while total absence of movement is a sign of fetal death.

Actography (a count of fetal movements by a woman herself) is an accessible, free, but subjective and insufficiently informative method of monitoring the intrauterine state of the fetus. A movement count and fetal HR auscultation were the main methods to evaluate the state of the fetus up to the middle of the XX century, when phonocardiography and electrocardiography appeared [18].

According to Cochrane Review group [19, 20], a daily fetal movement count and absence of movements were analyzed in 5 studies involving 71,458 women. It was proved (with low significance level) that daily actography has an insignificant effect or does not influence C-section frequency (1,076 women; relative risk (RR) 0.93, 95% confidence interval (CI) 0.60–1.44), the use of obstetrical forceps or vacuum extraction (1,076 women; RR 1.04, 95% CI 0.65–1.66). At the same time, the everyday fetal movement count decreases the level of anxiety in mothers-to-be (1,013 women; standardized MD 0.22, 95% CI from –0.35 to –0.10). Actograpy is proved to have little or no effect on the frequency of preterm delivery (1,076 births; RR 0.81, 95% CI 0.46–1.46). It was also mentioned that the fetal movement count increases the frequency of CTG to monitor the state of the fetus [20].

Since 2011, devices to monitor fetal movement (accelerometers) within 24 hours and remotely have been developed [13, 17]. Currently in Russia, a system of fetal movement registration is being developed, which consists of movement detectors (which register vibrations caused by fetal movement) and a recorder [17]. The result of the test is shown on the screen as a movement count regardless of movement amplitude.

CARDIOTOCOGRAPHY

The study of fetal cardiac function, in contrast to actography, has become a promising method due to higher information value. It is proved that with development of hypoxia, caused by endogenous and exogenous factors, cardiac function is the first to change [11, 22].

Cardiac function is an important indicator of the intrauterine state of the fetus, and it was the heart function that started systematic research of the functional state of the fetus. Cardiac performance is controlled by the central and peripheral nervous systems. Oxygenation anomalies in the nervous system lead to changes in the nature of impulses to the heart, and consequently, in the heart rate [11].

Study of the fetal heart rate dates back to 1818, when a Swiss scientist Francois Mayor suggested using auscultation of the fetal heart rate through a mother's anterior abdominal wall to define whether the fetus is dead or alive. The next step was heart rate auscultation with a stethoscope, suggested by J. Kargaradec. It was concluded for the first time that the intrauterine state of the fetus depends on its cardiac activity. In 1906, M. Kremer was the first to record fetal ECG through a mother's abdominal wall.

In 1950, the founder of electronic fetal heart rate (FHR) monitoring, Edward Hon (USA) declared a new principle of processing fetal ECG results recorded through a mother's anterior abdominal wall. The principle of such processing consisted in measuring each R-R-interval, then calculating instantaneous heart rate, and presenting it in form of a graph.

Cardiotocography (CTG), based on Doppler shift, was introduced in clinical practice in early 70s of the XX century and is still widely used. An electronic system in a heart rate monitor transforms changes in each R-R interval into instantaneous HR (bpm) [11]. Initially, parameters to visually evaluate fetal cardiac activity were developed; later, scores for CTG evaluation were suggested; among the most widely spread are the Ficher's scale (1976), the modified Kreb's scale, and the scale of International Federation of Gynecology and Obstetrics (1987). It is worth noting that the use of this method allowed

to increase the accuracy of fetal state evaluation only to 73–76%. In 1970, G. Dawes and C. Redman declared the beginning of the development of software aimed at automatically analyzing CTG data, based on determination of short-term variation (STV) in real time. Using the method proposed by G. Dawes and C. Redman, the accuracy of evaluating the state of the fetus is 83.6% (E.R. Guzman et al., 1996), 72.8% (A.M. Vintzileos et al., 1993), and 67.8% (E.V. Poplavskaya, 2005) [24].

ULTRASOUND

Ultrasound fetometry accurately registers fetal growth restriction. Placenta description may reveal early preclinical stages of placental deficiency, but as an isolated marker, placentometry has rather low specificity. Evaluating the volume and quality of amniotic fluid is important in antenatal hypoxia diagnosis, as significant changes in amniotic fluid characteristics serve as an indirect sign of kidney perfusion and the state of fetal microcirculation [22, 27, 28].

Doppler ultrasound of the uterine artery, umbilical cord vessels, and intrafetal blood flow became a routine procedure in obstetrics. A combination of Doppler ultrasound and CTG allows to increase the efficiency of diagnosing the state of the fetus.

Absent or reversed diastolic blood flow is a sign of impairment in the fetal adaptive and compensatory response; it is manifested by centralization of the blood flow and development of disseminated intravascular blood coagulation with vasoconstriction of vessels in the gastrointestinal tract and kidneys, which leads to their heavy ischemic injuries. After birth, such injuries clinically manifest by necrotizing enterocolitis, oligoanuria, hematuria, proteinuria, intracranial hemorrhage, neurological complication, and respiratory distress syndrome (RDS) [22]. The interval between the moment of registering critical blood flow characteristics in the umbilical artery and fetal death varies from 0 to 71 days [22].

Currently, in critical circulatory anomalies, apart from measuring the umbilical artery pulsatility index, it is recommended to study blood flow in the middle cerebral artery and ductus venosus and determine the cerebro – placental ratio [15]. The frequency and volume of the tests to monitor the state of the fetus depend on the degree of blood flow anomalies. At the same time, the use of the methods based on the Doppler shift (ultrasound, ECG) is limited for safety reasons, as the impact of ultrasound on a developing fetus is yet to be defined [29, 30]. According to the ALARA (As Low As Reasonably Achievable) principle, the test must only be run when justified to ensure minimal impact in minimal time period to achieve an adequate result [27, 31]. Therefore, ongoing use of Doppler ultrasound for dynamic fetal monitoring is inappropriate.

PHONOCARDIOGRAPHY

At the end of the XIX century, fetal cardiac activity was first registered with the help of phonocardiography (PCG) (a method for evaluating cardiac activity and valvular apparatus based on recording and analyzing sounds appearing at heart beat). In 1891, E. Pestalozza demonstrated first phonograms at the X International Medical Congress in Berlin. In the middle of the 1950s in Russia, at the Research Institute of Obstetrics & Gynecology of the USSR Academy of Medical Sciences, under the supervision of N.L. Garmasheva, the first phonocardiograph was developed, allowing to simultaneously register fetal heart beat and uterine contractions [32]. The main parts of the phonocardiograph are microphone, transforming sound vibrations into electrical ones; frequency filters, combined with signal intensifiers; and a registering device. Using different types of microphones (rifle, stethoscope, logarithmic) and band-pass filters allows to identify significant acoustic phenomena and register sound vibrations in full as well as in a selected frequency band.

In the aerly 60s of the XX century, L.S. Persianinov et al. [33] studied a method of combined use of PCG and ECG to diagnose acute hypoxia during delivery and detect fetal heart beat anomalies. Accuracy evaluation of the method undertaken by V.N. Demidov and A.A. Aristov showed that in 80% of cases PCG may detect chronic fetal hypoxia (ECG and PCG register a reduced amplitude of QRS tone and voltage, a decrease of mechanical systole duration, and arrhythmia), and in 71% of cases, it may help to suspect umbilical cord pathology (nuchal cord, true knot, velamentous cord insertion) [34]. PCG has been used in a number of Russian institutions but did not gain popularity due to a lack of capacities for mathematical data processing to analyze the adequacy of cardiointervalograms and, consequently, a hardware – software complex for arranging a monitoring system.

In recent years, digital technologies have been developing quickly and methods for mathematical analysis have been introduced, which increases the capacities of PCG. Development of this method as an

alternative to ultrasound cardiography is considered relevant [12, 13]. Today PCG is actively used in cardiology to diagnose heart failure, cardiac defect, and pulmonary hypertension. New approaches to PCG application in the diagnosis of ischemic heart disease are being discussed. There are data on projects devoted to the use of this method for screening cardiovascular diseases [35].

Such advantages as noninvasiveness, safety, and relatively unexpensive equipment provide the basis for successful PCG implementation in modern perinatal medicine.

Taking into consideration the research and use of PCG to register and interpret fetal heart sounds, a number of issues may arise. Pregnancy term, subcutaneous tissue thickness, fetal and placental position, and mother's breathing patterns may influence signal intensity and amplitude. More often than with CTG, a signal loss or a change in its amplitude is explained by changes in the position and type of the fetus and application force of the detector to the mother's anterior abdominal wall. A large number of sounds on the part of the mother (abdominal aorta, uterine artery pulsation, breathing movement, abdominal sounds) and on the part of the fetus (fetal movement, breathing movement) create difficulties in signal filtration. The specifics of fetal blood circulation (patent foramen ovale, an arterial duct between main the pulmonary artery and the aortic arch, the absence of pulmonary circulation) change the PCG pattern [11, 28].

Thus, the main task of PCG adaptation to obstetrics is development of algorithms of external noise-cancelling and construction of an adequate cardiointervalogram, which allows to make conclusions about the intrauterine state of the fetus.

In Russia, there are successful phonography-based developments. For example, Diagnostika + LLC (Tomsk) has developed a software and hardware appliance FetalCare aimed at 24-hour fetal monitoring [36–39]. After detecting audio data, their primary processing takes place aimed at increasing sound quality. The result of PCG primary processing goes through an algorithm for detecting sounds similar to heart sounds, which generates an intensified audio signal and segmentation results of potential heart tones in PCG. This algorithm differs from other existing ones by its increased detection accuracy in the context of an amplitude change. To increase its reliability, external algorithms are in place, they classify the results by heart tone segmentation. At the end of the study, a graph of potential R-R intervals is processed by the algorithm for constructing an cardiointervalogram. The results of the study are presented as a report in PDF (Portable Document Format) [36–39]. Currently, on the basis of this equipment, software aimed at medical and technical support of a doctor and a pregnant woman is being developed. A doctor's workspace may exist as a separate software or become an element of a medical institution IT system. A telemedicine module based on artificial intelligence will allow to differentiate between technical and medical issues in the course of testing, producing an emergency signal in case of critical parameters and recommendations for frequently asked questions.

INTERPRETATION OF DATA RECEIVED IN PCG. CARDIOINTERVALOGRAM

The main component of heart rate evaluation of a fetus, a baby, or an adult is an interval between two heart beats. A cardiointervalogram allows to receive an integral estimate of cardioregulation with a minimal number of tested parameters: basal heart rate, heart rate variability, instantaneous frequency of oscillatory activity, accelerations and decelerations. It is not important which method is used for rhythm detection, as in PCG, like in CTG, standard parameters are used, therefore, for PNG we use standardized scales elaborated for CTG.

The following standard integral indices are analyzed. *Basal heart rate* is a parameter of interaction between parasympathetic and sympathetic nervous systems (sympathetic system is dominant at early pregnancy terms, thus fetal HR is higher, after reaching a certain level of parasympathetic maturity, the balance is achieved and the average HR decreases).

Heart rate variability (irregularity in the heartbeat) is the result of an interaction between parasympathetic and sympathetic divisions of the autonomic nervous system. The difference in the cardiac interval duration is on average 20–30 msec (or 2–3 bpm). HR anomalies, occurring from beat to beat and having specific direction and amplitude, appear on CTG in the form of HR oscillations. Basal HR variability is characterized by short-term and long-term oscillations.

Instantaneous oscillations (STV) reflect frequency differences, calculated on average every 1/16 of a minute. STV is controlled by the parasympathetic nervous system, measured in msec, and is a sensitive indicator of fetal tissue oxygenation. STV is only evaluated and interpreted with computer processing of the record. STV predictive value is being discussed

and was proved, for example, in a cohort study of 28, 000 emergency childbirths in Great Britain [25].

Opposite results were received in the original prospective TRUFFLE study, which evaluated whether low STV index in combination with Doppler pulsatility index in the ductus venosus at early stages of IUGR may increase 2-year survival rate among infants without neurological anomalies in contrast to computer-assisted CTG only counting STV. The result of the research was a clinical recommendation not to conduct preterm delivery given low STV until circulation in the ductus venosus becomes normal, as such an algorithm did not improve prognosis [26].

Long-term oscillations (long-term variation, LTV) are cyclic variations in basal HR with a frequency of 3–5 cycles per minute with an amplitude from 5 to 20 bpm on average, which depend on the state of the fetus and are controlled by the sympathetic nervous system. Changes in LTV are characteristic of fetal oxygenation and its compensatory responses to stress.

STV and LTV change with a decrease in oxygen level in the blood. Given hypoxemia development, first, HR variability increases (since in oxygen deficiency, suprarenal cortex is activated, blood pressure rises, and a response to baroreceptor signals is generated). Then with hypoxemia aggravation in combination with acidemia, HR variability decreases due to functional CNS depression. STV reacts to hypoxia earlier than LTV.

CONCLUSION

Currently in obstetrics, there is no one best optimal method for constant monitoring of the intrauterine state of the fetus. In one of the conclusions of the TRUFFLE study, everyday standard CTG was proven inefficient in the high-risk group of antepartum fetal death. Monitoring the intrauterine state of the fetus by phonocardiography may become that missing link that will allow to decrease perinatal mortality. It is beyond argument that phonocardiography is more than just a cardiointervalogram. It is yet to prove its diagnostic efficacy at different degrees of fetal abnormalities. The efforts will be justified if a combination of acoustic and mathematical analysis allows to increase the capabilities of predictive perinatal medicine.

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Authors' information

Repina Ekaterina S. – Teaching Assistant, Obstetrics and Gynecology Division, Siberian State Medical University, Tomsk, repinaekaterina.ssmu@gmail.com, http://orcid.org/0000-0003-2881-6135

Kosteley Yana V. – Senior Lecturer, Department of Economic Mathematics, Computer Science and Statistics, Tomsk State University of Control Systems and Radioelectronics, Tomsk; Engineer, Programmer, Diagnoskika+ LLC, Tomsk, iana.v.kostelei@tusur.ru, http://orcid.org/0000-0003-0775-350X

Bureev Artem Sh. – Director of Diagnostika+ LLC, Tomsk, artem_bureev@mail.ru.

Yuriev Sergey Yu. Dr. Sci. (Med.), Professor, Obstetrics and Gynecology Division, Siberian State Medical University; Director, Perinatal Health Center LCC, Tomsk, sergeiyuriev@gmail.com, https://orcid.org/0000-0002-1343-5471.

Petrov Ilia A. – Dr. Sci. (Med.), Professor, Obstetrics and Gynecology Division, Siberian State Medical University, Tomsk, obgynsib@gmail.com, ORCID 0000-0002-0697-3896

Tikhonovskaya Olga A. – Dr. Sci. (Med.), Professor, Obstetrics and Gynecology Division, Siberian State Medical University, Tomsk, tikhonovskaya2012@mail.ru, ORCID 0000-0003-4309-5831

Mikheenko Galina A. – Professor, Obstetrics and Gynecology Division, Siberian State Medical University, Tomsk, mchnk@mail.ru, ORCID 0000-0002-3869-1906

(✉) **Repina Ekaterina S.,** repinaekaterina.ssmu@gmail.com

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Metabolic potential of gut microbiota in helminth infections as a way to achieve bronchial asthma control

Sokolova T.S., Malchuk V.N., Zaytseva A.D., Fedorova O.S., Karpova M.R.

Siberian State Medical University
2, Moscow Trakt, Tomsk, 634050, Russian Federation

ABSTRACT

The aim of the review was to analyze modern experimental studies and clinical trials aimed at assessing metabolic activity of gut microbiota in bronchial asthma (BA) and helminth infections.
Being one of the most common chronic heterogeneous respiratory diseases, bronchial asthma secures its place among global health problems of great socioeconomic importance. In recent years, a lot of data has been accumulated indicating that the state of gut microbiota is an important factor determining the state of human health and affecting immune mechanisms underlying the development of allergic diseases in childhood. Dysbiosis of gut microbiota is due not only to changes in its composition, but also to disturbances in its metabolism. In accordance with the "gut – lung axis" concept, maintaining healthy gut microbiota and correcting its disorders, including strategies aimed at activating synthesis of short-chain fatty acids in the intestine, may become a new way to prevent and treat chronic respiratory diseases in childhood. In turn, experimental and epidemiological studies have shown the immunomodulatory activity of helminths. It is assumed that their impact on the composition and function of gut microbiota is one of the mechanisms by which helminths influence the immune response of the host and the course of BA.

Keywords: gut microbiota, helminth infection, short-chain fatty acids, bronchial asthma

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Метаболический потенциал микробиоты на фоне гельминтной инвазии как инструмент управления бронхиальной астмой

Соколова Т.С., Мальчук В.Н., Зайцева А.Д., Федорова О.С., Карпова М.Р.

Федеральное государственное бюджетное образовательное учреждение высшего образования «Сибирский государственный медицинский университет» Министерства здравоохранения Российской Федерации (ФГБОУ ВО СибГМУ Минздрава России)
Российская Федерация, 634050, г. Томск, Московский тракт, 2

РЕЗЮМЕ

Цель – провести анализ современных экспериментальных и клинических исследований, направленных на оценку метаболической активности микробиоты при бронхиальной астме (БА) и гельминтных инвазиях.

✉ *Sokolova Tatiana S.,* sokolova.ts@ssmu.ru

Бронхиальная астма относится к числу глобальных проблем здравоохранения, имеющих большую социально-экономическую значимость, является одним из самых распространенных хронических гетерогенных заболеваний дыхательных путей. В последние годы накоплено множество данных, указывающих на то, что состояние микробиоты кишечника является одним из важнейших факторов, определяющих состояние здоровья человека, в том числе влияющих на иммунные механизмы развития аллергических болезней в детском возрасте. Дисбиотическое состояние микробиоты кишечника обусловлено не только изменениями структуры, но и нарушениями ее метаболизма. В соответствии концепцией «ось кишечник – легкие» поддержание нормальной микробиоты кишечника, коррекция ее нарушений, в том числе стратегии, направленные на активацию синтеза короткоцепочечных жирных кислот в кишечнике, могут стать новым способом профилактики и лечения хронических респираторных заболеваний у детей. В свою очередь, в экспериментальных и эпидемиологических исследованиях показана иммуномодулирующая способность гельминтов. Предполагается, что воздействие на состав и функцию кишечного микробиома является одним из механизмов, посредством которых гельминты влияют на иммунный ответ организма хозяина и течение БА.

Ключевые слова: кишечная микробиота, гельминтные инвазии, короткоцепочечные жирные кислоты, бронхиальная астма

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INTRODUCTION

The increase in the prevalence of chronic noncommunicable diseases, including those of allergic etiology, is one of the main healthcare problems according to world statistics. Investigation of risk factors, development of preventive measures, and search for new approaches to the treatment of socially sensitive diseases are priorities for public health programs. Bronchial asthma (BA) is one of the most common chronic respiratory diseases affecting patients of all ages. Its course is accompanied by a significant decrease in the quality of life of patients and their families, as well as by heavy economic burden. That is why this problem remains highly relevant in the global agenda of medical science [1].

Epidemiological studies suggest a possible association between a high prevalence of allergies and reduced exposure to certain infectious agents and microbiota in childhood as a result of changes in dietary habits, improved hygiene conditions, inappropriate use of antibacterial drugs, and other factors, while living in rural areas, contacts with pets, and higher susceptibility to helminth infections exert a protective effect [2]. Currently, there are several hypotheses that explain the relationship between the prevalence of allergic diseases and environmental changes that have occurred in recent decades, such as urbanization, changes in housing and nutrition, and reduced microbial and parasitic exposure [3–5]. According to the hygiene hypothesis, insufficiency of infectious stimulation in childhood is associated with changes in the immunity that predispose to the development of allergies [3]. Later, the biodiversity hypothesis was put forward, which suggests that contacts with the natural environment enrich the human microbiome, reducing the risk of developing chronic noncommunicable diseases [5].

A lot of data have been accumulated indicating that the gut microbiota is one of the most important factors determining human health, including the effect on the immune mechanisms of the development of allergic diseases in childhood [6]. At the same time, recent studies demonstrate the significance of not only the taxonomic composition of microbiota, but also of its metabolic activity. Along with this, there are data on

the relationship between helminth-induced changes in the microbial composition and suppression of allergic inflammation in BA [7, 8]. Currently, the effects of microbiota – helminth interactions on asthma control remain largely unknown. The aim of this review was to analyze current experimental studies and clinical trials aimed at assessing the metabolic activity of gut microbiota in patients with BA and helminth infections.

MATERIALS AND METHODS

We analyzed scientific publications of the results of clinical trials and experimental studies aimed at investigating the effect of gut microbiota in asthma against the background of helminth infection. The search was carried out using the PubMed (https://pubmed.ncbi.nlm.nih.gov/) and eLIBRARY (https://www.elibrary.ru/) databases. The review presents original articles published from January 1, 2012 to July 01, 2022. The analysis was carried out according to the following algorithm.

Stage 1. Initial search for publications on the topic of gut microbiota and its metabolites in BA and (or) helminth infection. We used the following keywords in the search: short-chain fatty acids / metabolites / microbiota / asthma or short-chain fatty acids /metabolites / microbiota / helminths. We also searched for studies corresponding to the listed terms in the references in the selected publications.

Stage 2. The titles and abstracts of 1,833 articles selected during the initial keyword search were considered. Reviews and original articles that did not contain data on gut microbiota and its metabolites in BA and / or helminth infection were excluded. Therefore, 52 publications were selected for a detailed analysis.

Stage 3. Evaluation of full texts of the publications (n = 52). At this stage, articles that study the taxonomic composition of gut microbiota without assessing the level of metabolites were excluded. In addition, articles studying the use of a special diet in the experimental model were excluded. Therefore, 9 publications describing gut microbiota and its metabolites in asthma and 6 articles describing gut microbiota and its metabolites in helminth infection were included in the review.

GUT MICROBIOTA AND ITS METABOLIC ACTIVITY IN BA

Recently, interactions between gut and lung microbiota (the gut – lung axis) and their effect on immunity have been actively studied [9, 10]. In accordance with this concept, maintaining normal gut microbiota or correcting its disturbances can become a tool for prevention and treatment of respiratory diseases. Gut microbiota is a key modulator of immune, metabolic, and cellular functions that responds to inflammatory signals associated with BA; it may mediate disease susceptibility, severity, and phenotype [11].

The results of experimental and epidemiological studies demonstrate that establishment of gut microbiota at an early age plays a key role in the development of BA. Low diversity of gut microbiota in the first month of life is associated with development of the disease at school age [12]. The results of other prospective studies also indicate that low biodiversity and dysbiosis of gut microbiota in infancy are associated with a risk of developing BA in childhood [10, 13, 14].

The analysis of the taxonomic composition of gut microbiota showed that a high risk of BA is associated with a low representation of the *Faecalibacterium*, *Bifidobacterium, Roseburia, Alistipes, Ruminococcus,* and *Dialister* genera and a higher content of *Veillonella* [13, 15]. Another study also showed an association of underrepresented *Bifidobacterium, Faecalibacterium,* and *Akkermansia* genera with a high risk of developing BA [16]. However, in a Canadian cohort study of more than 300 children, the risk of developing BA was associated with a decrease in the relative abundance of *Veillonella* bacteria [14]. Studies have also shown that the composition of gut microbiota differs in patients depending on the severity and phenotype of BA [17]. It was noted that in patients with severe BA, an increase in the *Streptococcus* and *Escherichia-Shigella* genera was revealed [18]. Despite the differences in the gut microbiota composition in various studies, there is an association between low microbiota diversity and abundance of short-chain fatty acid (SCFA)-producing bacteria in children with BA.

One of the potential health biomarkers often considered in microbiome research is relative abundance of bacteria producing SCFAs, such as acetate, propionate, and butyrate [19, 20]. The main producents of butyrate are bacteria of the *Faecalibacterium* genus and the *Ruminococcaceae* and *Lachnospiraceae* families; propionate is mainly produced by *Bacteroides, Propionibacterium, Roseburia,* and *Selenomonas*, while acetate is mostly produced by *Bifidobacterium, Clostridium, Ruminococcus,* and *Lactobacillus* [21]. A decrease in the synthesis of butyrate and other SCFAs leads to a lack of energy supply and degenerative changes in intestinal epithelium. The permeability of the intestinal

barrier for food and bacterial antigens increases, which contributes to the development of chronic inflammatory bowel diseases. This greatly exacerbates the imbalance in the gut microbiota [22]. It is important to note that the anti-inflammatory effects of SCFAs are not limited to the gut. The species diversity of the gut microbiota with an increase in the content of bacteria that ferment plant fibers with the formation of SCFAs is associated with a decrease in T helper 2 cell-mediated allergic airway inflammation [23].

A number of experimental studies with modeling allergic airway inflammation showed that its milder course was associated with ingestion of SCFAs (butyrate, propionate, acetate) or a high-fiber diet [23–27]. It has been shown that oral administration of butyrate to mice was associated with a decrease in the number of eosinophils and neutrophils in bronchoalveolar lavage fluid and an increase in CD25 + FoxP3 + regulatory T cells (Treg) in the lung tissue [25, 27]. It has also been found that the administration of SCFAs to mice during pregnancy had a protective effect against the development of allergies in the offspring [25, 26]. Another study showed that propionate or a high-fiber diet could attenuate house dust mite-induced airway inflammation in mice by activating G protein-coupled receptor 41 (GPR41) [23]. The anti-inflammatory effect of butyric acid and other SCFAs is realized mainly due to the inhibition of histone deacetylase (HDAC) and nuclear transcription factor (NF-κB) and stimulation of Treg, providing a decrease in the production of proinflammatory cytokines and a shift in the Th1 / Th2 balance toward Th1 [28–31].

In the Canadian Healthy Infant Longitudinal Development (CHILD) cohort study, M.-C. Arrieta et al. showed that a reduced concentration of acetate in stool samples of children at the age of three months was associated with a risk of developing BA [14]. Another prospective study showed that infants with high levels of acetate in stool samples were less likely to be diagnosed with food allergies, and a reduced risk of developing BA was noted with high levels of butyrate and propionate [25]. In children with BA, stool samples showed a decrease in butyrate-producing bacteria, including *Faecalibacterium* and *Roseburia spp.,* and a lower level of butyrate compared to the control group [15]. In adult patients with BA (n = 44), regardless of the phenotype of the disease, a significant decrease in the total content of SCFAs in stool samples and a decrease in the absolute concentrations of individual acids and total content of isoacids were revealed compared to the controls [32].

In a randomized, placebo-controlled trial including patients with BA (n = 17), inulin supplementation for one week was shown to improve asthma control as measured by the Asthma Control Questionnaire and reduce eosinophil counts and sputum HDAC9 gene expression [33]. Despite evidence that oral SCFA attenuates allergic inflammation in experimental studies, ways to successfully prevent the development of allergies in humans remain unclear. At present, other metabolites of intestinal bacteria with proinflammatory and anti-inflammatory potential have been studied, such as biogenic amines, polyunsaturated fatty acids (PUFAs), and oxylipins [34–36]. Studies of metabolites in various biological samples (serum, urine, stool samples) in adults and children with BA demonstrate the association of the disease with changes in the levels of certain metabolites, such as tyrosine, tryptophan, sphingolipids, phospholipids, bile acids, PUFAs, SCFAs, etc. [36, 37]. Taken together, these results point to the need to evaluate the metabolic activity of the gut microbiota along with its species diversity.

EPIDEMIOLOGICAL STUDIES ON THE RELATIONSHIP BETWEEN ALLERGIES AND HELMINTHS

Epidemiological studies found that in regions with high prevalence of helminth infections, not only the prevalence of BA in the population varies, but also the severity of its clinical manifestations. This is thought to be due to the modulating effect of helminths on the human immunity [38–40]. The effect of helminth infection on the course of allergic diseases is realized through various mechanisms and depends on the type of the parasite and the duration and intensity of the infection [40].

Studies in different regions have shown a positive relationship between infection with the nematode *Ascaris lumbricoides* and the prevalence and uncontrolled course of BA, especially in childhood [40–43]. In patients suffering from ascariasis, an increase in the level of immunoglobulin (Ig) E, total and specific to the allergens of *Blomia tropicalis* and *Dermatophagoides pteronyssinus*, was found [43]. Researchers have noted similar effects in patients infected with *Strongyloides stercoralis and Toxocara* [44, 45]. On the contrary, numerous studies have shown a negative relationship between helminth infection *(A. lumbricoides, T. trichuria, Opisthorchis felineus, Ancylostoma, Schistosoma)* and skin test sensitivity or the level of specific IgE to

various allergens [46–49]. Researchers have noted that patients with *Necator americanus* infection have a milder course of BA [40, 50].

The results of epidemiological studies demonstrate a decrease in the risk of allergic diseases in residents of areas endemic for helminth infections [47, 51]. Scientists have observed lower levels of interleukin (IL)-5 and IL-4 and an increase in the production of anti-inflammatory IL-10 in *S. mansoni-infected* asthmatic patients compared to patients not affected by helminth infection [52]. The effect of *O. felineus* infection on the course of BA is characterized by a change in the immune response toward suppression of Th2-dependent mechanisms due to an increase in the expression of genes encoding IL-10 and tumor necrosis factor-β and a decrease in the level of IL-4 and IL-5 [53]. A number of studies have established an association between anthelmintic therapy and the progression of clinical symptoms of allergy and an increase in immune reactivity [53–55].

METABOLIC ACTIVITY OF MICROBIOTA AND HELMINTH INFECTION

Changes in the abundance and diversity of gut communities vary depending on the helminth species. It has been noted that the presence of helminth infections is associated with an increase in microbial diversity and a rise in the concentration of SCFAs in the large intestine [56, 57]. Influencing the composition and function of the gut microbiome is hypothesized to be one of the mechanisms by which helminths affect host immunity [8].

Experimental studies and clinical trials have shown that helminth infection affects the concentration of SCFAs in the intestine and blood serum. An increase in the total level of SCFAs, acetate, and propionate in the stool samples was revealed to be characteristic of *Heligmosomoides polygyrus infection* in the experimental animals compared to the control group. However, no statistically significant difference in the butyrate content was found [58]. The study by M.M. Zaiss et al. (2015) also demonstrated an association of *H. polygyrus* infection in mice with an increase in the total SCFA and acetate levels [7].

A. suum infection is associated with an increase in the content of propionate and butyrate and a trend toward an increase in the concentration of acetate [7]. It has been experimentally shown that transplantation of helminth-modified microbiota in the absence of experimental helminth infection reduces the level of proinflammatory cytokines in recipient mice in a BA model. This can be considered as a potential approach to the prevention of exacerbations of this pathology [7]. It has also been shown that the effect of helminth *infection* on SCFA levels depends on the diversity of the gut microbiota [59]. For example, the concentration of acetate and butyrate in the fecal samples of the laboratory mice infected with *Hymenolepis diminuta* was higher than in the animals with helminth infection against the background of administrated antimicrobial drugs [59].

There are few publications on the assessment of the metabolic activity of gut microbiota against the background of helminth infections in humans which all show contradictory results. This may be due to limited sample sizes, differences between the studied cohorts, and the type of parasitic infection. When assessing the composition of gut microbiota and its metabolites in the stool samples from patients with *S. stercoralis* infection, an increase in microbial alpha diversity and a decrease in beta diversity were revealed along with a change in the abundance of certain types of microorganisms and a decrease in the concentration of SCFAs compared to the participants without helminth infection [60, 61]. According to the results of another study, patients with celiac disease (n=8) and *N. americanus* infection showed no change in the level of SCFAs, but a trend toward an increase in the studied metabolites was noted [7].

Liver flukes can have a significant impact on the gut microbiota and its metabolites. In a study involving children suffering from *O. felineus* infection, an increase in the content of certain bacteria involved in the production of SCFAs and possessing anti-inflammatory potential was revealed (*Lachnospira, Ruminiclostridium, Eubacterium eligens, Faecalitalea, Barnesiella*) [62]. Another experimental study showed that the chronic stage of *O. felineus* infection was associated with an increase in the levels of fatty acids in the blood serum [63]. Infection with another trematode species, *O. viverrini*, in the laboratory animals caused an increase in the abundance of *Methanobrevibacter, Akkermansia,* and *Burkholderia-Paraburkholderia* in the stool samples [64]. However, studies evaluating the level of intestinal metabolites, including SCFAs, have not been performed in patients with *O. felineus* infection.

CONCLUSION

The conducted systematic review demonstrates the growing interest in the research of microbial metabolites in the context of the “gut – lung axis”

concept and indicates the need to assess not only the taxonomic composition, but also the functional activity of the gut microbiota. The results of modern studies have shown that the main microbiota factors associated with BA are a decrease in the diversity and metabolic potential of the gut microbiota, mainly due to a decrease in the production of SCFAs, and a cooccurring increase in the representation of certain opportunistic bacteria. Experimental studies provide evidence for the effectiveness of a high-fiber diet or oral SCFA in reducing allergic airway inflammation and reducing the risk of developing BA. However, clinical data on the potential of this diet and SCFA for asthma control are currently insufficient.

The analysis of research results indicates that helminths and intestinal bacteria may interact to promote immune homeostasis through anti-inflammatory metabolites, such as SCFAs. Harnessing the immunomodulatory potential of helminths while avoiding side effects associated with infection represents a potential option for managing BA. Currently, the effects of microbiota – helminth interactions on asthma control remain largely unknown and further research is needed to confirm this hypothesis. The disclosure of the role of the gut microbiota and its metabolites as factors of pathogenetic influence and modification of the course of BA in the context of helminth infection presents a prospect for the development of new preventive and therapeutic strategies for BA control.

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Authors' information

Sokolova Tatiana S. – Cand. Sci. (Med.), Associate Professor, Division of Intermediate-Level Pediatrics with a Couse in Pediatric Diseases of the General Medicine Department, Siberian State Medical University, Tomsk, sokolova.ts@ssmu.ru, https://orcid.org/0000-0002-1085-0733

Malchuk Viktoria N. – Post-Graduate Student, Division of Intermediate-Level Pediatrics with a Couse in Pediatric Diseases of the General Medicine Department, Siberian State Medical University, Tomsk, malchuk.viktoriya@mail.ru, https://orcid.org/0000-0003-0083-3398

Zaytseva Anastasiya D. – 4th-year Student, Pediatric Department, Siberian State Medical University, Tomsk, anastasiazaytseva022@gmail.com, https://orcid.org/0000-0001-6837-324X

Fedorova Olga S. – Dr. Sci. (Med.), Head of the Division of Intermediate-Level Pediatrics with a Couse in Pediatric Diseases of the General Medicine Department, Siberian State Medical University, Tomsk, olga.sergeevna.fedorova@gmail.com, https://orcid.org/0000-0002-7130-9609

(✉) **Sokolova Tatiana S.,** sokolova.ts@ssmu.ru

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A clinical case of myocardial infarction after coronary artery bypass grafting using the internal mammary artery

Zakharyan E.A.[1], Shatov D.V.[1], Grigoriev P.E.[2], Radkovskaya M.S.[1]

[1] V.I. Vernadsky Crimean Federal University (CFU), Medical Academy named after S.I. Georgievsky, 5/7, Lenina Av., Simferopol, Republic of Crimea, 295006, Russian Federation

[2] Sevastopol State University 33, Universitetskaya Str., Sevastopol, 299053, Russian Federation

ABSTRACT

Coronary artery bypass grafting (CABG) is the most preferred method of myocardial revascularization in multivessel coronary artery disease and severe progressive forms of the disease. The material of choice for CABG of the left anterior descending artery (LADA) is the internal mammary artery (IMA). However, even when using IMA as a conduit for CABG, one should be aware of a possibility of graft failure, which indicates a need for constant vigilance in this category of patients. Endovascular interventions on coronary arteries make it possible to efficiently and safely revascularize an occluded bypass graft, minimizing existing risks and improving both the quality of life of patients and their subsequent survival. The article considers a clinical case of the development of recurrent anterior myocardial infarction in the patient due to occlusion of the mammary graft to the LADA.

Keywords: coronary artery bypass grafting, revascularization, internal mammary artery, stenting, myocardial infarction

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Клинический случай стентирования маммарного шунта у пациента с повторным инфарктом миокарда передней стенки левого желудочка

Захарьян Е.А.[1], Шатов Д.В.[1], Григорьев П.Е.[2], Радковская М.С.[1]

[1] Крымский федеральный университет (КФУ) им. В.И. Вернадского, Медицинская академия им. С.И. Георгиевского
Россия, 295006, Республика Крым, г. Симферополь, бульвар Ленина, 5/7

[2] Севастопольский государственный университет (СевГУ)
Россия, 299053, г. Севастополь, ул. Университетская, 22

РЕЗЮМЕ

Коронарное шунтирование является наиболее предпочтительным методом реваскуляризации миокарда при многососудистом поражении и наличии тяжелых прогрессирующих форм заболевания. Материалом выбора

✉ *Shatov Dmitry V.,* dmitrii_shatov@mail.ru

для коронарного шунтирования передней межжелудочковой ветви левой коронарной артерии является внутренняя грудная артерия. Однако даже при использовании шунта из внутренней грудной артерии, следует помнить о его возможной дисфункции, что диктует необходимость постоянной настороженности у данной категории пациентов. Эндоваскулярные вмешательства на коронарных артериях позволяют эффективно и безопасно, сводя к минимуму существующие риски, провести реваскуляризацию окклюзированного шунта, обеспечивая улучшение как качества жизни больных, так и последующую их выживаемость. В статье рассмотрен клинический случай развития повторного инфаркта миокарда передней стенки левого желудочка в связи с окклюзией маммарного шунта к передней межжелудочковой ветви левой коронарной артерии.

Ключевые слова: коронарное шунтирование, реваскуляризация, внутренняя грудная артерия, стентирование, инфаркт миокарда

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

Источник финансирования. Авторы заявляют об отсутствии источника финансирования.

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INTRODUCTION

According to the World Health Organization, cardiovascular diseases are the leading cause of death in the world, among which coronary artery disease (CAD) permanently occupies a leading position [1]. Coronary artery bypass grafting (CABG) is the preferred method of revascularization in severe progressive forms of the disease and multivessel myocardial damage, taking into account the consent of the patient and the anatomical features of lesion localization [2].

A meta-analysis of the effectiveness of CABG and percutaneous coronary intervention (PCI) in ischemic heart failure with the participation of 54,173 patients (CABG (n = 29,075) and transluminal balloon angioplasty (n = 25,098)) in 2002–2019 has shown the best long-term results after major surgical interventions. The risk of death, myocardial infarction, and repeat revascularization procedures was lower in the group that underwent CABG than in the group of patients after PCI, in the absence of a statistically significant difference in the occurrence of stroke [3].

The internal mammary artery (IMA) is currently the gold standard conduit for bypassing the left anterior descending artery (LADA). The study by F.D. Loop et al. discussed the prospects for the use of IMA graft based on 10-year survival and cardiac events, as a result of which the internal mammary artery bypass (IMAB) to the LADA was defined as the gold standard of coronary revascularization [4]. Despite the high clinical effectiveness of CABG, there is a group of patients with recurrent clinical manifestations of coronary artery disease after surgery, including those who underwent surgeries using IMA for grafting.

B.D. Morchadze et al. analyzed the results of repeat revascularization surgeries in 92 patients with coronary artery disease. The main reasons for recurrent angina pectoris in the general group were shunt dysfunction (77%) and progression of atherosclerosis in native coronary arteries (CA) (23%) [5]. According to the literature, the main causes of mammary graft dysfunction include thrombosis, progression of atherosclerosis, shunt rupture, as well as its compression by a tumor, artificial pacemaker or post-traumatic hematoma [6–10].

This report describes a case of recurrent myocardial infarction of the anterior wall of the left ventricle (LV) due to occlusion of a mammary graft to the LADA.

CLINICAL CASE

A 60-year-old patient was admitted to the Regional Vascular Disease Center for the treatment of patients with acute coronary syndrome with complaints of intense burning pain behind the sternum radiating to the left half of the chest, left arm, interscapular region, severe weakness, and cold sweat. He had considered himself ill for 11 years, when, after a myocardial infarction of the anterior wall of the LV, CABG of

the right coronary artery (RCA) was performed, using IMAB to the LADA with the LV aneurysm plication. For a long time, the patient had noted arterial hypertension (maximum blood pressure 180/100 mm Hg). Previous surgical interventions included appendectomy in 2006 and cholecystectomy in 2017. Clinician-observed, the patient's general condition was of moderate severity; the patient was alert. The heart sounds were muffled. The heart rhythm was regular, 56 beats per minute; no noises were heard. Blood pressure in both arms was 160 / 90 mm Hg. Breathing was vesicular, 18 breaths per minute, no abnormal breath sounds were heard. The abdomen was soft and painless.

Electrocardiography (ECG) revealed sinus rhythm, bradycardia, and left axis deviation. Blockade of the anterior – superior fascicle of the left bundle branch was also revealed. The examination showed signs of anterior ST-elevation myocardial infarction (Fig. 1). The rapid diagnostic test for the qualitative detection of troponin I in the whole blood was positive.

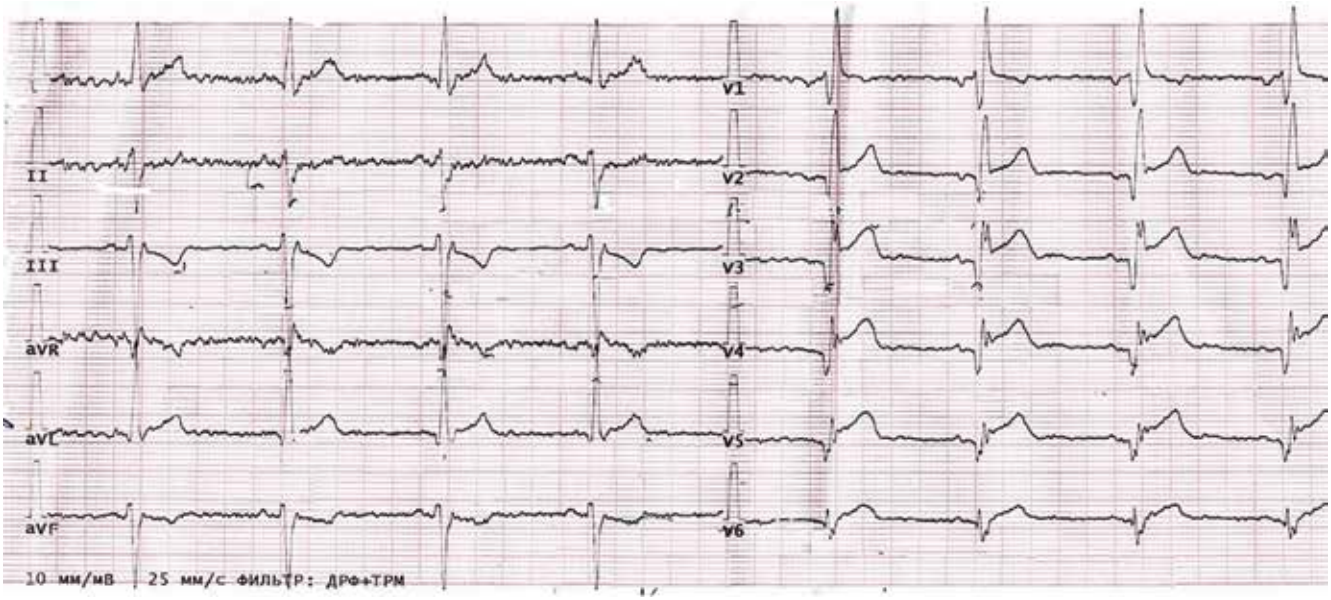

Fig. 1. Electrocardiogram before coronary angiography. Here and in Fig. 2, 3: speed 25 mm / s, voltage 10 mm / mV

According to ECG, the heart cavities were not dilated; there were no abnormalities in the pulmonary artery; the aorta, the cusps of the aortic and mitral valves were compacted, with the sufficient cusp opening. Moderate concentric LV myocardial hypertrophy was revealed. The septa appeared to be continuous. There was hypokinesis of the anterior LV segments. The global myocardial contractility was moderately reduced (LV ejection fraction 40%). Diastolic dysfunction of the LV myocardium was of the relaxation type. At the time of the study, the distance between the pericardial layers along the LV contour was noted to be 5 mm.

The patient underwent coronary angiography. Atherosclerosis and calcification of the coronary arteries were found. Stenosis of the orifice of LADA was 98%. The patient had chronic occlusion of the proximal third of the LADA. The middle third of the circumflex branch of the LCA had irregular contours. Stenosis of the proximal third of the RCA was 70%. There was chronic occlusion of the distal third of the RCA. The graft to the RCA was functioning. The proximal third of the graft to the LADA was occluded. In the area of occlusion, recanalization and predilation with balloons were performed: 1.0 x 15 mm and 2.5 x 15 mm, pressure 6–8 atm for both, respectively. A BioMatrix Flex stent 3.0 x 24 mm, under the pressure of 14 atm, was introduced and installed in the predilation zone. A check-up examination revealed that the functioning stent fully extended, occlusion of the graft to the LADA was eliminated, the vessel was patent, TIMI 3 flow.

The patient had no complications in the early postoperative period. The ECG showed positive changes (Fig. 2, 3). The patient was discharged 9 days later in improving condition.

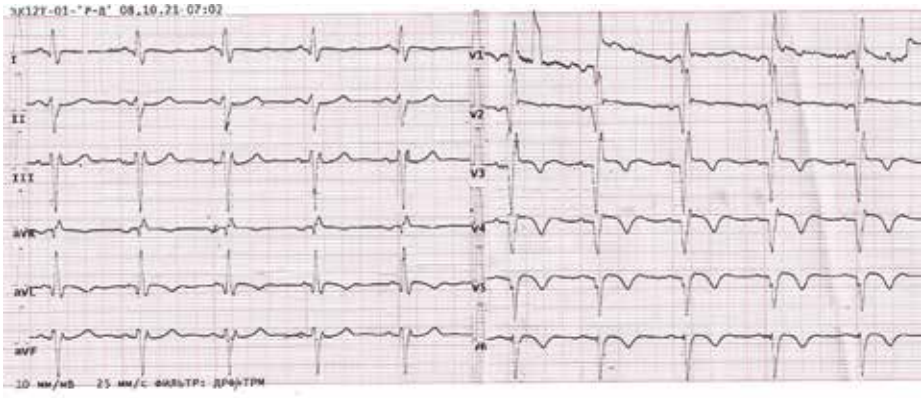

Fig. 2. Electrocardiogram 17 hours after percutaneous coronary intervention

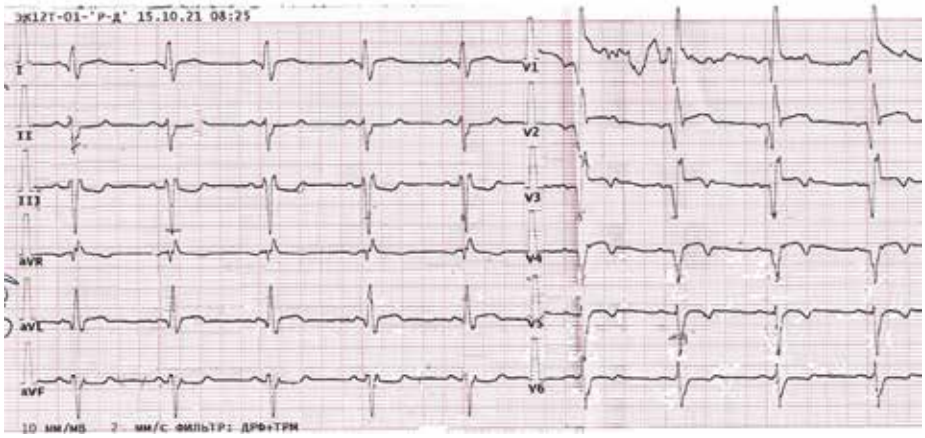

Fig. 3. Electrocardiogram 1 week after percutaneous coronary intervention

DISCUSSION

In this case, the cause of acute insufficiency of the mammary graft to the LADA was acute thrombotic occlusion, which led to repeat myocardial infarction of the anterior LV wall. PCI is the best procedure for revascularization in such cases. However, performing PCI using IMAB has additional risks compared to its performance in the native coronary bed. Due to the tortuous course of the IMA, there is a risk of its unintentional perforation or dissection with the development of adverse events during the procedure [6–8, 11]. Repeat open revascularization is indicated in a case of early graft failure (fresh anastomosis, complicated anatomy or tortuous graft course, as well as resternotomy for non-coronary reasons) [12]. At the same time, the acute onset, age, the presence of diabetes mellitus, low LV ejection fraction, and the development of myocardial infarction are the risk factors for poor outcomes in this category of patients [13].

To date, the use of IMA as a conduit remains the method of choice for LADA bypass due to its greater durability, the anatomical proximity of the artery to the heart, less further thickening of the intima, as well as a higher quality of life after surgery in comparison with the use of such transplants as radial or gastroepiploic arteries and great saphenous vein [14, 15]. Differences in the perioperative state of grafts and long-term patency may be caused by their different characteristics, in particular, tissue heterogeneity when using the great saphenous vein. However, arterial grafts can also be heterogeneous. For example, the IMA has an enhanced endothelial function and produces more nitric oxide and other relaxing factors, while radial and gastroepiploic artery grafts are more prone to spasms [16].

The study by D.P. Taggart highlighted that atherosclerotic lesions of the IMA graft were rarely noted. It was found that 10–15 years after CABG, the patency of the IMA was about 90–95%, while the saphenous vein was affected in about 50% of cases after 5–10 years with severe atheroma formation [17]. However, even when using IMA, possible shunt

occlusion should be kept in mind, which dictates the need for constant vigilance in all patients who have undergone such revascularization surgery. As a rule, repeat surgery is associated with a high operational risk, especially in debilitated, decompensated patients, as well as in the presence of concomitant diseases. Endovascular interventions allow to provide revascularization of the occluded shunt effectively and safely, minimizing the existing risks and securing the improvement in both the quality of life and subsequent survival of patients.

Repeat endovascular revascularization is safe and effective even in the presence of unfavorable prognostic factors (elderly population, manifesting type 2 diabetes mellitus, chronic heart failure). In the long term (3 years later), the intervention still displays high anti-ischemic efficacy, which ensures regression of LV myocardial remodeling and improvement in intracardiac hemodynamics [18].

This strategy is reflected in the ESC / EACTS Guidelines on Myocardial Revascularization (2018), where indications for repeat revascularization in patients with shunt occlusion are extensive myocardial ischemia and severe clinical symptoms that do not respond to conservative therapy. It is noted that repeat CABG is associated with higher risks of intraoperative mortality, therefore, PCI is the method of choice. The IMA is the preferred vessel for repeat CABG if it has not been used before [19].

Thus, in the long term after surgical intervention, there remains a risk of atherosclerosis progression both in native arteries and in grafts, which determines the need for careful monitoring of the patient's condition after CABG during follow-up. The use of PCI in patients after previous CABG is the method of choice.

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Authors' information

Zakharyan Elena A. – Cand. Sci. (Med.), Associate Professor, Department of Internal Medicine No. 1, Medical Academy named after S.I. Georgievsky of V.I. Vernadsky CFU, Simferopol, locren@yandex.ru, https://orcid.org/0000-0002-7384-9705

Shatov Dmitry V. – Cand. Sci. (Med.), Associate Professor, Department of General Surgery, Anesthesiology, Resuscitation and Emergency Medicine, Medical Academy named after S.I. Georgievsky of V.I. Vernadsky CFU, Simferopol, dmitrii_shatov@mail.ru, https://orcid.org/0000-0003-2248-5400

Grigoriev Pavel E. – Dr. Sci. (Biology), Associate Professor, Professor of the Department of Psychology, Institute of Education and Humanities, Sevastopol State University, Sevastopol, mhnty@yandex.ru, https://orcid.org/0000-0001-7390-9109

Radkovskaya Marina S. – Student, Medical Academy named after S.I. Georgievsky of V.I. Vernadsky CFU, Simferopol, mari_feod@mail.ru, https://orcid.org/0000-0002-0053-7575

(✉) **Shatov Dmitry V.,** dmitrii_shatov@mail.ru

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Application of multicolor flow cytometry in liquid biopsy of breast cancer

Kaigorodova E.V.[1, 2], Grishchenko M.Yu.[2, 3]

[1] *Cancer Research Institute, Tomsk National Research Medical Center (NRMC) of the Russian Academy of Sciences*
5, Kooperativny Str., Tomsk, 634050, Russian Federation

[2] *Siberian State Medical University*
2, Moscow Trakt, Tomsk, 634055, Russian Federation

[3] *Tomsk Regional Cancer Dispensary*
115, Lenina Av., Tomsk, 634050, Russian Federation

ABSTRACT

As a result of the clinical study NCT04817501 “Phenotypic characterization of circulating tumor cells (CTCs) in tumors of the female reproductive system”, we developed a method for preoperative prediction of a recurrence risk in patients with stage T1 endometrial cancer (Patent No. 2762493 of 21.12.2021).

The article presents a clinical case of the use of multicolor flow cytometry in liquid biopsy of breast cancer (BC). CTCs were detected in the blood of a patient with T2N0M0 BC, stage IIA before the initiation of treatment. Using multicolor flow cytometry, various CTC phenotypes were studied and the Her2/neu and ki-67 markers were determined. These markers were also studied in the biopsy and surgical material of the BC tissue using immunohistochemistry. As a result of the study, it was shown that the molecular profile of CTCs in the blood taken before fine needle aspiration biopsy coincided with that of cancer cells in the BC tissue. In addition, the calculated risk of tumor progression before biopsy predicted recurrence of cancer in this patient 20 months before its occurrence. The obtained results show the practical utility of multicolor flow cytometry in liquid biopsy of cancers. The ability to evaluate CTCs by various molecular parameters can be useful for diagnosing, predicting, monitoring, and determining treatment strategies for cancer patients.

Keywords: liquid biopsy, breast cancer, multicolor flow cytometry, Her2/neu, ki-67, circulating tumor cells

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✉ *Kaigorodova Evgenia V.,* zlobinae@mail.ru

Применение многоцветной проточной цитометрии в жидкостной биопсии рака молочной железы

Кайгородова Е.В.[1, 2], Грищенко М.Ю.[2, 3]

[1] *Научно-исследовательский институт (НИИ) онкологии, Томский национальный исследовательский медицинский центр (НИМЦ) Российской академии наук*
Россия, 634050, г. Томск, пер. Кооперативный, 5

[2] *Сибирский государственный медицинский университет (СибГМУ)*
Россия, 634050, г. Томск, Московский тракт, 2

[3] *Томский областной онкологический диспансер (ТООД)*
Россия, 634050, г. Томск, пр. Ленина, 115

РЕЗЮМЕ

В результате проведения клинического исследования NCT04817501 «Фенотипический спектр циркулирующих опухолевых клеток (ЦОК) при опухолях женской репродуктивной системы» разработан способ дооперационного прогнозирования риска рецидива у больных раком эндометрия Т1 стадии (патент № 2762493 от 21.12.2021).

Представлен клинический пример применения многоцветной проточной цитометрии в жидкостной биопсии рака молочной железы (РМЖ). В крови больной РМЖ T2N0M0 IIA стадии до начала лечения были выявлены ЦОК. Методом многоцветной проточной цитометрии исследованы различные фенотипы ЦОК и определены маркеры Her2/neu и Ki-67. В биопсийном и операционном материале ткани РМЖ методом иммуногистохимии были также исследованы данные маркеры. В результате показано, что молекулярный профиль ЦОК в крови, взятой до процедуры тонкоигольной биопсии, совпадал с молекулярным профилем опухолевых клеток ткани РМЖ. Кроме этого, рассчитанный риск развития опухолевой прогрессии до биопсии спрогнозировал возникновение рецидива у данной пациентки за 20 мес до его появления. Полученные результаты показывают практическую пользу многоцветной проточной цитометрии в жидкостной биопсии онкологических заболеваний. Возможность оценки ЦОК по различным молекулярным параметрам может быть полезной для диагностики, прогноза, мониторинга и определения стратегии лечения больных раком.

Ключевые слова: жидкостная биопсия, рак молочной железы, многоцветная проточная цитометрия, Her2/neu, Ki-67, циркулирующие опухолевые клетки

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

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INTRODUCTION

Breast cancer (BC) occupies a leading place in terms of incidence and is among the five deadliest cancers in the world and in Russia [1]. It is a well-known fact that the main reasons for failure to treat BC are recurrence of the disease and its hematogenous metastasis.

Circulating tumor cells (CTCs) are involved in both recurrence and metastasis of cancer. The CTC population is known to be heterogeneous [2–5]. The presence of CTCs is not always accompanied by metastasis formation, apparently because not all cancer cells that have entered the circulation have properties sufficient for it [2, 6]. Even localized tumors without clinically visible metastases have been shown to be sources of CTCs [3, 7, 8]. It is estimated that 3.2×10^6 tumor cells detach from one gram of tumor tissue per day, but most of them quickly proceed to apoptosis due to the loss of adhesion to the extracellular matrix, hemodynamic shear forces, or immune system attacks [9, 10].

Liquid biopsy was introduced as a new diagnostic concept in 2010 to analyze CTCs in the blood of cancer

patients and has now expanded to analyze circulating tumor-derived factors, in particular circulating tumor DNA (ctDNA), as well as extracellular vesicles (EVs), microRNAs, mRNAs, long non-coding RNAs, circulating extracellular proteins, and tumor-educated platelets (TEPs) [11–25].

Tomsk NRMC in collaboration with Tomsk Regional Cancer Dispensary carried out a clinical study NCT04817501 "Phenotypic characterization of circulating tumor cells (CTCs) in tumors of the female reproductive system", following which a method for preoperative prediction of a recurrence risk in patients with stage T1 endometrial cancer was developed (Patent No. 2762493 of 21.12.2021) [26]. The predictive model was based on multicolor flow cytometry data on the number of different CTC populations, including circulating cancer stem cells, CTCs with markers of epithelial – mesenchymal transition (EMT), and atypical / hybrid cell populations.

The aim of the study was to show the practical application of multicolor flow cytometry and the efficiency of the developed predictive model in the diagnosis and prognosis of breast cancer.

CLINICAL CASE

In March 2021, patient P, 48 years old, turned to an oncologist with complaints of a neoplasm in the left mammary gland. The examination by a mammologist revealed that the mammary glands were symmetrical; the nipples and halos were without abnormalities; abundant serous discharge from the nipple was observed; a tumor (4 cm) in the upper inner quadrant of the left mammary gland was detected by palpation.

No skin flattening was noted. Regional lymph nodes were not enlarged. The patient was referred for mammography, bone scintigraphy, and computed tomography (CT) of the chest. In addition, venous blood was taken from the patient to study the presence and molecular profile of CTCs using multicolor flow cytometry.

In the course of the study, using fluorochrome-conjugated monoclonal antibodies to CD45, Epcam, CK, muc16, CD44, CD24, CD133, Ncadherin, Her2/neu, Ki67, and NucBlu Live reagent, various populations of CTCs were identified. Liquid biopsy showed that 72% of CTCs were Her2/neu positive and 35% were Ki-67 positive (Fig. 1).

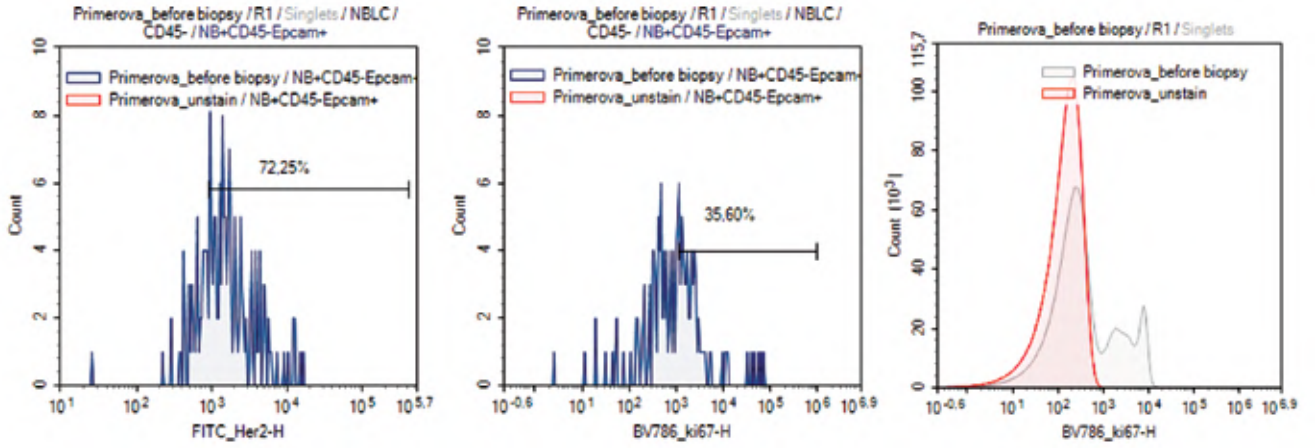

Fig. 1. Results of flow cytometry on the assessment of various populations of CTCs in the blood of patient P., 48 years old, before the biopsy

In addition, atypical / hybrid Epcam+CD45+ cells at a concentration of 19.3 cells / mm³ were detected in the blood of this patient. We calculated the risk of tumor progression using the model proposed in patent No. 2762493, based on data on the number of different CTC populations (the number of Epcam+CD45- cells, CTCs with the Epcam+CD45-CD44-CD24-Ncadherin+ phenotype, the number of circulating cancer stem cells without Epcam expression on the membrane with the Epcam(m)-CD45-CD44+CD24- phenotype (cells / ml), the number of atypical / hybrid forms of CTCs with the Epcam+CD45+ phenotype (cells / ml)). The risk of recurrence in this patient was 75%.

Mammography on the MG Adani machine (radiation exposure was 0.003 mSv) revealed accumulation

of pathological microcalcifications at the border of the inner quadrants of the LV. The area of changes was 44 × 19 × 26 mm. Impression: cancer of the left mammary gland manifested by microcalcifications. BI-RADS 5.

Chest CT of 31.03.2021 (CT Simens device, dose 4.80 mGy) revealed a mass in the left breast. No focal pathological lesions were found in the lungs.

Bone scintigraphy of 5.04.2021 using the pirfotechum radiopharmaceutical with an activity of 370 mBq and an effective radiation dose of 0.80 mSv did not reveal any scintigraphy signs of a focal skeletal lesion.

According to the ultrasound findings on the US Philips IU22 apparatus, a mass in the left mammary gland (34 × 11 × 17 mm) was found (BI-RADS 5). Axillary lymphadenopathy on the left was detected. Signs of diffuse fibrocystic breast disease were noted. The echotexture of the liver was not disturbed. Biopsy of the mass using a biopsy gun was performed. Impression of the histologic examination of the biopsy material (No. 8469-71\21) of 06.04.2021: non-specific invasive breast carcinoma (ICD-O code 8500/3), G 2 (3 + 2 + 1), with structures of ductal carcinoma *in situ*, G 2.

Immunohistochemistry of the biopsy material using the Leica Bond-Max immunohistochemistry staining system and antibodies against estrogen receptor (clone 6F11, Leica), progesterone receptor (clone 16, Leica), c-erB-2 (Her2/neu) (Polyclonal Rabbit, Dako), and Ki-67 (clone SP6, Cell Marque) showed positive homogeneous staining of tumor cells for estrogen receptors and heterogeneous staining for progesterone receptors (Fig. 2, *a, b*), Her2/neu 3+ membrane staining (Fig. 2, *c*); about 30% tumor cells were positive for the Ki-67 marker (Fig. 2, *d*), which corresponded to luminal B2 BC.

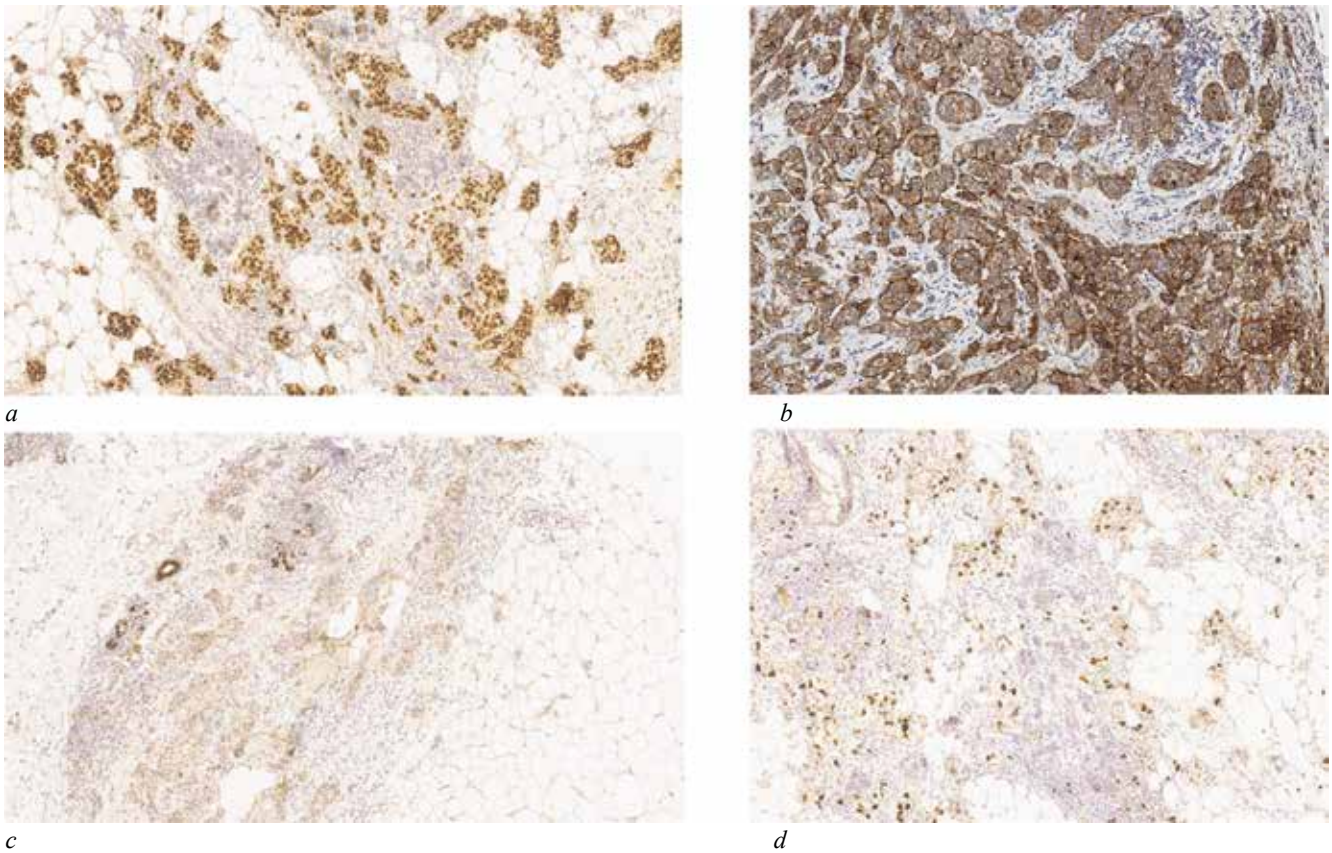

Fig. 2. Photographs of the immunohistochemical study of the biopsy material obtained from patient P., 48 years old. Immunohistochemical staining of breast cancer tissue using antibodies: *a* – against Estrogen receptor (clone 6F11, Leica), the image obtained using the digital slide scanner Aperio AT2, Leica at medium magnification; *b* – against progesterone receptor (clone 16, Leica); the image obtained using the digital slide scanner Aperio AT2, Leica at medium magnification; *c* – against c-erB-2 (Her2/neu) (Polyclonal Rabbit, Dako); the image obtained using the digital slide scanner Aperio AT2, Leica at high magnification; *d* – against Ki-67 (clone SP6, Cell Marque), the image obtained using the digital slide scanner Aperio AT2, Leica at medium magnification.

According to the results of the study, patient P., 48 years old, was diagnosed with cancer of the upper inner quadrant of the left breast (ICD code C50.2) T2N0M0, stage IIA. This patient underwent 6 courses of neoadjuvant chemotherapy (DCHP). In September 2021, she underwent a subcutaneous mastectomy on the left with a biopsy of the sentinel lymph nodes at Cancer Research Institute of Tomsk NRMC. Impression of sentinel lymph node cytology (slide no.: 2124cito 28.09.2021) showed the presence of lymphoid elements. The impression of the pathomorphological examination of the surgical material (No. 27486-5051): invasive carcinoma of no special type G2 (ICD-O code 8500/3). No metastatic lesion was detected in the sentinel lymph node. Curative pathomorphosis according to the RCB grading system – RCB-I, according to G.A. Lavnikova – III degree, pathological type ypT1N0Mx. No tumor was detected along the resection margins.

Until May 2022, the patient received transtuzumab, then tamoxifen. On 15.07.2022, according to the chest CT, areas of compaction in the lungs without dynamics were identified. The impression of the ultrasound examination of 15.07.2022: incomplete involution of the right breast. Residual signs of fibrocystic breast disease on the right. Condition after a subcutaneous mastectomy on the left, sentinel lymph node biopsy, expander installation (September 2021). No echoscopy evidence of disease progression was obtained. Diffuse changes in the liver were noted. Chronic cholecystitis. Bone scintigraphy of 21.11.2022 did not reveal any scintigraphy signs of non-proliferative focal skeletal lesions. The impression of the ultrasound examination of 18.11.2022: axillary lymphadenopathy on the left. Fine-needle aspiration biopsy was performed. The cytological examination of the lymph node puncture showed the presence of metastasis.

Thus, according to the results of the examination 13 months after the surgery, data for regional BC recurrence were obtained. In December 2022, the patient underwent axillary lymphadenectomy. The impression of the pathomorphological study (No. 39063-76/22 of 26.12.2022): metastasis of invasive breast carcinoma (ICD-O code 8500/6) to the lymph node, with invasion of the tumor into the capsule of the lymph node and extracapsular spread into the perinodal adipose tissue, signs of lymphovascular invasions. Data for neural invasion were not found. Currently, the patient has independently sought care at Blokhin National Medical Research Center of Oncology, where she is receiving radiation therapy and chemotherapy with anastrozole and transtuzumab.

CONCLUSION

Thus, the use of multicolor flow cytometry in liquid biopsy of BC made it possible to identify heterogeneous populations of CTCs. As a result of the study, it was shown that the molecular profile of CTCs in the blood taken before fine needle aspiration biopsy coincided with the molecular profile of tumor cells in the BC tissue. In addition, our calculated risk of tumor progression before biopsy predicted a recurrence of the disease in this patient 20 months before its occurrence. The obtained results show the practical utility of multicolor flow cytometry in liquid biopsy of cancers. The ability to evaluate CTCs by various molecular parameters can be useful for diagnosing, prognosing, monitoring, and determining a treatment strategy for cancer patients.

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Authors’ information

Kaigorodova Evgenia V. – Dr. Sci. (Med.), Associate Professor, Leading Researcher, Department of General and Molecular Pathology, Cancer Research Institute, Tomsk NRMC; Professor, Biochemistry and Molecular Biology Division with a Course in Clinical Laboratory Diagnostics, Siberian State Medical University, Tomsk, zlobinae@mail.ru, http://orcid.org/0000-0003-4378-6915

Grishchenko Maksim Yu. – Cand. Sci. (Med.), Chief Physician, Tomsk Regional Cancer Dispensary; Associate Professor, Surgery Division with a Course in Mobilization Training and Disaster Medicine, Siberian State Medical University, Tomsk, Grishenko83@mail.ru

(✉) **Kaigorodova Evgenia V.,** zlobinae@mail.ru

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ЖУРНАЛ «БЮЛЛЕТЕНЬ СИБИРСКОЙ МЕДИЦИНЫ» ПРЕДСТАВЛЕН НА СЛЕДУЮЩИХ РЕСУРСАХ

unpaywall	Unpaywall – браузерное расширение. Оно находит бесплатные копии научных статей, оригиналы которых находятся под paywall. В открытой базе данных сервиса есть более 31 миллиона научных публикаций
Baidu 学术	Baidu – китайская компания, предоставляющая веб-сервисы, основным из которых является поисковая система с таким же названием – лидер среди китайских поисковых систем
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WIKIDATA	Викиданные – это бесплатная и открытая база знаний, которую могут читать и редактировать как люди, так и машины
SJR SCImago Journal & Country Rank	SCImago Journal & Country Rank – это общедоступный портал, который включает научные показатели журналов и стран, разработанные на основе информации, содержащейся в базе данных Scopus® (Elsevier B.V.). Эти показатели можно использовать для оценки и анализа научных областей.
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НейроАссистент	НейроАссистент – сервер научного издательства предназначен для авторов, редакторов и рецензентов научных журналов. Сервис позволяет автоматизировать работу с текстами научных статей с помощью искусственного интеллекта

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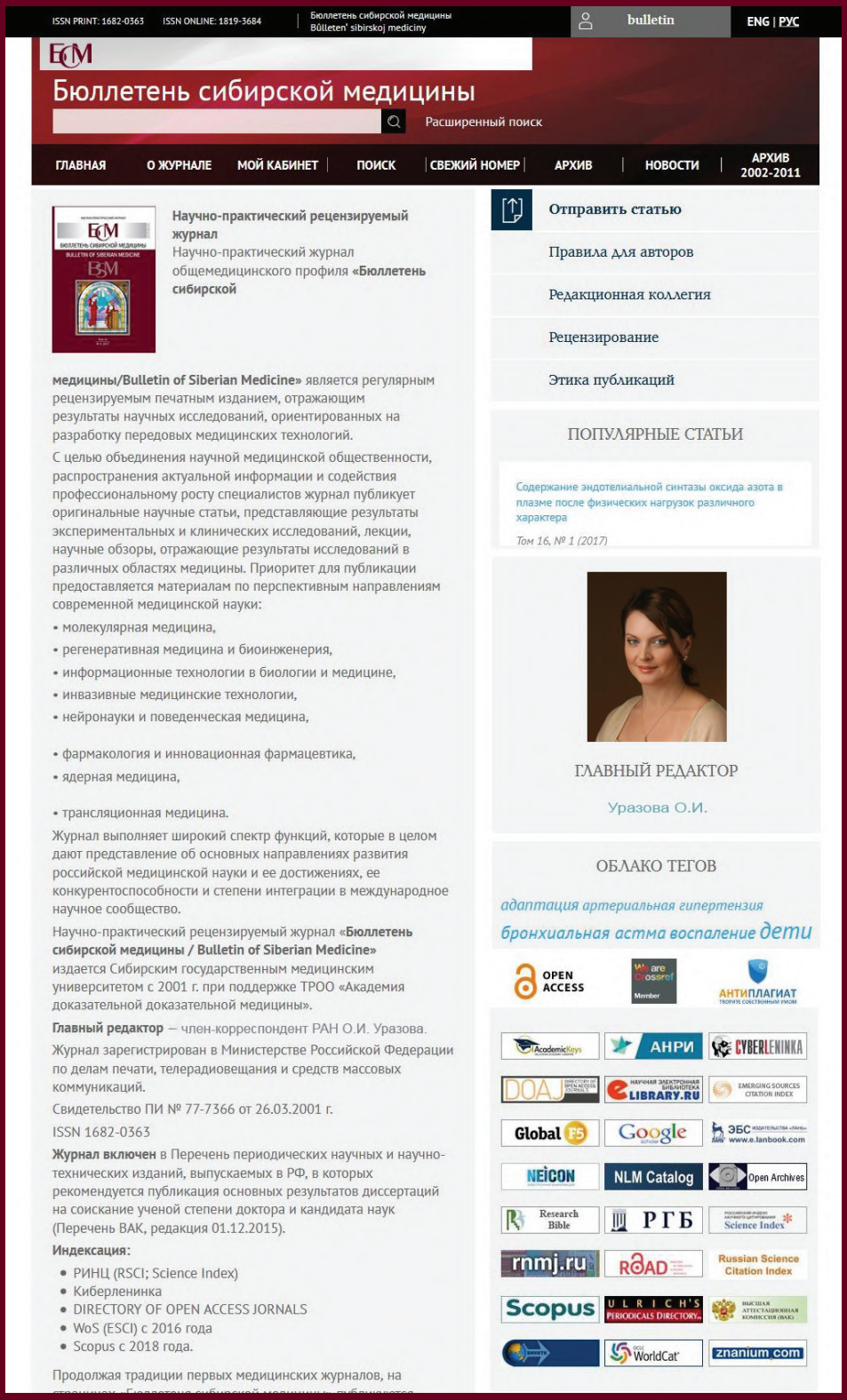

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