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Protective effect of the *Prunella grandiflora* L. extract in relation to the toxic effect of etoposide through the example of *Drosophila melanogaster*

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ABSTRACT

The data on the protective properties of the *Prunella grandiflora L.* extract were obtained when used together with the anticancer drug etoposide on the experimental strain of *Drosophila melanogaster*. The combined use of etoposide and 10% extract of *P. grandiflora* decreased mortality in *D. melanogaster* individuals to 15% and doubled the average individual fertility compared to the use of this cytostatic drug without the extract. Using the SMART method, the presence of the antigenotoxic effect was identified, which manifests itself through the absence of chromosomal aberrations.

Key words: medicinal plants, antigenotoxic effect, *Drosophila melanogaster,* protective properties, etoposide, SMART.

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Протекторный эффект экстракта *Prunella grandiflora* L. относительно токсического воздействия этопозида на примере *Drosophila melanogaster*

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РЕЗЮМЕ

Получены данные о протекторных свойствах экстракта *Prunella grandiflora* L. (черноголовка крупноцветковая) при совместном его использовании с противораковым препаратом «Этопозид» на эксперименталь-

✉ *Antosyuk Olga N.,* e-mail: antosuk-olga@mail.ru.

ной линии животных *Drosophila melanogaster.* При совместном применении этопозида и 10%-го экстракта *P. grandiflora* показано снижение летальности у особей *D. melanogaster* до 15% и увеличение средней индивидуальной плодовитости в два раза в сравнении с использованием данного цитостатика без экстракта. Методом SMART установлено наличие антигенотоксического эффекта, который проявляется в отсутствии хромосомных аберраций.

Ключевые слова: лекарственные растения, антигенотоксический эффект, *Drosophila melanogaster,* протекторные свойства, этопозид, SMART.

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INTRODUCTION

Due to the high prevalence of cancer in the human population, the search for medicinal drugs, protectors, and adaptogens is an extremely important area of research. Cytostatics used in chemotherapy are characterized by the presence of both the main (antitumor) effect and many side effects, in particular, general toxic and genotoxic ones. To reduce side effects, various protectors are being studied, most of which are extracts from medicinal plants with a complex of various components [1].

When using an extract from medicinal plants as a protector, it is important to establish whether it itself has any toxic and genotoxic effect. In this regard, it is necessary to analyze the extract separately and together with a cytostatic drug. The use of medicinal plant extracts is widespread, a lot of such studies are carried out in India, China, and other countries where traditional medicine is common [2–4]. However, the search for protectors based on raw plant materials grown in Russia is relevant.

In this aspect, plants of the genus *Prunella* L. growing in the Ural Region are of considerable interest. The genus *Prunella* belongs to the family Lamiaceae Juss. which representatives have high biological activity and can be used to obtain valuable medicinal raw materials. *Prunella vulgaris* L. (common self-heal) is an official medicinal plant in Chinese medicine [5]. The extract from the aerial parts of *P. vulgaris* has antioxidant [6, 7], anti-inflammatory, antibacterial, antifungal [8, 9], and antitumor properties [10].

To prove these data, an article appeared in 2019 showing that *P. vulgaris* root extract inhibits *in vitro* and *in vivo* carcinogenesis in human breast carcinoma cells [11]. In Russia, *P. vulgaris* is still not a pharmacopoeial species, but in recent years it has been mentioned in the literature as a plant producing the most important classes of biologically active substances (BAS) [12–14] and has been studied as a component in pharmaceutical preparations [15]. Medicinal properties of *Prunella grandiflora* (L.) Scholler (large-flowered self-heal) are poorly studied, its extract shows antifungal and antibacterial properties and has biological activity during hypoxia [16, 17]. However, according to our assumptions, the *P. grandiflora* extract may show protective properties against anticancer drugs, as some peculiar features were revealed in the content of the main groups of BAS.

In particular, rosmarinic acid (70–89%) was found to dominate among the phenolcarboxylic acids in *P. grandiflora*. It was observed that, regardless of the harvest year, the content of rosmarinic acid was higher in *P. grandiflora* than in *P. vulgaris* [12, 18]. Rosmarinic acid has antitumor, antiproliferative [19], and anticyclooxygenase activity [20] and can protect against cancer and radiation sickness [21]. Thereafter, the aim of the study was to identify the protective properties of *P. grandiflora* in relation to the toxic and genotoxic effects of etoposide.

MATERIALS AND METHODS

The *P. grandiflora* herb was collected in the flowering phase in the Krasnoufimsk district of the Sverdlovsk region to the north of the Mariyskiy Ust-Mash village, on the Mokraya Mountain (N 56°09’22.0’’, E 058°32’19.6’’) in 2018. The plants were dried in

well-ventilated rooms. The dried raw material was ground to the size of particles passing through a 1mm sieve. A weighed portion of large-flowered self-heal in the amount of 0.8 g was extracted in 10ml of 70% alcohol for 24 hours. Then, 2.4 ml of 10% extract was added to 17.6 ml of nutrient medium. Ethyl alcohol (70%) was added to the nutrient medium in the 10 : 90 ratio, respectively, or 2.4 ml of ethyl alcohol per 17.6 ml of the nutrient medium.

We used a 20 mg / ml solution of etoposide for injections (Vero-pharm Ebave, Russia) at the concentration of 800 μg / kg of the nutrient medium. The chosen concentration of the cytostatic agent demonstrated a pronounced genotoxic effect [22]. The Oregon R laboratory line was used to assess the viability and overall mortality. For crossing, individuals were selected that were grown under the standard laboratory conditions at the temperature of 24 °C, with moderate humidity and light, in the nutrient medium containing 250 ml distilled water, 25 g glucose, 25 g yeast, and 2 g agar. For the study, the *D. melanogaster* species were used that eclosed from the puparium within no later than 6 hours (virgin females). To evaluate fertility, 25 individual pairs were placed in 25 test-tubes with a hollow lid, filled with agar medium and covered with yeast.

The laid eggs (F_1) were collected daily from the surface of the lids using a dissecting needle and placed on Petri dishes with an agar layer for further development. From the total number of eggs laid per day, the percentage of eggs that did not develop at the early stage (< 6 hours, white color) and at the late stage (> 6 hours, brown color) was calculated. To determine the mortality rate of the larvae in the parental generation of *D. melanogaster*, they were grown on the nutrient media with the extract, etoposide or the extract and etoposide combined in the amount of 300 individuals each. The overall mortality was determined by the number of individuals that died at the larval and pupal stages. At the larval stage, the difference between the number of individuals placed on the nutrient medium and the number of puparia was determined, which indicates death of unpupated larvae. Lethality at the pupal stage was identified by the presence of filled puparia in the sample, which indicates death of individuals within the puparium.

The genotoxic effect was determined using the SMART (Somatic Mutation And Recombination Test) technology. For this, females from the yellow mutant line (yellow body color, the yellow gene located on the X chromosome) were crossed with males from the white singed 3 mutant line (white eyes and singed bristles on the body, the genes are also located on the X chromosome), placing them on the test medium for 72 hours. After 72 hours, the parental generation of the flies was removed, and hybrid offspring (F_1) developed from the eggs they laid. Hybrid females of the wild phenotype (brown and gray body, straight bristles, red eyes) were used for the analysis, since the males had the yellow phenotype inherited from maternal individuals following the criss-cross phenomenon. The bristles on the female bodies were examined, and the number of bristles that were not typical of the normal phenotype in color and shape were noted, namely, yellow and / or singed ones. The area containing such bristles was recorded in the table as a single spot “y” (yellow) or “sn” (singed), or a double spot “y sn”. Statistical analysis was performed using Statistica (data analysis software system) v. 8.0., Serial Number: JPZ803I371720ARCN-6, StatSoft, Inc. When comparing and analyzing the samples, the Mann – Whitney test and the chi-square test with Yates’ correction were used.

RESULTS AND DISCUSSION

To determine the general toxic effect, two parameters were used: determination of the overall mortality of *D. melanogaster* species and their average individual fertility. The assessment of survival and mortality rates was carried out in three groups of the *D. melanogaster* species receiving the extract and etoposide separately or together during the entire period of development. After 10 days, the survival rate of the *D. melanogaster* individuals in the group grown on the nutrient medium containing the 10% *Prunella grandiflora* extract and etoposide was 72% (Fig. 1).

Figure 1 shows that the combined use of etoposide and the extract reduced the mortality rate by 20% compared to the control group and by 15% compared to the group receiving the cytostatic drug without the extract. When the *P. grandiflora* extract alone was added to the nutrient medium, no decrease in the mortality rate was found as opposed to the controls. Under the effect of 70% alcohol, the mortality rate was 36.33%. Thus, this concentration of alcohol does not have a pronounced general toxic effect and is suitable as an extract base. Consequently, the analysis of the overall mortality of individuals grown on the nutrient medium containing the 10% extract of *Prunella grandiflora L.* and etoposide showed a positive effect on their survival.

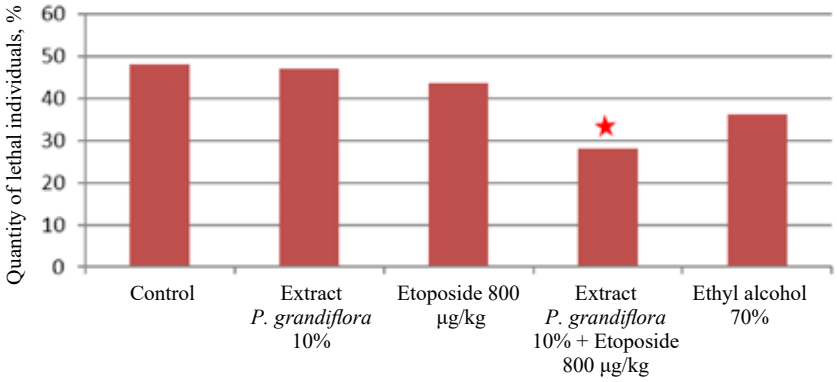

Fig. 1. The overall lethality of individuals of the Oregon-R line of *Drosophila melanogaster*, grown on the nutrient medium with addition of various components: * values that significantly differ from the corresponding parameters in the control group, $p < 0.001$ (chi-square test)

The parameters of viability of the Oregon-R line were assessed, such as the average individual fertility (AIF), and the frequency of early and late lethality of the offspring at the embryonic stage (up to 6 hours of development – early embryonic lethality (EEL), after – late embryonic lethality (LEL)). Figure 2 demonstrates the improvement in fertility rates when etoposide and the extract were used together and when the extract was used alone.

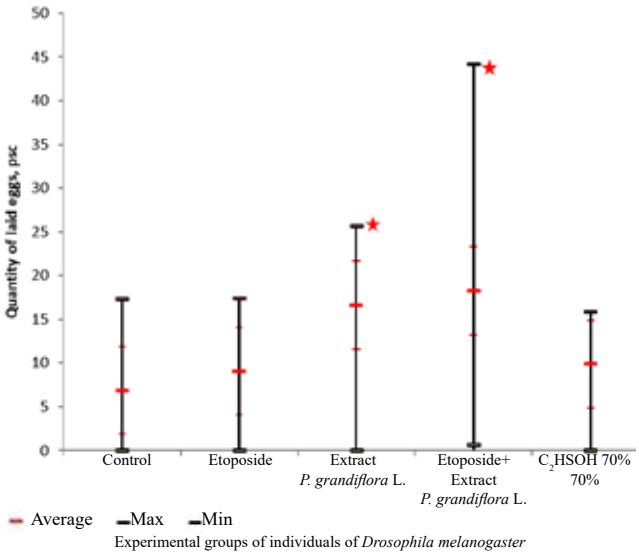

Fig. 2. Average individual fertility of the Oregon-R line of *Drosophila melanogaster* grown on the nutrient medium with addition of various tested substances: * values that significantly differ from the corresponding parameters in the control group, $p < 0.05$ (Mann – Whitney test)

A decrease in the general toxic effect was registered under the effect of the extract, which manifested itself through a change in the AIF indicator: when receiving etoposide, the AIF was 9.03; when the cytostatic and the extract were combined, it went up to 18.27. In addition, the comparative characteristics of AIF in the studied flies showed that the maximum number of laid eggs was recorded when the extract was used with etoposide. Therefore, the extract neutralizes the toxic effect on fertility potential. However, such pronounced effect does not apply to the mortality rate of the offspring at the embryonic stage. Perhaps this is explained by the short duration of the positive effect of the extract for changing the lethality index F_1. It should be noted that the results of AIF obtained using 70% alcohol, which is the base of the extract, are comparable to those obtained using etoposide.

The genotoxic effect of etoposide was also analyzed using SMART lines. An increase in the frequency of mutations and recombinations with a rise in the concentration in the nutrient medium was observed (Table). Table 1 shows that the extract itself does not change the frequency of mutations and recombinations, thus, it does not show genotoxic properties, while etoposide has a clear genotoxic effect, which complies with the results of other studies [23].

The genotoxicity of 70% alcohol manifests itself in the form of single singed spots, and therefore, it can be assumed that its action specifically damages the chromosome in the periventricular region. The absence of females with yellow spots allows to suppose that the eliminated chromosome region was not exposed to the active effect of alcohol. At the same time, the chi-square value in the experimental group receiving etoposide 400 µg / kg, as opposed to the controls, was close to the critical one.

Table 1

Characteristics of somatic mosaicism in *Drosophila melanogaster* using yellow (y) and singed (sn) markers

Test groups	Sample	Number of individuals with mutant spots					(χ2)	(*p*)
		y	sn	y sn	Other mutant phenotypes	Sample proportion, %		
Control	573	1	2	0	0	0.52	–	–
Etoposide (400 μg / kg)	499	0	11	0	0	2.2	4.615	0.032
Etoposide (800 μg / kg)	196	0	0	0	9	4.59	13.199	‹0.001
P. grandiflora extract, 10%	176	0	2	0	0	1.14	0.118	0.731
Alcohol, 70%	227	0	7	0	0	3.08	6.68	0.010
P. grandiflora extract, 10% + etoposide (800 μg / kg)	230	1	5	1	0	3.04	6.55	‹0.011

In addition, when using etoposide at the concentration of 800 μg / kg of nutrient medium, a large number of non-characteristic mutant recessive phenotypes was recorded that resulted from the pseudodominance phenomenon, which indirectly demonstrates the effect of this cytostatic agent on the frequency of chromosomal aberrations. According to A.N. Sortibran et al., an increase in chromosomal rearrangements does indeed take place [24].

When the extract and etoposide were used together, no change was found in the genotoxic properties of etoposide in relation to the frequency of spot occurrence, however, the antigenotoxic effect was recorded in relation to the frequency of chromosomal aberrations. Therefore, it can be asserted that the extract is selective for the genotoxic properties of etoposide. Since extracts of other medicinal plants were used both in high and low concentrations for the manifestation of antigenotoxic properties [25], it seems reasonable to test this hypothesis with respect to the *P. grandiflora* extract used in this study.

CONCLUSION

The discovery of a positive effect of the 10% *P. grandiflora* extract on antigenotoxicity, overall lethality, and viability of the Oregon-R *D. melanogaster* individuals exposed to etoposide and the extract makes further testing of this extract reasonable. The data obtained allow to consider the possibility of using the *P. grandiflora* extract as a component in the diet of patients undergoing certain therapeutic treatment.

REFERENCES

1. Burkhart J., Walchli C., Heusser P., Weissenstein U., Baumgartner S., Andres A. *In vitro* investigation into the potential of a mistletoe extract to alleviate adverse effects of cyclophosphamide. *Altern. There Health Med.* 2010; 16 (3): 40–48.
2. Liang C., Pan H., Li H., Zhao Y., Feng Y. *In vitro* anticancer activity and cytotoxic screening of phytochemical extracts from selected traditional Chinese medical plants. *J. Buon.* 2017; 22 (2): 543–551.
3. Siew Y.Y., Yew H.C., Neo S.Y., Seow S.V., Lew S.M. Evaluation of anti-proliferative activity of medicinal plants used in Asian traditional medicine to treat cancer. *J. Ethnopharmacol*. 2019; 235: 75–87. DOI: 10.1016/j.jep.2018-12.040.
4. Daddan J.R., Sreenivasulu B., Umanahesh K., Peddanna K., Rao D.M. *In silico* studies on anti-stress compounds of ethanolic root extract of *Hemidesmus indicus* L. *Curr. Pharm. Biotechnol.* 2020; 21 (6): 502–515. DOI: 10.2174/1389201002166619211152754.
5. Chen Y.H., Guo Q.S., Wang C.Y. Textual research on change of medicinal parts and herbal medicine of *Prunella vulgaris*. *J. Chin. Mater. Med*., 2010; 35 (2): 242–246. DOI: 10.4268/cjcmm20100228.
6. Feng L., Jia X., Zhu M-M., Chen Y., Shi F. Antioxidant activities of total phenols of *Prunella vulgaris* L. *in vitro* and in tumor – bearing mice. *Molecules.* 2010; 15 (12): 9145–956. DOI: 10.3390/molecules15129145.
7. Mrudula G., Mallikarjuna Rao P., Jayaveera K.N., Arshad M.D., Rehmann S.A. Antistress and antioxidant effects of *Prunella vulgaris* leaves. *Pharmacologyonline.* 2010; 2: 952–962.
8. Dmitruk S.I. Anti-inflammatory properties, antibacterial and antifungal activity of the extract from the aerial part of *Prunella vulgaris* L. *Plant resources*. 2001; 35 (4): 92–97 (in Russ.).
9. Dmitruk S.I., Dmitruk V.S. Technological factors and pharmacological properties of *Prunella vulgaris* L. extract. *Bulletin SB RAMS*. 2001; 21 (3): 36–39 (in Russ.).
10. Feng L., Jia X., Shi F., Chen Y. Identification of two polysaccharides from *Prunella vulgaris* L. and evaluation on their anti – lung adenocarcinoma activity. *Molecules.* 2010; 15 (8): 93–103. DOI: 10.3390/molecules15085093.
11. Gao W., Liang H., Li Y., Liu Y., Xu Y. Root extract of *Prunella vulgaris* inhibits *in vitro* and *in vivo* carcinogenesis in MCF-5 human breast carcinoma cells via suppression of angiogenesis, induction of apoptosis, cell cycle arrest and modulation of PI3K/AKT signalling pathway. *JBUON.* 2019; 24 (2): 549–554.
12. Pluhatar Yu.V., Shevchuk O.M., Logvinenko L.A. Species of the genus Prunella L. as sources of valuable biologically ac-

tive substances. *Agrarian Bulletin of the Urals*. 2017; 8 (162): 37–45 (in Russ.).
13. Shamilov A. A. Plants of the genus Prunella: chemical composition, species pharmacological action. *Proceedings of Voronezh State University, Series: Chemistry. Biology. Pharmacy*, 2017; 4: 152–160 (in Russ.).
14. Volodin V.V., Gruzdev B.I., Martynenko V.A., Kanev V.A. Plants-producers of the main classes of biologically active substances. Syktyvkar: Komi Republic printing house, 2014: 206 (in Russ.).
15. Tsurkan A.A., Golembiovskaya E.I. Study of lipophilic substances of the common self-heal (*Prunella vulgaris* L.). Development, research and marketing of new pharmaceutical products. Collection of scientific papers; Pyatigorsk, 2013; 68: 217–19 (in Russ.).
16. Plant resources of Russia: wild flowering plants, their component composition and biological activity. A.L. Budantsev, editor. SPb.; M.: KMK; 2011: 4: Families of *Caprifoliaceae – Lobeliaceae*. 236–239 (in Russ.).
17. Shamilov A.A., Arlt A.V., Ivashev M.N. Activity of extracts from the large-flowered self-heal herb in hypoxic hypoxia. *International Journal of Applied and Fundamental Research*, 2013; 5: 132–33 (in Russ.).
18. Alekseeva L.I., Bolotnik E.V. Rosmarinic acid and antioxidant activity of *Prunella grandiflora* and *Prunella vulgaris* (Lamiaceae). *Plant World of Asian Russia*. 2013; 1 (11): 121–125 (in Russ.).
19. Makino T., Ono T., Muso E., Yoshida H., Honda G., Sasayama S. Inhibitory effects of rosmarinic acid on the proliferation of cultured murine mesangial cells. *Nephrol. Dial. Transplant.* 2000; 15 (8): 1140–145. DOI: 10.1093/ndt/15.8.1140.
20. Patrick P., Kalidas S. Inhibitory effects of rosmarinic acid extracts on porcine pancreatic amylase *in vitro. Asia Pacific J. Clin. Nutr*. 2004; 13 (1): 101–106.
21. Lu Y., Foo L.Y. Polyphenolics of *Salvia* – a review. *Phytochem.* 2002; 59 (2): 117–140. DOI: 10.1016/s0031-9422(01)00415-0.
22. Antosyuk O.N., Gorskaya A.V., Evaluation of the genetic activity of drugs used in antitumor therapy using the example of *Drosophila melanogaster*. *Russian Journal of Biopharmaceuticals*. 2020; 12 (1): 50–56 (in Russ.). DOI: 10.30906/2073-8099-2020-12-1-50-56.
23. Frei H., Wurgler F.E. Induction of somatic mutation and recombination by four inhibitors of eucoriotic topoisomerases assayed in the wing spot test of *Drosophila melanogaster*. *Mutagenesis.* 1996; 11 (4): 315–325. DOI: 10.1093/mutage/11.4.315.
24. Sortibran A.N., Tellez M.G., Rodrigeres-Arnaiz R. Genotoxic profile of inhibitors of topoisomerases I (campothehin) and II (etoposide) in a mitotic recombination and sex-chromosome loss somatic eye assay of *Drosophila melanogaster. Mutat. Res*. 2006; 604 (1-2): 83–90. DOI: 10.1016/j.mrgentox.2006.01.003.
25. Shahani S., Rostamnezhad M., Ghaffapi-rad V., Gasemi A., Pourfallah T.A., Hosseinimehr S.J. Radioprotective effect of Achillea millefolium L. against genotoxicity, induced by ionizing radiation in human normal lymphocytes. *Dose Response.* 2015; 13 (1): 1559325815583761. DOI: 10.1177/1559325815583761.

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Medical Center for Remote Monitoring of COVID-19 patients: organization experience and efficiency assessment

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ABSTRACT

Aim. To summarize the experience of organizing and evaluating the efficiency of the Medical Center for Remote Monitoring of patients with COVID-19 and community-acquired pneumonia in Tomsk.

Materials and methods. The project of the Medical Center for Remote Monitoring was developed on the basis of data from theoretical and empirical analyses of the current state of the healthcare system in the Tomsk region and the epidemiological situation with regard to COVID-19. The Center efficiency was assessed based on the analysis of quantitative and time indicators of the load on the emergency medical service and outpatient service. Statistical processing of the results was carried out using the Statistica 12.0 software package.

Results. On October 20, 2020, in Tomsk, on the premises of the Emergency Medical Unit,, a Medical Center for Remote Monitoring was established. It was aimed at providing remote consultations for patients with COVID-19 and community-acquired pneumonia. According to its algorithm, operators of the Center assessed a patient's condition on a point scale using standardized speech modules to make a decision on the tactics of their support. During the second wave of COVID-19, when the Center operated, a statistically significant decrease in the load on the ambulance service (average number of calls per day, average waiting time for all calls and coronavirus-related calls) as well as on the outpatient service (average number of house calls per day, including calls for acute respiratory diseases) was registered.

Conclusion. The work of the Medical Center for Remote Monitoring based on the described model is associated with a decrease in the load on the healthcare system in an unfavorable epidemiological situation due to high COVID-19 incidence. This experience can be spread to other regions of Russia and adapted for other categories of citizens.

Key words: telemedicine technologies, remote monitoring, new coronavirus infection, COVID-19, lean technologies, effectiveness, emergency medical care, outpatient service.

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Медицинский центр удаленного мониторинга пациентов с COVID-19: опыт организации и оценка эффективности

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РЕЗЮМЕ

Цель. Обобщение опыта организации и оценка эффективности деятельности Медицинского центра удаленного мониторинга (далее Центр) для пациентов с COVID-19 и внебольничными пневмониями в г. Томске.

Материалы и методы. Разработка проекта Центра осуществлялась на основе данных теоретического и эмпирического анализа текущего состояния системы здравоохранения Томской области и эпидемиологической ситуации по COVID-19. Эффективность деятельности подразделения оценивалась по результатам анализа количественных и временных показателей нагрузки на систему оказания скорой медицинской помощи и амбулаторно-поликлиническую службу. Статистическая обработка результатов проводилась с помощью пакета прикладных программ Statistica 12.0.

Результаты. В г. Томске на базе ОГАУЗ «Станция скорой медицинской помощи» 20.10.2020 организован Центр, алгоритмом работы которого предусмотрена оценка операторами состояния пациента по балльной шкале с использованием стандартизованных речевых модулей для принятия решения о тактике его сопровождения. На фоне работы подразделения в период «второй волны» COVID-19 зарегистрировано статистически значимое снижение уровня показателей нагрузки на систему оказания скорой медицинской помощи (среднее количество обращений в сутки, среднее время ожидания по всем вызовам и вызовам, связанными с COVID-19, и амбулаторно-поликлиническую службу (среднее количество вызовов на дом в сутки, в том числе по поводу острых респираторных заболеваний).

Заключение. Работа Центра по представленной модели ассоциирована со снижением нагрузки на систему здравоохранения в период неблагоприятной эпидемиологической ситуации по COVID-19. Данный опыт может быть распространен на другие регионы России и адаптирован под иные категории граждан.

Ключевые слова: телемедицинские технологии, удаленный мониторинг, новая коронавирусная инфекция, COVID-19, бережливые технологии, эффективность, скорая медицинская помощь, амбулаторно-поликлиническая служба.

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

Active implementation of telemedicine has been one of the significant global trends in healthcare development in recent years. The new coronavirus (COVID-19) pandemic has given a new impetus to the use of remote technologies in the field of health protection, which came to be seen not only as an auxiliary tool to reduce financial and time costs of medical care provision and increase its accessibility for residents of remote areas, but also as an integral part of comprehensive measures to combat COVID-19, contributing to social distancing and prevention of cross-infection, reducing the load on the healthcare system, and saving personal protective equipment [1–3].

An all-round revision of organizational approaches to medical care delivery, involving the expansion of existing and the launch of new telemedicine programs, affected the primary healthcare system, the system of specialty care, and the work of palliative care institutions. One of the examples may be experience of Toronto Cardiac Clinic (Canada), where an expanded remote monitoring program for heart failure patients was launched in March of 2020. It intended to replace the majority of face-to-face meetings with a doctor with video conversations or phone calls and to use the Medly mobile application for remote clinical support [4]. Other examples include restructuring of the telemedicine program at the Federico II University Hospital of Naples (Italy), where 75% of outpatient visits of patients with chronic liver diseases were replaced with remote appointments during the lockdown [5], and provision of online antenatal care in China [6].

In the context of the pandemic, the care for COVID-19 patients has become an independent field of application of remote monitoring technologies. However, insufficient knowledge about the clinical and epidemiological aspects of COVID-19, nonexistence of procedures for handling such patients remotely and apparent resource constraints have set a challenging task for public health officials – to develop effective solutions from scratch by trial and error. In the absence of clear regulation, the implemented activities differed significantly with respect to the groups of patients, the tools used, and the categories of medical professionals involved. These activities encompassed opening telemedicine centers for COVID-19 treatment and consulting patients with negative PCR test results [7], COVID-19 virtual monitoring clinics for obstetric patients [8]; organizing of centralized hotlines for screening of patients with suspected COVID-19 [9]; setting up telerehabilitation units for patients who have had this infection [10].

Now Russia has already gained significant experience in using remote technologies to combat COVID-19. At the beginning of the pandemic, according to the requirements specified in the Order of the Ministry of Health of the Russian Federation "On the temporary procedure for organizing the work of medical organizations in order to implement measures to prevent and reduce the risks of spread of the new coronavirus infection COVID-19", which is a key industry document governing medical care delivery in the conditions of the COVID- 19 spread [11], remote consultation centers for anesthesiology and resuscitation were established for adults and children. They operated in the doctor-doctor format both at the federal and regional levels.

It is worth noting that until November 23, 2020, this order did not regulate the issues of remote medical care delivery directly to patients with the new coronavirus infection, i.e. in the doctor-patient format. For this reason, relevant units were established in the regions and functions were assigned to them taking into account solely regional specifics, such as resource constraints; the existing experience in using remote monitoring technologies and the general level of computerization of the industry; the existing system of interaction between healthcare organizations and laboratories, territorial bodies of the Russian Federal Service for Surveillance on Consumer Rights Protection and Human Well-Being (Rospotrebnadzor), and the Statutory Health Insurance Fund, etc.

Taking into account the ongoing pandemic and the risks of such epidemiological threats in the future, the analysis of unique regional practices and foreign experience of using remote monitoring technologies for COVID-19 patients seems to be a relevant area of research, the results of which can serve as the basis for development of appropriate guidelines and operating procedures both at regional and federal levels. The Tomsk region was one of the first to organize centralized remote care for this category of patients.

The aim of this article was to summarize the experience of organizing a Medical Center for Remote Monitoring of patients with COVID-19 and community-acquired pneumonia on the territory of Tomsk and assess its effectiveness.

MATERIALS AND METHODS

The information base used for the development of the basic provisions for the Medical Center for

Remote Monitoring of patients with COVID-19 and community-acquired pneumonia (hereinafter referred to as the Center) encompassed the data derived from theoretical and empirical analyses of the current state of the healthcare system in the Tomsk region and the existing epidemiological situation regarding the new coronavirus infection COVID-19.

The population of the Tomsk region as of 01.01.2020 accounted for 1,079,271 people, of which 597,819 people reside in Tomsk. At the time of the project launch, there were 72 state healthcare organizations in the region (69 of them were regionally governed), including 53 institutions in the city of Tomsk (50 of them were regionally governed). The capacity of outpatient organizations accounted for 16,816 visits per shift, the bed / population ratio was 85.4 per 10,000 people. In accordance with the requirements specified in the Order of the Ministry of Health of the Russian Federation "On the temporary procedure for organizing the work of medical organizations in order to implement measures to prevent and reduce the risks of spread of the new coronavirus infection COVID-19" and regional enactments approved on its basis in the Tomsk region, all the necessary measures were taken to adapt the medical infrastructure to the pandemic (re-profiling of hospitals, allocation of respiratory emergency medical teams (EMT) and individual medical workers in outpatient organizations to provide medical care for patients with symptoms of acute viral respiratory infections (AVRI)).

To assess the epidemiological situation regarding COVID-19, the research used data from "stopcoronavirus.rf", an official Internet resource designed to inform the population about the coronavirus infection (COVID-19). Effectiveness of the Center was assessed by analyzing quantitative performance indicators as well as by studying the dynamics in indicators of the load on the EMT and outpatient service.

Indicators were monitored in the period from 01.07.2020 to 19.01.2021 based on the operational data of the Healthcare Department of the Tomsk Region (HDTR) and subordinate healthcare institutions. Statistical processing of the research results was carried out using the Statistica 12.0 software package. Data are presented as the median and the interquartile range *Me* (Q_1; Q_3).

RESULTS

To assess the potential load on the healthcare system associated with the progression of the COVID-19 pandemic, we analyzed average monthly values of a daily increase in confirmed cases of the new coronavirus infection in the Tomsk region in the period from July 2020 to January 2021 (Fig. 1).

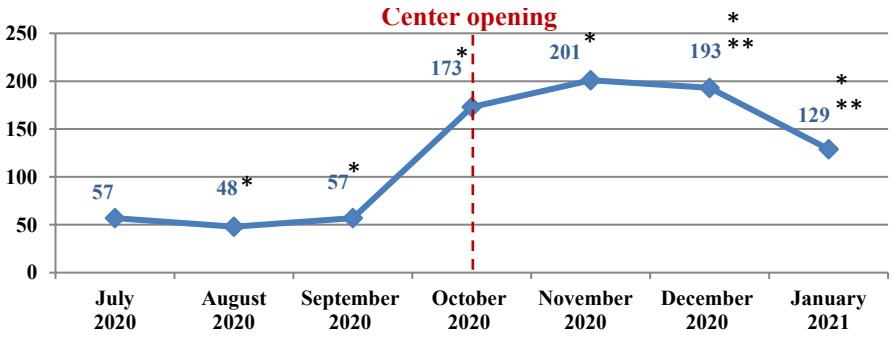

Fig. 1. Dynamics of average monthly values of the daily increase in confirmed cases of the new coronavirus infection in the Tomsk region: * $p < 0.0001$ in comparison with the previous month, ** $p < 0.0001$ in comparison with October 2020

The time trend of this parameter was characterized by rather low values in the summer months, an increase in September 2020 and a drastic increase in October 2020. The maximum values of the indicator were recorded in November 2020, after which the trend curve began to decline. All changes between the average levels of the time series were significant in contrast to the previous period ($p < 0.0001$). It is worth noting that, despite a significant decrease in the average number of infected people per day in December 2020 against November 2020, the December level remained significantly higher than the October one ($p < 0.0001$), i.e. in the first months after the opening, the Center operated in a worsening epidemiological situation regarding COVID-19, under the conditions of the so-called second wave of the new coronavirus infection.

For the Tomsk region and for most regions, the intensity of the second wave of COVID-19 jeopardized the stability of the healthcare system. Almost all areas

of work with citizens were on the verge of destabilization, from information support (HDTR regional hotline for AVRI, influenza, and the coronavirus infection operating in the region experienced serious overloads) to the emergency medical care delivery.

Despite the taken measures involving the engagement of medical workers and deployment of additional capacities to provide care for people with COVID-19, a drastic increase in the number of affected people provoked an obvious shortage of resources, which was determined by a rise in the number of COVID-19 cases among medical workers and their sickness leaves. The situation was aggravated by increased panic among the population due to the inability to reach medical organizations and increased waiting time for a doctor and EMT. Moreover, while in the districts of the Tomsk region, the problems were less pronounced due to the small size and low density of the population, in the regional center, the situation was critical and required immediate decisions, one of which was the establishment of the Center.

By the decision of the operational headquarters, the project for the establishment of the Center was developed urgently. The algorithm of work of the Center was approved by the order of the HDTR of 19.10.2020 No. 1085 "On the organization of work of the Medical Center for Remote Monitoring". The Center was set up on the premises of the Emergency Medical Unit (EMU) and started functioning on 20.10.2020.

The organizational structure of the Center included the Director, operators (graduate students of the medical university and resident doctors who performed the main scope of work on remote consulting of patients), and chief on-call doctors (mainly specialists over 65 years old as well as those with contraindications for working directly in medical organizations, whose main task was to provide methodological support for operators and advice for citizens who inquired about treatment issues, for example, to explain doctor's prescriptions, etc.). Everyone employed in the Center underwent advanced training in provision of medical care for COVID-19 patients at Siberian State Medical University in the amount of 36 hours. Based on the results of the first month of the Center's operation, the functionality of senior operators was added. Their duties included advising ordinary operators in case of difficulties, induction training of new employees, and writing internal reports on the activities of the unit.

The main task of the Center was to provide remote consultations for patients with COVID-19 and community-acquired pneumonia, who were already put under observation on an outpatient basis or in a day care hospital. It was done in order to reduce the load on polyclinics and EMT and to make resources available to serve patients in need of initial medical examination. Patients were informed about the activities of the Center by means of specially designed leaflets, containing contact information, reasons for contacting the Center, and advice on preparing for a remote consultation, as well as via the media and social networks.

The key reasons for contacting the Center included: deterioration of a patient's condition; the emergence of symptoms in asymptomatic patients; lack of face-to-face or remote monitoring of the patient's condition by a healthcare organization in which the patient was kept under observation. The internal processes of the Center were organized using lean management methods and tools, such as work standardization, visualization, and value stream mapping.

It is worth noting that the work of the operators was carried out in the medical information system of the Tomsk region (MIS TR): patient identification, creation of a medical record for the provided service "Consultation of the operator of the Medical Center for Remote Monitoring", which was also available to a doctor who followed up on a patient in the outpatient setting. That ensured the continuity of information between the Center and outpatient clinics.

Operation of the Center in a test format (the first week) proceeded during the daytime with a subsequent switch to a round-the-clock service. The main task of the operator was to assess the patient's condition in order to make a decision on further patient management tactics. The project team members developed speech modules for adults and children, that included grading of the severity of the patient's condition based on their inclusion in risk groups, complaints, and a number of objective parameters available for measurement by the patient independently (blood pressure level, respiration and heart rate, glucose meter readings in patients with diabetes mellitus, etc.).

So, with a total score of less than 7, the patient's condition was assessed as stable, and oral recommendations were given regarding continuation of the prescribed treatment. If there were questions not related to the clinical aspects of the patient's condition, for example, the need to obtain reference information on the rules of self-isolation, working hours of healthcare facilities in high alert mode, etc., the patient's call was forwarded to the HDTR hotline for AVRI, influenza, and coronavirus infection for informational support.

With a total score of 7–14, the patient management tactics involved transfer of the appeal to an outpatient clinic in order to organize a personal doctor's visit. The appeal was transferred by an operator in the medical information system of the Tomsk region (MIS TR). For this purpose, each polyclinic set up "virtual respiratory rooms" and appointed employees responsible for handling the transferred calls. During the period of the maximum incidence rate (from November 23, 2020), these appeals, according to the appropriate agreement, were transferred by the Center to the emergency medical service of a private organization (Medika–Tomsk LLC) and processed within two hours.

A total score of 15–26 in adults and 15–30 in children required transfer of the appeal to the EMU to organize a visit of the EMT and provide emergency medical care.

Regardless of the patient management option, the patient was informed about the need to contact the Center in case of deterioration of the general condition and if they had additional questions.

The general scheme of the Center's operation is shown in Fig. 2.

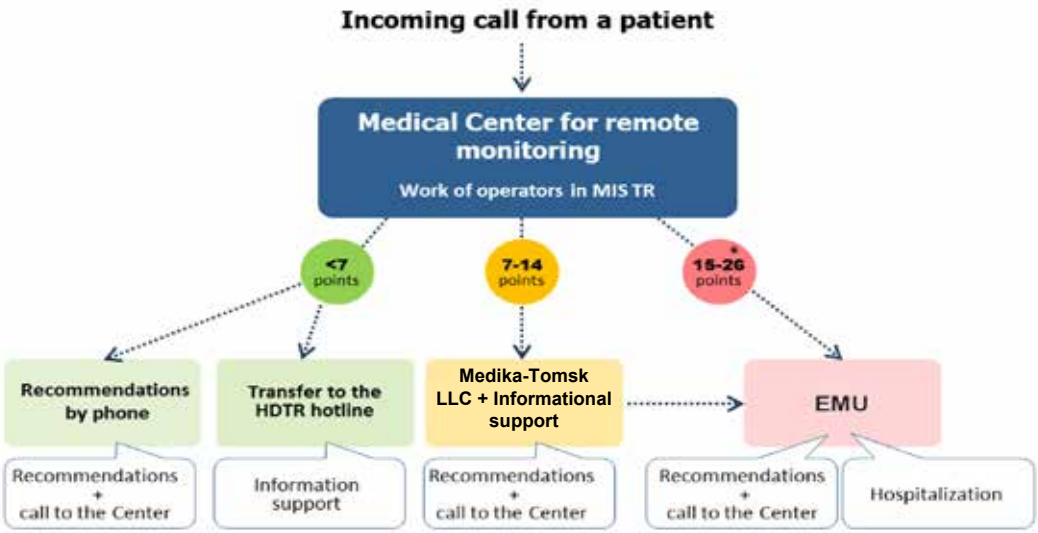

Fig. 2. The general scheme of the Center's operation

The control over the appeals transferred to outpatient clinics and EMU was carried out on a daily basis by a senior on-call doctor in the Center in the MIS TR. For each unhandled call, the operator made an outgoing call to the patient in order to clarify the severity of their condition and make a decision on patient management tactics in accordance with the above-described scoring system. The Director of the Center daily carried out a summary analysis of its performance and sent this information to the HDTR and the operational headquarters of the Tomsk region to ensure the ability to quickly respond to emerging problems and develop proposals for further improvement of the Center's performance.

Analysis of the Center's performance indicators. From 20.10.2020 to 19.01.2021, the Center received 70,883 calls. 94 calls were transferred to EMU, 1,514 calls – to outpatient clinics, and general recommendations were given during 69,275 calls. 4,801 calls in total (both during and after a remote consultation) were redirected to the HDTR hotline for AVRI, influenza, and coronavirus infection.

The average duration of one consultation in the test mode of the Center reached 11.05 (7.12; 15.08) minutes, and due to the measures implemented to achieve the target interaction between the Center and healthcare organizations, it decreased to 5.5 (4.95; 5.92) minutes by November 2020 and thereafter did not change significantly.

Reasons for contacting the Center were the following: deterioration of the condition – 7,936 calls (11.2%); a doctor failed to come / call – 3,577 calls (5.1%); asymptomatic COVID-19 course – 475 calls (0.7%); newly detected COVID-19 – 2,621 calls (3.7%); onset of symptoms in asymptomatic patients – 5,404 calls (7.6%); problems with a sickness leave certificate – 7,319 calls (10.3%); problems with the PCR test, including the inability to find out its results – 13,530 calls (19.1%); contact with a COVID-19

positive person – 4,966 calls (7.0%); other reasons – 25,055 calls (35.3%). The "other reasons" category included a wide range of issues not related to assessment of COVID-19 patient condition, such as receiving subsidized medicines; forced time extension for receiving high-tech medical care; the need for volunteer help; negative result of a COVID-19 PCR test for the employer; the possibility of receiving free drugs, etc.

Analysis of the load on the EMT. The data of the load parameter analysis on the EMT at the time of the Center's operation are set out in Table 1 and Figures 3 and 4.

Table 1

Dynamics of the load parameters on the EMT, *Me* (Q_1; Q_3)

Month, year	Number of EMT calls, units/day	Number of served EMT calls, units/day	Number of EMT calls transferred to the next day, units	Waiting time for all EMT calls, min.	Number of served calls related to COVID-19, units/day	Waiting time for EMT calls related to COVID-19, min.
July, 2020	1,044.00 (966.00; 1,129.00)	547.00 (522.00; 567.00)	0.00 (0.00; 2.00)	70.03 (56.03; 85.03)	104.00 (91.00; 113.00)	158.03 (102.03; 204.03)
August, 2020	974.00* (868.00; 1,053.00)	548.00 (528.00; 569.00)	0.00 (0.00; 0.00)	53.03* (41.03; 62.03)	88.00* (74.00; 97.00)	73.02* (63.03; 118.04)
September, 2020	1,176.50* (1,045.00; 1,295.00)	589.50* (558.00; 614.00)	0.00* (0.00; 24.00)	74.53* (51.03; 90.03)	130.00* (117.00; 145.00)	157.82* (68.25; 239.32)
October, 2020	1,857.00* (1,559.00; 2,035.00)	566.00 (548.00; 608.00)	187.00* (139.00; 208.00)	123.04* (101.03; 172.03)	192.00* (182.00; 209.00)	282.03* (216.03; 349.03)
November, 2020	1,225.00* (977.00; 1,618.00)	547.00 (523.00; 596.00)	31.00* (0.00; 164.00)	99.04* (68.03; 125.03)	193.50 (176.00; 204.00)	139.53* (95.04; 230.03)
December, 2020	942.00* (862.00; 982.00)	545.00 (517.00; 592.00)	0.00* (0.00; 0.00)	51.03* (38.03; 55.03)	171.00* (145.00; 185.00)	70.04* (46.03; 90.03)
January, 2021	994.00 (913.00; 1,083.00)	531.00 (520.00; 583.00)	0.00 (0.00; 0.00)	45.05 (37.05; 59.06)	124.00* (111.00; 133.00)	62.06 (40.05; 96.06)

* $p < 0.05$ in comparison with the previous month (here and in Table 2)

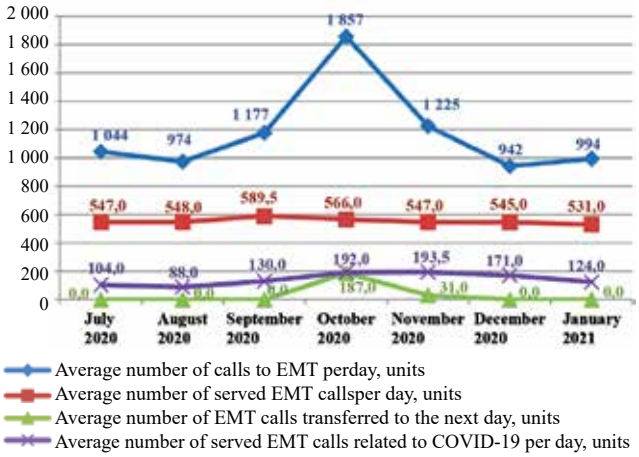

Fig. 3. Dynamics of quantitative indicators of the load on the EMT

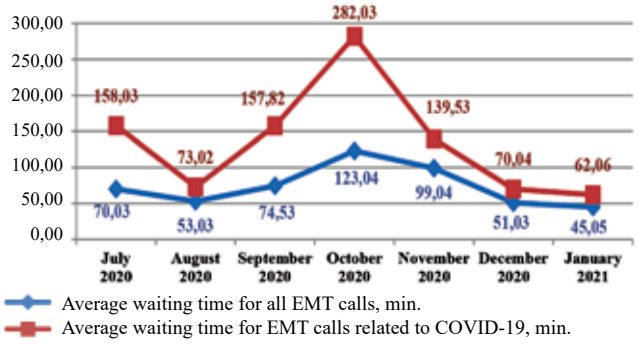

Fig. 4. Dynamics of time indicators of load on the EMT

The average number of calls to EMT per day in August 2020 significantly decreased as opposed to the previous month, and from September, there had been a significant parameter increment with a peak value in October 2020, which reflected a drastic increase in the load on the EMT caused by the second wave of the pandemic. However, in November and December 2020, a significant decrease in the average number of calls to EMT was recorded as opposed to the previous periods, despite the unfavorable epidemiological situation with the COVID-19 incidence. In January 2021, the parameter level stabilized, showing no sig-

nificant changes in comparison with December 2020. A significant decrease in the average number of calls beginning from November 2020 confirmed effective performance of the Center, which took upon itself the reception and processing of part of the calls, earlier received directly by the EMU.

The average number of served EMT calls per day during the researched period did not show statistically significant dynamics (except for a significant increase in September 2020 in comparison with the level in August 2020). No further parameter increment in the conditions of the deteriorating epidemiological situation as well as its subsequent decrease due to the opening of the Center can be explained by the limited maximum number of EMT on duty per day, related among other things to COVID-19 cases found among medical professionals of EMU during the peak of the pandemic. For example, throughout the study, the daily number of EMT on duty varied from 35 to 42.

During the period of active work of the Center (November 2020), there was also no significant decrease in the dynamics of the average number of served EMT calls related to COVID-19 per day for a similar reason. A subsequent significant decrease is mainly associated with a natural decrease in the load on the EMT due to a decline in the number of cases.

At the same time, the average number of EMT calls transferred to the next day, as an indicator of an obvious failure of the adaptive capabilities of the system under the conditions of the extreme load, significantly decreased during the first weeks of the Center performance (by November 2020) after a rise in the incidence rate in October 2020. Moreover, these calls have not been registered since December 2020.

The time parameters of the EMT work had big sensitivity in terms of reflecting the load on the EMT against the background of the Center operation. An average waiting time for all EMT calls after a significant increase in September and October 2020, compared to the previous months of the Center operation, significantly decreased in November and December of the same year, while the average number of COVID-19 cases continued to increase. A similar trend was observed in the analysis of the average waiting time for EMT calls related to COVID-19.

Analysis of the load on the outpatient clinics. The data of the analysis of the load on outpatient organizations against the background of Center operation are set out in Table 2.

Table 2

Dynamics of load parameters on the outpatient service, *Me* (Q_1; Q_3)

Month, year	Average number of house calls, units/day	Average number of house calls regarding acute respiratory diseases, units/day
July, 2020	1,186.00 (355.00; 1,280.00)	308.000 (117.00; 345.00)
August, 2020	1,060.00* (288.00; 1,191.00)	278.000 (125.00; 352.00)
September, 2020	1,391.50* (560.00; 1,571.00)	567.000* (282.00; 711.00)
October, 2020	2,562.00* (1,177.00; 2,965.00)	1,034.000* (557.00; 1,356.00)
November, 2020	2,249.00 (730.00; 2,465.00)	904.500 (365.00; 1,048.00)
December, 2020	1,723.00* (656.00; 1,900.00)	594.000* (272.00; 663.00)
January, 2021	708.00* (552.00; 1,449.00)	299.000* (228.00; 452.00)

Dynamics of the parameters of the load on the outpatient clinics as a whole showed the same trends as parameters of the load on the EMT, but without a significant difference between the levels in October and November 2020. This can be explained by the fact that in the first weeks of the Center operation, its activities were designed exclusively for providing remote consultations for patients with confirmed diagnosis of COVID-19 infection with clinical manifestations of the disease to determine patient management tactics. At the same time, a large number of visits to the outpatient clinics was associated with the need to monitor the condition of asymptomatic carriers and people after COVID-19 exposure. Transfer of these functions to the Center in November 2020 significantly reduced the burden of the outpatient clinics, which was confirmed by a significant decrease in the values of both analyzed parameters under the conditions of the increased COVID-19 incidence.

DISCUSSION

Performing a retrospective assessment of the experience in establishing the Center, the following factors can be pointed out that have made a significant contribution to its successful performance. First, the existence of a medical university in the region, which made it possible to urgently recruit the main team of operators from graduate students and resident doctors. Publicly available sources describe different approaches to staffing of such organizations: from engaging

volunteers [7] to official employment of specialists as medical personnel, which happened in many Russian regions [12–14].

The legal basis for the performance of duties by operators in the Tomsk region was a civil law contract. We believe that in the context of the rapidly growing pandemic and the need to quickly create a new structure, this legal basis is optimal. On the one hand, it provides legal guarantees for both parties in the contract. On the other hand, it allows for flexible management of the Center structure by changing the proportion of doctors and operators and the number of employees depending on top-priority tasks. The second factor was high level of IT development in the industry. The third factor was regional experience of using lean technologies in healthcare, which was repeatedly described in our previous articles [15–19]. The use of proven lean management tools made it possible to quickly set up and standardize the main processes of the Center already at the start of its work.

In the Tomsk region, the Center was organized on the premises of EMU. This site was selected due to suitable floor spaces and the ability to ensure prompt transfer of the most complicated calls requiring emergency medical care delivery. As alternative options for the placement of such structures, a Medical Prevention Center [12], a Physical Therapy and Sports Medicine Center [20], and a Clinical Hospital [13] can be considered.

In some regions of the Russian Federation, health authorities decided not to use the centralized format of organizing medical assistance for this category of patients using telemedicine technologies, developing them instead on the premises of all medical organizations providing primary health care [14]. Obviously, the issue of location does not have a universal solution and shall be resolved in each particular case individually, taking into account the regional specifics, such as available resources and epidemiological situation. In our opinion, centralization would be preferable if the region has a sufficient level of IT development and widespread implementation and sufficient functional capabilities of regional medical information systems. In addition to the traditional advantages, this approach is associated with resource saving and increased manageability and enables to create a single input channel for information about the most urgent problems arising in the process of medical and informational support of patients included in monitored groups. A detailed analysis of these groups can form the basis for both selective prompt response measures and long-term systemic improvements.

Despite the apparent obviousness, the issues of the effectiveness of such centers are not so unambiguous. In theory, introduction of a new structure and involvement of additional staff should relieve the existing healthcare system in the fight against COVID-19. However, the risks of the opposite effect are always high in reality: creation of an additional load on the primary care and the EMT system due to transfer of irrelevant cases (overestimation of the severity of the patient's condition, transfer of a call to an outpatient clinic for which only informational support is needed), lack of continuity in the transfer of information, and duplication of functions of the telemedicine center and outpatient clinics following an imperfect interaction scheme.

The analysis of the Center performance given in this article confirms its effectiveness both for reducing the load on the system of emergency medical care delivery and for optimizing the work of the outpatient service in the region. In addition to the general factors described above, a number of organizational decisions also contributed to the improvement of the Center performance.

As mentioned earlier, the vast majority of calls were processed by providing general recommendations on the phone, i.e. they did not require face-to-face contact between a patient and a medical professional, and the category "other calls" had the largest share in the structure of reasons to contact the Center. To ensure high-quality counseling by operators on this block of issues, the project team carried out a preliminary analysis of the most common reasons for contacting call centers of hospitals, EMU, or HDTR, which could potentially be processed by the Center. Response measures were thought out and appropriate scripts were developed for quick response to frequently asked questions.

As a temporary measure aimed at ensuring the availability of medical care during the periods of the most dramatic rise in disease incidence, handling of house calls was outsourced to private medical organizations. It is worth mentioning that the majority of the available scientific studies on the use of telemedicine technologies in relation to COVID-19 patients mention the clinical efficacy and safety of these tools [21] and describe individual quantitative indicators of work of such units [7, 8]. At the same time, there are practically no publications that would assess the systemic effects of their performance, which increases the value of our results.

In our opinion, the prospects for the Center operation are associated with the possibilities of its adaptation for remote monitoring of patients with chronic non-infectious diseases. Currently, the region is already conducting a comprehensive review involving the use of the resources of the Center for these purposes. Scientific substantiation and assessment of the effectiveness of the measures implemented in this area will be the object of our further research.

CONCLUSION

The conducted research shows a positive systemic impact provided by the Center activities, which is manifested through a decrease in the load on the primary health care system and EMT during the unfavorable epidemiological situation following high COVID-19 incidence. The experience of the Tomsk region in organizing a system for remote monitoring of patients with COVID-19 and community-acquired pneumonia can be recommended for use in other regions of the Russian Federation, taking into account the proven effectiveness. It is advisable to adapt the solutions used within the described project to deliver remote consultations to other categories of citizens.

REFERENCES

1. Baudier P., Kondrateva G., Ammi C., Chang V., Schiavone F. Patients' perceptions of teleconsultation during COVID-19: A cross-national study. *Technological Forecasting and Social Change*. 2021; 163: 120510. DOI: 10.1016/j.techfore.2020.120510.
2. Wong A., Bhyat R., Srivastava S., Lomax L.B., Appireddy R. Patient care during the COVID-19 pandemic: use of virtual care. *Journal of Medical Internet Research*. 2021; 23 (1): e20621. DOI: 10.2196/20621.
3. Hare N., Bansal P., Bajowala S.S., Abramson S.L., Chervinskiy S., Corriel R., Hauswirth D.W., Kakumanu S., Mehta R., Rashid Q., Rupp M.R., Shih J., Mosnaim G.S. Work group report: COVID-19: Unmasking telemedicine. *Journal of Allergy and Clinical Immunology: In Practice*. 2020; 8 (8): 2461–2473. DOI: 10.1016/j.jaip.2020.06.038.
4. Wali S., Margarido M.G., Shah A., Ware P., McDonald M., O'Sullivan M., Duero Posada J., Ross H., Seto E. Expanding telemonitoring in a virtual world:A case study of the expansion of a heart failure telemonitoring program during the COVID-19 pandemic. *Journal of Medical Internet Research*. 2021; 23 (1): e26165. DOI: 10.2196/26165.
5. Guarino M., Cossiga V., Fiorentino A., Pontillo G., Morisco F. Use of telemedicine for chronic liver disease at a single care center during the COVID-19 pandemic: Prospective Observational Study. *Journal of Medical Internet Research*. 2020; 22 (9): e20874. DOI: 10.2196/20874.
6. Wu H., Sun W., Huang X., Yu S., Wang H., Bi X., Sheng J., Chen S., Akinwunmi B., Zhang C.J.P., Ming W.K. Online antenatal care during the COVID-19 pandemic: opportunities and challenges. *Journal of Medical Internet Research*. 2020; 22 (7): e19916. DOI: 10.2196/19916.
7. Kim D.S., Chu H., Min B.K., Moon Y., Park S., Kim K., Park S.H., Kim Y.D., Song M., Choi G.H., Lee E. Telemedicine Center of Korean Medicine for treating patients with COVID-19: a retrospective analysis. *Integrative Medicine Research*. 2020; 9 (3): 100492. DOI: 10.1016/j.imr.2020.100492.
8. Krenitsky N.M., Spiegelman J., Sutton D., Syeda S., Moroz L. Primed for a pandemic: Implementation of telehealth outpatient monitoring for women with mild COVID-19. *Semin Perinatol*. 2020; 44 (7): 151285. DOI: 10.1016/j.semperi.2020.151285.
9. Crane S.J., Ganesh R., Post J.A., Jacobson N.A. Telemedicine consultations and follow-up of patients with COVID-19. *Mayo Clin. Proc*. 2020; 95 (9S): S33–34. DOI: 10.1016/j.mayocp.2020.06.051.
10. Iannaccone S., Castellazzi P., Tettamanti A., Houdayer E., Brugliera L., de Blasio F., Cimino P., Ripa M., Meloni C., Alemanno F., Scarpellini P. Role of rehabilitation department for adult individuals with COVID-19: the experience of the San Raffaele Hospital of Milan. *Arch. Phys. Med. Rehabil*. 2020; 101 (9): 1656–1661. DOI: 10.1016/j.apmr.2020.05.015.
11. On the temporary procedure for organizing the work of medical organizations in order to implement measures to prevent and reduce the risks of the spread of the new coronavirus infection COVID-19: Order of the Ministry of Health of the Russian Federation of March 19, 2020 No. 198n. URL: http://docs.cntd.ru/document/564482310 (access date: 18.02.2021) (in Russ.).
12. Order of the Moscow Department of Healthcare of April 6, 2020 No. 356 "On the use of telemedicine technologies when organizing consultations on the issues of the coronavirus infection COVID-19 and the selection of personnel for medical organizations in the city of Moscow." URL: http://docs.cntd.ru/document/564612722 (access date: 18.02.2021) (in Russ.).
13. Order of the Ministry of Health of the Sverdlovsk Region of April 16, 2020 No. 621-p "On the use of telemedicine technologies when organizing consultations on the issues of the coronavirus infection COVID-19" (as amended on December 4, 2020). URL: http://docs.cntd.ru/document/570743231 (access date: 18.02.2021) (in Russ.).
14. Order of the Health Committee of the Government of St. Petersburg of December 4, 2020 No. 876-r "On the organization of medical care delivery using telemedicine technologies to patients with the new coronavirus infection COVID-19, ARVI, influenza and community-acquired pneumonia in St. Petersburg." URL: http://docs.cntd.ru/document/573047265 (access date: 18.02.2021) (in Russ.).
15. Deev I.A., Boykov V.A., Kanonerker L.M., Kobyakova O.S., Kulikov E.S., Novikova I.Y., Osihov I.A., Sivolobova T.V., Taranov A.A., Titova M.A., Shibalkov I.P. Application of lean management in medical examination of children in Tomsk region. *Manager of Health Care*. 2019; (9): 30–36 (in Russ.).
16. Deev I.A., Kobyakova O.S., Shibalkov I.P., Protasova L.M., Boykov V.A., Baranovskaya S.V., Suvorova T.A., Babeshina M.A. Optimization of patient flow routing as a basis for improving organizational efficiency of outpatient care (experience of the Tomsk region). *The Siberian Journal of Clinical and Experimental Medicine*. 2020; 35 (4): 95–102 (in Russ.). DOI: 10.29001/2073-8552-2020-35-4-95-102.

17. Deev I.A., Kobyakova O.S., Boykov V.A., Shibalkov I.P., Baranovskaya S.V., Protasova L.M., Shnaider G.V., Suvorova T.A. Results of the Standard's implementation on organizing outpatient care delivery in the Tomsk region. *Social aspects of population health* [serial online]. 2020; 66 (6): 1. URL: http://vestnik.mednet.ru/content/view/1210/30/lang,ru/ (in Russ.) DOI: 10.21045/2071-5021-2020-66-6-1.
18. Suvorova T.A., Deev I.A., Kobyakova O.S., Boykov V.A., Baranovskaya S.V., Koshel A.P., Taukina N.V., Shibalkov I.P. Application of lean production tools in the organization of medical examinations of certain groups of the adult population. *Vestnik Roszdravnadzora*. 2020; 5 (2): 4–9 (in Russ.).
19. Deev I.A., Kobyakova O.S., Babeshina M.A., Baranovskaya S.V., Boykov V.A., Masunov V.N., Milkevich M.N., Rodionov N.V., Suvorova T.A., Taranov A.A., Shibalkov I.P. Applying lean manufacturing technologies to optimize ambulance operations amid the spread of COVID-19. *Manager of Health Care*. 2021; (1): 24–32 (in Russ.).
20. Order of the Department of Health of the Vladimir Region of November 13, 2020 No. 774 "On remote monitoring of the health status of patients with the new coronavirus infection COVID-19." URL: http://docs.cntd.ru/document/570985152 (access date: 18.02.2021) (in Russ.).
21. Martínez-García M., Bal-Alvarado M., Santos Guerra F., Ares-Rico R., Suárez-Gil R., Rodríguez-Álvarez A., Pérez-López A., Casariego-Vales E.; en nombre del Equipo de Seguimiento Compartido TELEA-COVID Lugo; Equipo TELEA COVID-19 (Lugo). Monitoring of COVID-19 patients by telemedicine with telemonitoring. *Rev. Clin. Esp*. 2020; 220 (8): 472-479. DOI: 10.1016/j.rce.2020.05.013.

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New experimental possibilities for statin-associated myopathy diagnosing

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ABSTRACT

Aim. To identify the relationships between structural proteins of myocytes, as well as indicators of antioxidant defense and metabolites of glycolysis against the background of using statins in animals in the experiment for clarifying the diagnosis of statin-associated myopathy.

Materials and methods. The study was conducted on outbred male rats, which were divided into 3 groups. The control group consisted of intact animals, and there were two experimental groups: group 1 – animals with induced hypercholesterolemia, group 2 – animals with induced hypercholesterolemia treated with simvastatin. In the muscles of animals from the studied groups, the content of structural proteins of the sarcomere, titin and nebulin, was analyzed, and the concentration of glycolysis metabolites (pyruvic acid and lactate) and the activity of antioxidant defense enzymes (superoxide dismutase (SOD) and catalase) were determined.

Results. The use of simvastatin in the animals led to a decrease in the content of NT- and N2A-titin isoforms and an increase in the content of the T2-proteolytic fragment. Complete absence of nebulin was also noted, which reflects the presence of dystrophic changes in the muscle tissue. Long-term administration of simvastatin caused metabolic changes in the rats, characterized by impaired supply of cells with molecular oxygen. However, as opposed to the animals with hypercholesterolemia that were not given statins, a decrease in hypoxia-induced shifts was observed. Abnormalities in the performance of the antioxidant defense (AOD) system and multidirectional changes in the activity of the antioxidant pair “SOD – catalase” were noted. The correlation analysis revealed a positive relationship between the content of the NT-titin isoform and the SOD activity and negative correlations between the content of the N2A-titin isoform and the level of lactate, as well as between the T2-proteolytic fragment of titin and the level of lactate.

Conclusion. The study revealed a complex set of biochemical changes in the muscles that underlie myotoxicity of statins during their long-term use. Additional biochemical parameters were found, such as SOD activity and lactate concentration, changes in which, along with the determination of titin and nebulin concentrations, indicating tissue hypoxia, will make it possible to more accurately diagnose statin-associated myopathy.

Key words: statins, statin-associated myopathy, simvastatin, hypercholesterolemia, skeletal muscle, titin, nebulin.

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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Новые возможности диагностики статиновой миопатии в эксперименте

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РЕЗЮМЕ

Цель. Выяснить взаимосвязи между структурными белками миоцитов, а также показателями антиоксидантной защиты и метаболитами гликолиза на фоне применения статинов у животных в эксперименте для уточнения постановки диагноза «статиновая миопатия».

Материалы и методы. Исследование проводилось на беспородных самцах крыс, которых разделили на три группы. Контрольная группа – интактные животные и две экспериментальные группы: группа 1 – животные с индуцированной гиперхолестеринемией, группа 2 – животные с индуцированной гиперхолестеринемией, получавшие симвастатин. В мышцах животных исследуемых групп был проведен анализ содержания структурных белков саркомера – титина и небулина, а также определена концентрация метаболитов гликолиза (пировиноградной кислоты и лактата) и активность ферментов антиоксидантной защиты (супероксиддисмутазы (СОД) и каталазы).

Результаты и обсуждение. Применение симвастатина у животных приводило к уменьшению содержания NT- и N2A-изоформ титина, увеличению содержания протеолитического фрагмента Т2, также отмечалось полное отсутствие небулина, что отражает наличие дистрофических изменений в мышечной ткани. Длительное введение симвастатина вызывало у крыс метаболические изменения, характеризующиеся нарушением обеспечения клеток молекулярным кислородом. Однако, по сравнению с животными с гиперхолестеринемией, которым статины не вводили, наблюдалось уменьшение гипоксических сдвигов. Отмечены нарушения в работе системы антиоксидантной защиты, разнонаправленные изменение активности антиоксидантной пары «СОД – каталаза». Проведенный корреляционный анализ выявил положительную зависимость между содержанием NT – изоформы титина и активностью СОД, отрицательные корреляционные зависимости между содержанием N2A-изоформы титина и уровнем лактата, Т2-протеолитического фрагмента титина и уровнем лактата.

Заключение. Выявлен сложный комплекс биохимических изменений в мышцах, лежащих в основе миотоксичности статинов при их длительном применении. Обнаружены дополнительные биохимические показатели – активность СОД и концентрация лактата, изменения которых, наряду с определением титина и небулина, свидетельствующие о гипоксическом повреждении ткани, позволят более точно диагностировать статиновую миопатию.

Ключевые слова: статины, статиновая миопатия, симвастатин, гиперхолестеринемия, скелетные мышцы, титин, небулин.

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

To date, statins are actively prescribed to patients with cardiovascular diseases as effective lipid-lowering agents [1]. However, the use of this group of drugs can cause statin-induced muscle damage – statin-associated myopathy, which can manifest itself in different ways: from mild myalgia to life-threatening rhabdomyolysis [2]. The development of muscle damage symptoms is the main reason for patients' refusing from statin therapy, and, according to some authors, their number reaches 75% within 2 years from the start of therapy [3].

The diagnosis of statin-associated myopathy is complicated by the fact that in some cases it is asymptomatic, or the symptoms are not pronounced and are mostly subjective. There is currently no universal diagnostic test that would make it possible to make such a diagnosis. One of the methods for diagnosing myopathies is to determine the activity of creatine phosphokinase (CPK) in the blood serum. A drastic increase in the CPK activity in blood is an indicator of damage to myocytes. However, often in statin-associated myopathy, an increase in its activity may either not be observed, or is insignificant [4]. Therefore, identification of additional biochemical parameters that would make it possible to increase the efficiency of diagnosing statin-associated myopathy is relevant.

Current literature provides experimental research data proving that the development of muscular dystrophy is accompanied by a decrease in the content of giant sarcomeric proteins titin and nebulin in myocytes due to their increased proteolysis. At the same time, the elasticity and contractility of muscle tissue decrease [5].

In our earlier studies, metabolic changes in erythrocytes and skeletal muscles of rats were presented against the background of prolonged use of simvastatin, which proved the prooxidant effect of statins.

The aim of the study was to identify the relationship between the structural proteins of myocytes, as well as indicators of antioxidant defense and metabolites of glycolysis against the background of statin use in animals in the experiment for clarifying the diagnosis of statin-associated myopathy.

MATERIALS AND METHODS

The study was carried out on outbred male rats aged 12–14 months and weighing 300–350g. The animals were kept in accordance with the Order of the Ministry of Healthcare of the Russian Federation No. 708N of 23.08.2010 "On approval of the rules of laboratory practice" and sanitary rules of the joint venture 2.2.1.3218-14 "Sanitary and epidemiological requirements to the equipment and maintenance of experimental biological clinics (vivariums) of 29.08.2014". During the experiment, the animals were divided into control and experimental groups.

The control group (35 rats) adhered to a standard vivarium diet for 3 months and received 0.5 ml of distilled water through the esophageal tube once a day.

The experimental groups consisted of 70 rats. Essential hypercholesterolemia was induced in these animals by feeding them with a diet rich in animal fats (ghee) and fast-digesting carbohydrates (cane sugar, semolina) for 3 months. It was diagnosed by the level of total cholesterol (CS) on the "Bayer" analyzer (Germany) after the specified period. Then these animals were divided into 2 equal groups. The animals of group 1 received food without added drugs for 2 months, and once a day they received 0.5 ml of distilled water through the esophageal tube. The animals of group 2, in contrast to group 1, received simvastatin (Zocor, 20 mg) once a day for 2 months, (0.0012 g / 100 g of body weight) in the form of an aqueous suspension through the esophageal tube.

The animals were eliminated from the experiment by decapitation. All manipulations were in compliance with generally accepted ethical standards (Protocol No. 21/15 of the Local Independent Ethics Committee of Rostov State Medical University of 10.12.2015).

Fragments of animal skeletal muscles were used for the study. A muscle tissue homogenate was prepared in the ratio of 1 g of tissue : 9 ml of chilled saline, centrifugation was carried out at 3,000 rpm, and the supernatant was used to determine the concentration of glycolysis metabolites and the activity of antioxidant enzymes.

The pyruvic acid (PVA) concentration was determined by the formation of a colored compound upon interaction with 2.4-dinitrophenylhydrazine [6]. The lactate concentration was determined by the color reaction of paraoxidiphenyl with acetaldehyde formed from lactate in the presence of sulfuric and phosphoric acids and copper ions [7]. The activity of superoxide dismutase (SOD) was determined by a method based on the ability of the enzyme to inhibit autooxidation of adrenaline in an alkaline medium at pH = 10.2 [8]. The catalase activity was determined by the decrease in the substrate – hydrogen peroxide per unit time – by the reaction with ammonium molybdate [9].

The study of the content of giant sarcomeric proteins (titin and nebulin) was carried out by sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE) with the addition of agarose according to R. Tatsumi, A. Hattori (1995) [10] modified by I.M.Vikhlyantsev, Z,A. Podlubnaya (2017) [11] at the Institute for Theoretical and Experimental Biophysics of the Russian Academy of Sciences (Pushchino).

Statistical processing of the obtained results was performed using the STATISTICA 10.0 and Excel Microsoft software. The significance of differences in the considered parameters of the compared groups was assessed using the Student's t-test after checking the distribution for normality using the Shapiro – Wilk test. When conducting the correlation analysis, the

parametric Pearson's coefficient was used, since the samples were in compliance with normal distribution. Differences were considered statistically significant at $p \leq 0.05$. Data are presented as the mean and the standard error of the mean ($M \pm m$).

RESULTS AND DISCUSSION

Keeping animals of the experimental groups on a diet enriched with animal fats and carbohydrates led to a statistically significant increase in cholesterol levels compared to the control group. Administration of simvastatin to the animals of group 2 for two months contributed to a decrease in the level of cholesterol in the blood serum to 1.637 mmol / l, which did not differ significantly from the values in the control group.

To assess the structural and functional state of the skeletal muscles of the animals that were injected with simvastatin for a long time, changes in the qualitative and quantitative composition of titin and nebulin in *m. biceps* of animals of the studied groups were studied (Table 1). The levels of titin and nebulin were evaluated in relation to the content of myosin heavy chains.

Table 1

Changes in the content of titin and nebulin in the muscle tissue of animals in the studied groups, $M \pm m$

Groups Parameters	Control group, $n = 35$	Experimental group
		group 2, $n = 35$
T2-fragment	0.113 ± 0.002	0.137 ± 0.0024 $p < 0.001$
N2A-isoform	0.136 ± 0.002	0.094 ± 0.0025 $p < 0.001$
NT-isoform	0.026 ± 0.0015	0.016 ± 0.0017 $p < 0.001$
Nebulin	0.031 ± 0.0023	Absent

Note. p – significance level of differences relative to the parameters in the control group.

The levels of titin and nebulin in *m. biceps* of the animals in group 1 did not differ significantly from those in the control group. At the same time, studies showed that long-term use of simvastatin in the animals with induced hypercholesterolemia caused a decrease in the content of the NT-titin isoform by 38.46% ($p < 0.001$) and N2A-titin isoform by 30.88% ($p < 0.001$). In addition, against the background of simvastatin use in the animals, an increase in the level of the T2-proteolytic fragment by 1.2 times was noted in their muscle tissue, as well as complete absence of nebulin relative to the animals that did not receive the drug.

The obtained results are in line with the studies of modern scientists who prove that myopathies of various origins are accompanied by a decrease in the content of structural proteins of the sarcomeric cytoskeleton, such as titin (primarily its NT-isoforms) and nebulin, and, as a consequence, lead to deterioration of contractile properties of the muscles [5]. Based on this, it can be assumed that a decrease in the level of titin and nebulin with prolonged use of simvastatin is an indicator that reflects the presence of degenerative processes in the muscle.

To elucidate the nature of myotoxicity of statins, an analysis of the parameters of energy metabolism and the activity of antioxidant defense enzymes in the muscle tissue of the animals in the studied groups was carried out. Glycolysis metabolites – pyruvic acid and lactate – are indicators of the efficiency of molecular oxygen supply to the cells. As a result of the study, in the muscles of the animals in group 1, an increase in the concentration of lactate by 73.23% ($p < 0.001$) and a significant increase in the concentration of pyruvic acid by 247.11% ($p < 0.001$) were noted relative to the control group (Table 2).

It can be assumed that the increase in the concentration of glycolysis metabolites may be associated with an excess of fast-digesting and rapidly oxidized carbohydrates in the diet of the rats in this group. At the same time, a very high level of lactate indicates development of tissue hypoxia, which triggers a cascade of metabolic disorders in the muscle tissue of the animals. According to D.A. Kruse (1997), a drastic increase in the concentration of lactate can serve as a marker of the pathological process severity [12].

Table 2

Levels of glycolysis metabolites in the muscle tissue of animals in the studied groups, $M \pm m$

Groups Parameters	Control group, $n = 35$	Experimental group	
		group 1, $n = 35$	group 2, $n = 35$
Lactate (μmol / ml protein)	3.96 ± 0.447	6.86 ± 0.657 $p < 0.001$	4.64 ± 0.491 $p > 0.05$ $p_1 < 0.01$
Pyruvic acid (μmol / ml protein)	2.25 ± 0.024	7.81 ± 0.570 $p < 0.001$	3.28 ± 0.269 $p < 0.001$ $p_1 < 0.001$

Note. p – significance level of differences relative to the parameters of the control group; p_1 – significance level of differences relative to the parameters of group 1.

In the group of animals with induced hypercholesterolemia against the background of simvastatin use (group 2), we observed a decrease in the concentration of lactate by 32.36% ($p_1 < 0.01$) and a fall in the level of pyruvic acid by 58.0% ($p_1 < 0.001$), respectively, as opposed to the animals

in group 1. Relative to the control group, the concentration of pyruvic acid increased by 45.77% ($p < 0.001$), and there was also a slight increase in the concentration of lactate by 17.17% ($p > 0.05$).

Based on the obtained data, it can be concluded that in the animals of group 2, a tendency to normalization of carbohydrate metabolism was observed against the background of simvastatin use, compared with the animals of group 1. However, compared with the animals of the control group, the concentration of glycolysis metabolites still remained elevated. On the one hand, preservation of an increased level of glycolysis metabolites may reflect the inferiority of adaptive mechanisms and require additional measures aimed at reducing hypoxia-induced shifts. On the other hand, attention is drawn to the pronounced increase in the level of pyruvic acid, as opposed to the lactate concentration. According to the experimental data of Z.I. Mikashinovich (1989), an increase in the level of pyruvic acid under conditions of hypoxia improves microcirculation in tissues and contributes to a decrease in pathobiochemical shifts [13].

SOD and catalase play an important role in the antioxidant defense of almost all cells in the body, including myocytes. As a result of the study, in the group of animals with induced hypercholesterolemia, an increase in the catalase activity by 82.66% ($p < 0.001$) was noted, and the SOD activity did not significantly change relative to the control group (Table 3).

Table 3

Activity of SOD and catalase in the muscle tissue of animals in the studied groups, $M \pm m$

Groups Parameters	Control group, $n = 35$	Experimental group	
		group 1, $n = 35$	group 2, $n = 35$
Superoxide dismutase [conv. unit / mg protein]	0.446 ± 0.049	0.500 ± 0.046 $p > 0.05$	0.219 ± 0.024 $p < 0.001$ $p_1 < 0.001$
Catalase [mKat / mg protein]	1.494 ± 0.211	2.729 ± 0.162 $p < 0.001$	2.786 ± 0.438 $p < 0.001$ $p_1 > 0.05$

Note. p – significance level of differences relative to the parameters of the control group; p_1 – significance level of differences relative to the parameters of group 1.

The revealed increases in catalase activity in the animals with hypercholesterolemia with unchanged SOD activity, according to O.I. Dotsenko et al., are possibly associated "with the activation of peroxisomal reactions or other parallel processes that require increased catalase activity" [14]. Administration of simvastatin to the animals with experimental hypercholesterolemia contributed to a decrease in the SOD activity by 56.2% ($p_1 < 0.001$), while the catalase activity remained practically unchanged relative to the parameters of the animals that did not receive simvastatin. Relative to the parameters of animals in the control group, a significant decrease in the SOD activity by 50.89% ($p < 0.001$) was revealed, and the activity of catalase increased by 86.47% ($p < 0.001$).

According to the literature, such a multidirectional change in the activity of the antioxidant pair "SOD – catalase" is also a sign of hypoxia development in the muscle tissue [14]. The antioxidant pair "SOD – catalase" is characterized by cross regulation, when high activity of one of the enzymes inhibits the activity of the other by the feedback mechanism. A decrease in the SOD activity poses a potential danger of oxidative damage to the most important macromolecules, due to a decrease in disproportionation of the superoxide anion radical and its transformation into a more aggressive hydroxyl radical by the Haber – Weiss reaction.

A correlation analysis was carried out to reveal the relationship between the parameters of carbohydrate energy metabolism, the activity of antioxidant defense enzymes, and giant sarcomeric proteins (titin and nebulin) in the muscle tissue of animals with induced hypercholesterolemia against the background of long-term use of simvastatin. As a result, a positive correlation was found between the content of the NT-titin isoform and the SOD activity, $r = 0.34$, $p = 0.05$ ($p \leq 0.05$). Negative correlations were also noted between the content of the N2A-titin isoform and the level of lactate, $r = –0.35$, $p = 0.04$ ($p \leq 0.05$), and between the content of the T2-proteolytic fragment of titin and the level of lactate, $r = –0.35$, $p = 0.04$ ($p \leq 0.05$).

CONCLUSION

According to the literature, titin, a giant protein of the sarcomeric cytoskeleton, is a template for correct assembly of proteins [15]. The study showed that the use of simvastatin in animals with induced hypercholesterolemia leads to a decrease in the content of giant proteins – titin and nebulin – in myocytes due to their increased proteolysis. As a result of degradation of structural proteins, a decrease in the contractile ability of skeletal muscles occurs, which is observed in pathological conditions, such as muscular dystrophies [16]. On this basis, the levels of titin and nebulin in the muscle tissue can be used as an early marker of statin-associated myopathy.

The analysis of energy metabolism parameters and their relationship with the dynamics of titin isoforms in animals after prolonged administration of simvastatin made it possible to determine a number of indicators that informatively reflect destructive changes in the muscle tissue. The revealed additional biochemical indicators, such as the SOD activity and the lactate level, along with the determination of titin and nebulin concentrations, indicating hypoxic processes in tissues, will make it possible to more accurately diagnose statin-associated myopathy.

REFERENCES

1. Aleksanyan L.A., Silina E.G. Statins and the "burden" of civilization: proven benefits in atherosclerotic diseases. *Russian medical journal.* 2011; 19 (1): 186–190 (in Russ.).
2. Arora R., Liebo M., Maldonado F. Statin-induced myopathy: the two faces of Janus. *J. Cardiovasc. Pharmacol. Ther.* 2006; 11 (2): 105–112. DOI: 10.1177/1074248406288758.
3. Stroes E.S., Thompson P.D., Corsini A., Vladutiu G.D., Raal F.J., Ray K.K., Roden M., Stein E., Tokgözoğlu L., Nordestgaard B.G., Bruckert E., Becker G.D., Krauss R.M., Laufs U., Santos R.D., Hegel R.A., Hovingh G.K., Leiter L., Mach F., März W., Newman C.B., Wiklund O., Jacobson T.A., Catapano A.L., Chapman M.J., Ginsberg H.N., European Atherosclerosis Society Consensus Panel. Statin-associated muscle symptoms: impact on statin therapy-European Atherosclerosis Society Consensus Panel Statement on Assessment, Aetiology and Management. *Eur. Heart J.* 2015; 36 (17): 1012–1022. DOI: 10.1093/eurheartj/ehv043.
4. Law M., Rudnicka A.R. Statin safety: a systematic review. *Am. J. Cardiol.* 2006; 97 (8A): 52–60. DOI: 10.1016 / j. amjcard.2005.12.010.
5. Zinovyeva O.E., Samkhaeva N.D., Vikhlyantsev I.M., Ulanova A.D., Mikhailova G.Z., Shcheglova N.S., Kazakov D.O., Emelyanova A.Yu., Nosovsky A.M. Change in the content of titin and nebulin and their phosphorylation level in the quadriceps femoris muscle in chronic alcoholic myopathy. *Neurology, Neuropsychiatry, Psychosomatics.* 2019; 11 (1): 21–27 (in Russ.). DOI: 10.14412/2074-2711-2019-1-21-27.
6. Kamyshnikov V.S. Handbook of clinical and biochemical studies and laboratory diagnostics. 2nd ed., rev. and add. M.: MEDpress-inform, 2004: 911 (in Russ.).
7. Danilova L.A., Basharina O.B., Krasnikova E.N., Litvinenko L.A., Ramenskaya N.P., Fomenko M.O., Mashek O.N. Handbook of laboratory research. SPb.: Peter, 2003: 733 (in Russ.).
8. Gurevich V.S., Kontorshchikova K.N., Shatilina L.V. Comparative analysis of two methods for determining the activity of superoxide dismutase. *Laboratory Work*. 1990; (4): 44–47 (in Russ.).
9. Korolyuk M.A., Ivanova L.I., Mayorova I.G., Tokarev V.E. Method for determination of catalase activity. *Laboratory Work*. 1988; (1): 16–19 (in Russ.).
10. Tatsumi R., Hattori A. Detection of giant myofibrillar proteins connectin and nebulin by electrophoresis in 2% polyacrylamide slab gels strengthened with agarose. *Anal. Biochem.* 1995; 224 (1): 28–31. DOI: 10.1006/abio.1995.1004.
11. Vikhlyantsev I.M., Podlubnaya Z.A. Nuances of electrophoresis study of titin/connectin. *Biophys. Rev.* 2017; 9 (3): 189–199. DOI: 10.1007/s12551-017-0266-6.
12. Kruze D.A. Clinical significance of the determination of blood lactate. *Anesthesiology and Reanimatology.* 1997; (3): 77–83 (in Russ.).
13. Mikashinowich Z.I. General and particular patterns of metabolic changes in the endocrine organs and blood with varying severity of traumatic shock and acute blood loss: Dis. ... Dr. Biol. Sci. Rostov-on-Don, 1989: 409 (in Russ.).
14. Dotsenko O.I., Dotsenko V.A., Mishchenko A.M. The activity of superoxide dismutase and catalase in red blood cells and some tissues of mice under conditions of low-frequency vibration. *Physics of the Living.* 2010; 18 (1): 107–113 (in Russ.).
15. Rozhkov D.O., Zinovyeva O.Ye., Barinov A.N. Vikhlyantcev I.M., Ulanova A.D. Skeletal Muscle Condition in Chronic Non-Specific Pain in the Lower Back and Approaches to Therapy. *Effective Pharmacotherapy.* 2018; 2 (11): 24–32 (in Russ.).
16. Tskhovrebova L., Trinick J. Titin and nebulin in thick and thin filament length regulation. *Subcell Biochem.* 2017; 82: 285–318. DOI: 10.1007/978-3-319-49674-0_10.

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The role of free radical oxidation in the kidneys in the nephroprotective action of eplerenone, a mineralocorticoid receptor antagonist, in experimental diabetes mellitus

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ABSTRACT

Aim. To study the effect of eplerenone on the activity of free radical oxidation and renal function in rats with experimental diabetes mellitus induced by streptozotocin.

Materials and methods. Experiments were carried out on 36 male Wistar rats. Diabetes mellitus (DM) was simulated by a single intraperitoneal injection of streptozotocin at a dose of 65 mg / kg. Eplerenone was injected into the stomach at a dose of 50 mg / kg.

Results. It was found that eplerenone in experimental diabetic nephropathy (DN) significantly attenuated proteinuria: the concentration of protein in the urine became 4 times lower than in untreated DN ($p < 0.001$). In the kidneys, eplerenone therapy normalized the structure and function of renal glomeruli and restored the podocyte number, which was reduced by 37.8% in the DN model. Free radical oxidation (FRO) in the kidneys of rats treated with eplerenone increased – the concentration of thiobarbituric acid reactive substances rose by 1.5 times ($p = 0.009$), and changes in the activity of antioxidant enzymes, such as superoxide dismutase (a decrease by 2.4 times, $p = 0.002$), catalase (an increase by 1.8 times, $p < 0,001$), and glutathione peroxidase (an increase by 1.5 times, $p < 0.001$) were observed, as opposed to the values in the controls.

Conclusion. In streptozotocin-induced experimental diabetic nephropathy in rats, eplerenone had a nephroprotective effect, but increased oxidative stress in the kidneys. The increase in FRO could be determined by the nongenomic effect of aldosterone, which accumulates under conditions of prolonged mineralocorticoid receptor (MR) blockade. The nephroprotective effect of eplerenone can be associated with the weakening of the genomic effects of aldosterone, realized with the participation of MR.

Key words: eplerenone, free radical oxidation, diabetes mellitus, diabetic nephropathy.

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Роль свободнорадикального окисления в почках в нефропротекторном действии блокатора минералокортикоидных рецепторов эплеренона при экспериментальном сахарном диабете

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РЕЗЮМЕ

Цель. Изучить влияние эплеренона на активность свободнорадикального окисления и функции почек крыс при экспериментальном сахарном диабете, вызванном стрептозотоцином.

Материалы и методы. Эксперименты проведены на 36 самцах крыс линии Вистар. Сахарный диабет моделировали однократным внутрибрюшинным введением стрептозотоцина в дозе 65 мг/кг. Эплеренон вводили в желудок в дозе 50 мг/кг.

Результаты и обсуждение. Установлено, что эплеренон при экспериментальной диабетической нефропатии (ДН) существенно ослабляет протеинурию: количество белка в моче становится меньше в 4 раза, чем при нелеченой ДН ($p < 0,001$). В почках под влиянием терапии эплереноном нормализуются структура и функции почечных клубочков, в том числе восстанавливается количество подоцитов, уменьшенное при модели ДН на 37,8%. Активность свободнорадикального окисления (СРО) в почках крыс, получавших эплеренон, усиливается: увеличивается концентрация тиобарбитуратреактивных продуктов в 1,5 раза ($p = 0,009$) и изменяется по сравнению с показателями контроля активность антиоксидантных ферментов – супероксиддисмутазы (снижается в 2,4 раза, $p = 0,002$), каталазы (увеличивается в 1,8, $p < 0,001$) и глутатионпероксидазы (увеличивается в 1,5 раза, $p < 0,001$).

Заключение. При экспериментальной ДН, вызванной у крыс введением стрептозотоцина, эплеренон оказывает нефропротекторное действие, но усиливает оксидативный стресс в почках. Усиление СРО могло быть обусловлено негеномным мембранным действием альдостерона, компенсаторно накапливающегося в условиях длительной блокады минералокортикоидных рецепторов (МКР). Нефропротекторное действие эплеренона можно связать с ослаблением геномных эффектов альдостерона, реализуемых при участии МКР.

Ключевые слова: эплеренон, нефропротекторное действие, свободнорадикальное окисление, экспериментальная диабетическая нефропатия.

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

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INTRODUCTION

Oxidative damage to the kidneys makes a significant contribution to the development of diabetic nephropathy (DN) [1], therefore, the study of the mechanisms of peroxidation in the kidneys, the search for new targets for targeted antioxidant therapy, and the development of new pharmacological approaches to DN treatment on their basis are relevant.

There are four main mechanisms of oxidative stress development in the kidneys in DN: direct inhibition of cellular antioxidant systems by glucose and its metabo-

lites, accumulation of advanced glycation end products (AGEs), activation of protein kinase C, and activation of the renin-angiotensin-aldosterone system (RAAS) [2]. NADPH oxidase catalyzing the formation of reactive oxygen species (ROS) is activated in the kidneys [3]. When searching for new effective methods of drug therapy in DN, an important question remains unresolved: which mechanism contributes the most to the oxidative stress development? The answer to this question would contribute to targeted and more effective development of new pharmacological approaches to DN therapy.

We studied the effect of RAAS drug inhibition on oxidative damage to the kidneys and the course of DN in experimental diabetes mellitus (DM). For this purpose, eplerenone was chosen as a pharmacological tool, which is a non-steroidal aldosterone antagonist previously studied as a nephroprotector in DN [4].

The aim of the study was to investigate the effect of eplerenone on the activity of free radical oxidation and renal function in rats with experimental, streptozotocin-induced DM.

MATERIALS AND METHODS

Experiments were conducted on 36 male Wistar rats aged 2–3 months and weighing 300–350 grams. The study was carried out in accordance with Directive 86/609/EEC, the Declaration of Helsinki, and the "Rules for Work Using Experimental Animals". The study was approved by the local Ethics Committee at ASMU (Protocol No. 4 of 30.04.2020). The rats were placed in individual metabolic cages adapted for urine collection. The animals were kept under natural light conditions, at room temperature and could freely consume water and "Chara" feed produced by Assortiment-Agro LLC. All manipulations with animals were carried out from 9 AM to 12 AM.

According to the design of the experiment, the animals were divided into 3 groups: a control group and an experimental group each consisting of 13 animals and a group of intact rats containing 10 animals. In the control and experimental groups, the rats were administered intraperitoneally with 1 ml of streptozotocin solution (Applichem, Germany) at a single dose of 65 mg / kg in the citrate buffer to model DM. For more accurate modeling of type 2 DM, the rats were previously injected intraperitoneally with cytoflavin solution at the nicotinamide rate of 115 mg / kg. The administration of nicotinamide weakens streptozotocin-induced damage to the islets of Langerhans and allows for moderate hyperglycemia without massive cytolysis of pancreatic cells [5].

In the experimental group for DN treatment, eplerenone (Polpharma, Poland) was injected in the stomach at a dose of 50 mg / kg once a day daily for 3 weeks, starting from the 5th week after the streptozotocin injection. The dose of eplerenone was chosen according to the results of previous studies on the nephroprotective effect of the drug in the DM model [4]. In preliminary studies, we showed that typical signs of nephropathy in rats develop only by the end of the 4th week after streptozotocin administration [6].

To assess renal functions in rats of the control and experimental groups, the concentrations of glucose, protein, and creatinine in urine were determined and their excretion with urine was calculated prior to the start of DM modeling and then weekly. The glucose, protein, and creatinine levels in the urine were measured using the DIRUICS-T240 automatic biochemical analyzer (Dirui Industrial Co., Ltd., China) with biochemical kits (DIACON-DS, Russia).

After 8 weeks of the experiment, the animals were euthanized by ether, their kidneys were extracted and washed with isotonic sodium chloride solution. One kidney was used for morphological studies, the second one was used to study the activity of FRO. According to the methods given in the manual [7], the activity of FRO was evaluated by the concentration of thiobarbituric acid reactive substances (TBARS) and the activity of antioxidant enzymes, such as catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx), in the kidneys.

For morphological studies, Sakkura (Japan) devices were used. The kidneys were fixed in 10% neutral formalin solution. The material was dehydrated in isopropyl alcohol using the carousel-type machine TISSUE-TEK VIPTM6 (Japan). The material was poured into paraffin using the paraffin filling station TISSUE-TEK TEC 5 (Japan). 5–7 μm-thick histological sections were obtained using the semi-automatic rotary microtome Accu-Cut SRM (the Netherlands) and stained with hematoxylin and eosin and according to the Van Gieson's method in the TISSUE-TEK Prisma automated slide stainer (Japan). Neutral glycosaminoglycans were determined histochemically using Schiff reagent according to McManus. The slides were placed under film in the TISSUE-TEK Film coverslipper (Japan). Morphometric studies were performed using a computer image analysis system consisting of the Leica DME microscope (Germany), the Leica EC3 digital color camera (Leica Microsystems AG, Germany), a personal computer, and Video Test Morphology 5.2 software. The area of renal glomeruli and the area of

capillary lumina were measured, and after special computer processing of digital photos, the total area of the vascular bed, the area of the mesangium, and the number of podocytes in the glomerulus were calculated.

The results were processed statistically using the Statistica 13.3.1 software (license JPZ906I-448517FAACD-K). The results of the biochemical studies are presented by the median and the interquartile range (Me (25%; 75%)). The results of the morphometric studies were presented by the mean and the standard error of the mean ($M \pm m$). Statistical comparisons between the groups were carried out using the nonparametric Mann – Whitney U-test, comparisons within the group were performed using the nonparametric Wilcoxon test [8].

RESULTS

The experiments showed that during 8 weeks in rats of the control and experimental groups, the amount of excreted urine and renal excretion of glucose and creatinine statistically significantly exceeded the initial levels and did not differ between the groups (not shown in the tables and figures).

In the control and experimental groups during the first 28 days of DM modeling, renal excretion of protein significantly increased (Fig. 1): in the control group – from 2.7 (1.8; 8.7) to 11.0 (5.9; 15.3) mg / day ($p = 0.004$), in the experimental group – from 3.0 (0.6; 3.8) to 10.2 (9.9; 13.4) mg / day ($p = 0.002$). Statistically significant differences between the groups were not recorded. From the 5th to the 8th week of the experiment, protein excretion in the control group continued to increase and peaked by the end of the experiment – up to 36.0 (28.6; 43.2) mg / day, which was 13.3 times higher than before the experiment ($p = 0.001$). After the start of eplerenone administration, the increase in renal protein excretion stopped, and until the end of the experiment, the excretion did not change compared to the indicator measured on the 28th day. As a consequence, at the 6–8th week of eplerenone administration, renal protein excretion was statistically significantly lower than in the control group during the specified periods of time: after 6 weeks, it decreased by 2.3 times to 5.0 (3.2; 7.5) mg / day as opposed to 11.6 (5.3; 14.8) mg / day ($p = 0.018$), after 7 weeks – by 1.8 times to 7.7 (2.6; 10.3) mg / day as opposed to 13.8 (11.6; 16.0) mg / day ($p = 0.002$), after 8 weeks – by 4 times to 9.0 (3.5; 15.0) mg / day as opposed to 36.0 (28.6; 43.2) mg / day ($p < 0.001$).

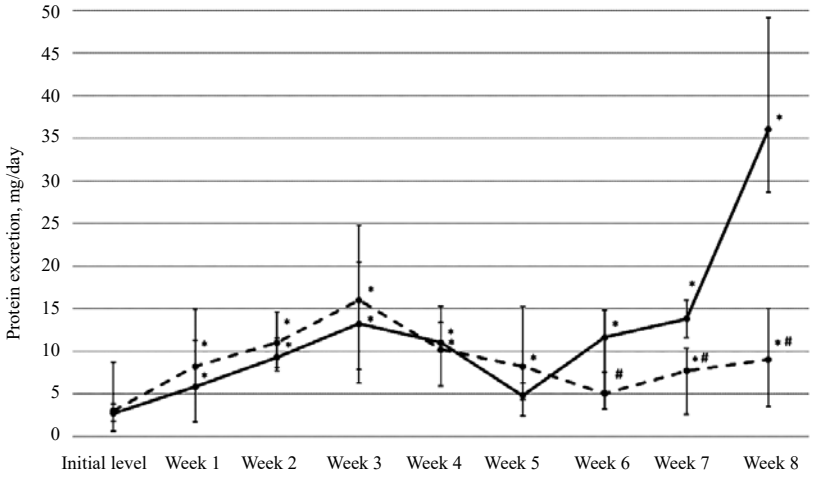
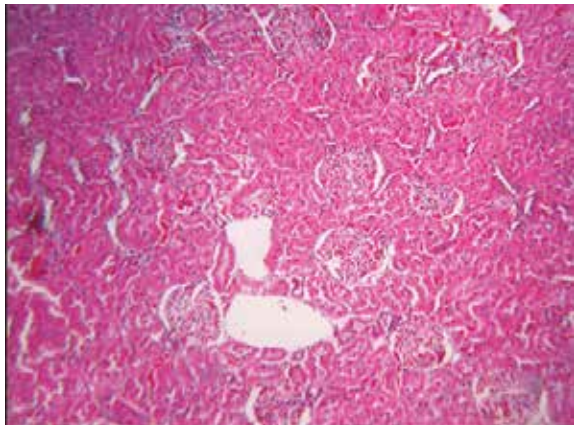


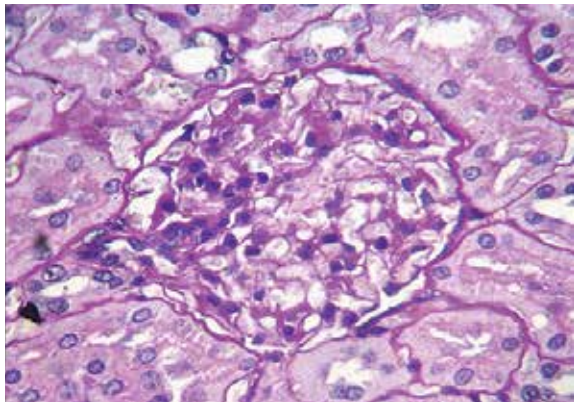
Fig. 1. Dynamics of protein excretion in urine: * indicates statistically significant differences compared to the initial level, the sign # indicates statistically significant differences between the groups

Morphological studies of the kidneys of experimental animals allowed to establish that in the control group, renal glomeruli were enlarged and the intercapillary space in the glomeruli increased due to the accumulation of periodic acid-Schiff (PAS)-positive material in the mesangium (Fig. 2). The lumina of the glomerular capillaries were narrowed, the basement membranes of the capillaries and the Browman's capsule were thickened. Podocytes increased in size, swelling of their nuclei occurred. In the renal interstitium, foci of nephrosclerosis were found; in these areas, the tubular basement membranes were thickened. The tubular nephrocytes were flattened, the tubular lumen was dilated. Most nephrocytes were in a state of hyaline-drop dystrophy. In some regions, lymphocytic-plasmacytic infiltration was identified.

In rats of the experimental group who received eplerenone, the area of renal glomeruli decreased (Fig. 3). A focal increase in the intercapillary space and deposition of PAS-positive material were weakly pronounced. The lumina of the glomerular capillaries were mostly wide, congestion of the capillaries was noted. Podocytes were small in size, with rounded small nuclei. In the renal interstitium, nephrosclerosis phenomena were minimal. The morphological structure of nephrocytes approached normal one, hyaline-drop dystrophy phenomena were absent at most sites. The intergroup comparison of quantitative morphometric parameters is presented in Table 1.

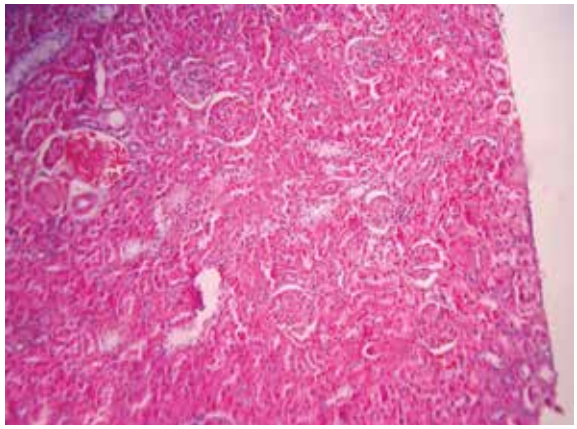


a

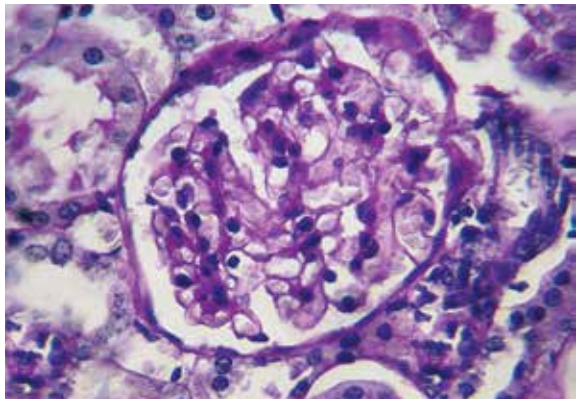


b

Fig. 2. Rat kidney tissue in the control group: *a* – increase in the size of glomeruli, staining with hematoxylin and eosin, ×100; *b* – increase in the intercapillary space and narrowing of the capillary lumina, McManus staining, ×1,200



a



b

Fig. 3. Rat kidney tissue in the experimental group: *a* – decrease in the size of glomeruli, staining with hematoxylin and eosin, ×100; *b* – decrease in the intercapillary space and dilation of the capillary lumina, McManus staining, ×1,200

Table 1

Morphometric parameters of renal glomeruli in experimental animals, *M* ± *m*

Parameters	Intact rats	Control group	Experimental group
Area of renal glomeruli (μm²)	6,174.7 ± 257.5	7,758.65 ± 329.5 $p_{int} < 0.001$	4,810.4 ± 202.6 $p_c < 0.001$ $p_{int} < 0.001$
Total area of the glomerular vessels (μm²)	2,900 ± 27.4	1,148.5 ± 107.65 $p_{int} < 0.001$	1,569.65 ± 282.05 $p_{int} < 0.001$
Area of glomerular capillary lumen (μm²)	47.5 ± 3.7	24.25 ± 1.65 $p_{int} < 0.001$	31.4 ± 2.4 $p_c = 0.004$, $p_{int} = 0.001$
Area of the glomerular mesangium (μm²)	4,738.7 ± 43.3	5,609.9 ± 823.1	3,733.3±505.9
Podocytes (particles)	10.2 ± 0.20	7.4 ± 0.6 $p_{int} < 0.001$	11.8 ± 0.5 $p_c < 0.001$

Note. p_{int} – level of statistical significance as opposed to intact rats, p_c – level of statistical significance in the experimental group as opposed to the control group (here and in Table 2).

Table 2

Activity of free radical oxidation in the kidneys of rats in experimental groups, *Me* (25%; 75%)

	Intact rats	Control group	Experimental group
TBARS concentration (μmol / mg)	6.1 (5.4; 6.9)	6.0 (4.8; 6.6)	8.5 (6.2; 9.9) $p_{int} = 0.005$, $p_c = 0.009$
CAT activity (%)	14.4 (10.2;15.6)	32.7 (23.0;37.7) $p_{int} < 0.001$	60.1 (50.5;64.0) $p_{int} < 0.001$ $p_c < 0.001$
SOD activity (%)	18.2 (13.0; 18.5)	8.8 (7.7; 10.3) $p_{int} = 0.003$	3.7 (3.4; 5.5) $p_{int} < 0.001$, $p_c = 0.002$
GPx activity (%)	38.5 (25.2; 41.6)	58.7 (57.5; 60.3) $p_{int} < 0.001$	89.5 (88.4; 91.2) $p_{int} < 0.001$ $p_c < 0.001$

In the kidneys of rats in the control group, CAT activity increased by 2.3 times, GPx – by 1.5 times, SOD activity decreased by 2.1 times as opposed to values in the intact animals (Table 2). The TBARS concentration did not change.

In the experimental group of animals receiving eplerenone, contrary to expectations, FRO increased: TBARS concentrations increased by 1.4 times compared to the value in the intact and control rats; CAT activity became 4.2 times higher than in the intact rats and 1.8 times higher than in the controls. SOD activity decreased by 2.4 times compared to the value in the control group and by 4.9 times compared to the intact rats. GPx activity was 2.3 times higher than in the intact animals and exceeded the value in the control group by 1.5 times.

DISCUSSION

The rats with the DN model developed oxidative stress. A month after the streptozotocin administration against the background of FRO, the activity of the main antioxidant enzymes (GPx and SOD) increased [9]. This appears to be compensatory in response to ROS generation. Two months after the DM modeling, the SOD activity decreased, whereas the GPx and CAT activity increased. It is possible that as FRO intensity progresses, the activity of SOD is depleted, and the GPx and CAT activity undergoes substrate stimulation by a large amount of ROS. The treatment cycle with eplerenone in experimental DM increased oxidative stress in the kidneys, but had a nephroprotective effect. This allows us to conclude that RAAS inhibitors normalize the functions of the renal glomeruli and their filtration barrier, regardless of the effect on FRO activity in the kidneys in DM.

FRO enhancement in the kidneys upon eplerenone administration can be explained by a compensatory increase in aldosterone production and a rise in its level in the kidneys [10]. Under the conditions of mineralocorticoid receptor blockade, aldosterone exhibits nongenomic activity in the form of membrane effects [11]. One of such effects is known to be activation of protein kinase C [12]. This enzyme triggers a cascade of ROS generating reactions [2].

The mineralocorticoid receptor antagonist eplerenone can weaken the genomic effects of aldosterone upon prolonged administration. The main reason for proteinuria development in DN is a decrease in the number of podocytes and weakening of their functions [13]. Numerous studies indicate a direct link between mineralocorticoid receptor activation and damage to podocytes [14]. In our study with the DN model, eplerenone normalizes the number of podocytes and their functional state and, therefore, reduces proteinuria.

CONCLUSION

In the DN model, the nephroprotective effect of eplerenone is determined by the blockade of the genomic effects of aldosterone and is not associated with the effect on FRO activity in the kidneys. Eplerenone enhances FRO in the kidneys following the accumulation of aldosterone and its nongenomic membrane effect.

REFERENCES

1. Sagoo M.K., Gnudi L. Diabetic nephropathy: Is there a role for oxidative stress? *Free Radic. Biol. Med.* 2018; 116: 50–63. DOI: 10.1016/j.freeradbiomed.2017.12.040.
2. Bhatti A.B., Usman M. Drug targets for oxidative podocyte injury in diabetic nephropathy. *Cureus.* 2015; 7 (12): e393. DOI: 10.7759/cureus.393.
3. Sharma K. Mitochondrial dysfunction in the diabetic kidney. *Adv. Exp. Med. Biol.* 2017; 982: 553–562. DOI: 10.1007/978-3-319-55330-6_28.
4. Ahn J.H., Hong H.C., Cho M.J., Kim Y.J., Choi H.Y., Eun C.R., Yang S.J., Yoo H.J., Kim H.Y., Seo J.A., Kim S.G., Choi K.M., Baik S.H., Choi D.S., Kim N.H. Effect of eplere-

none, a selective aldosterone blocker, on the development of diabetic nephropathy in type 2 diabetic rats. *Diabetes Metab. J.* 2012; 36 (2): 128–135. DOI: 10.4093/dmj.2012.36.2.128.
5. Spasov A.A., Voronkova M.P., Snigur G.L., Cheplyaeva N.I., Chepurnova M.V. Experimental model of type 2 diabetes mellitus. *Biomedicine.* 2011; (3): 12–18 (in Russ.).
6. Filinova S.O., Zharikov A.Yu., Bobrov I.P., Mazko O.N., Makarova O.G. The pathomorphological pattern of diabetic nephropathy in experimental diabetes mellitus. *Kazan Medical Journal.* 2019; 100 (1): 147–152 (in Russ.). DOI: 10.17816/KMJ2019-147.
7. Bryukhanov V.M., Zverev Ya.F., Lampatov V.V., Zharikov A.Yu., Talalaeva O.S. Methods of preclinical (experimental) studies on the effect of drugs on the renal function. Novosibirsk: Geo, 2013: 84 (in Russ.).
8. Khafizyanova R.Kh., Burykin I.M., Aleeva G.N. Mathematical statistics in experimental and clinical pharmacology. Kazan: Medicine, 2006: 374 (in Russ.).
9. Filinova S.O., Zharikov A.Yu., Mazko O.N., Makarova O.G., Balandovich B.A. Parameters of prooxidant and antioxidant states in the rat kidneys with experimental diabetes mellitus. Pathological Physiology and Experimental Therapy. 2020; 64 (1): 124–127 (in Russ.). DOI: 10.25557/0031-2991.2020.01.124-127.
10. Gupta G., Dahiya R., Singh Y., Mishra A., Verma A., Gothwal S.K., Aljabali A., Dureja H., Prasher P., Negi P., Kapoor D.N., Goyal R., Tambuwala M.M., Chellappan D.K., Dua K. Monotherapy of RAAS blockers and mobilization of aldosterone: A mechanistic perspective study in kidney disease. *Chemico-Biological Interactions.* 2020; 317: 108975. DOI: 10.1016/j.cbi.2020.108975.
11. Mihailidou A.S., Tzakos A.G., Ashton A.W. Non-genomic effects of aldosterone. *Vitam. Horm.* 2019; 109: 133–149. DOI: 10.1016/bs.vh.2018.12.001.
12. Eiam-Ong S., Chaipipat M., Manotham K., Eiam-Ong S. Aldosterone rapidly activates p-PKC delta and GPR30 but suppresses p-PKC epsilon protein levels in rat kidney. *Endocr. Regul.* 2019; 53 (3): 154–164. DOI: 10.2478/enr-2019-0016.
13. Podgórski P., Konieczny A., Lis Ł., Witkiewicz W., Hruby Z. Glomerular podocytes in diabetic renal disease. *Adv. Clin. Exp. Med.* 2019; 28 (12): 1711–1715. DOI: 10.17219/acem/104534.
14. Bai M., Chen Y., Zhao M., Zhang Y., He J.C., Huang S., Jia Z., Zhang A. NLRP3 inflammasome activation contributes to aldosterone-induced podocyte injury. *Am. J. Physiol. Renal. Physiol.* 2017; 312 (4): F556–F564. DOI: 10.1152/ajprenal.00332.2016.

Authors contribution

Zharikov A.Yu. – conception and design, analysis and interpretation of data, final approval of the manuscript for publication. Filinova S.O. – setting up of the experimental model. Mazko O.N., Makarova O.G. – setting up of the experimental model, carrying out of the biochemical laboratory studies. Bobrov I.P. – carrying out of the morphological studies. Bryukhanov V.M. – critical revision of the manuscript for important intellectual content.

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Patterns of conjunctival and scleral regeneration after intraoperative application of cyclosporin A solution in rabbits with steroid-induced glaucoma

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ABSTRACT

Aim. In an *in vivo* experiment, to study the effect of local intraoperative application of 0.05% cyclosporin A solution on the conjunctival and scleral regeneration after surgery on the rabbit eyes with steroid-induced glaucoma.

Materials and methods. At the first stage of the experiment, a model of steroid-induced glaucoma was reproduced for 29 male Californian rabbits by injecting 0.5 ml of a 0.4% solution of dexamethasone subconjunctivally in both eyes once a week for 3 months (12 subconjunctival injections for each rabbit). At the second stage of the experiment, after the development of steroid-induced glaucoma, the rabbits were divided into the main group, consisting of the subgroup "a" ($n = 8$) and the subgroup "b" ($n = 8$), and the comparison group ($n = 8$). In all animals, a penetrating incision of the conjunctiva and a non-penetrating incision of the sclera of one of the eyes were performed.s. A hemostatic sponge impregnated with 0.05% cyclosporin A solution was applied to the intervention area in the main group, in the subgroup "a" – for 3 minutes, in the subgroup "b" – for 6 minutes. In the comparison group, the cytostatic was not used.

Results. The use of 0.05% cyclosporin A solution led to a decrease in the infiltration of fibroblasts and inflammatory cells in the area of surgical injury. On the 4th day after the surgery, cell density in the intervention area in the subgroup "a" with 3-minute application of cytostatic – antimetabolite solution was 2.7 times lower ($p = 0.043$) than in the comparison group, while exceeding the values in the subgroup "b" by 3.2 times ($p = 0.036$). The number of fibroblasts in the subgroups "a" and "b" was 3.6 ($p = 0.043$) and 12.8 times ($p = 0.031$) less than in the comparison group, and a shift in the cellular composition of the infiltrate towards the fibroblastic population occurred only on the 14th day after the surgery.

Conclusion. Intraoperative application of 0.05% cyclosporin A solution significantly slows down the course of regeneration, reducing infiltrative inflammation in the intervention area, which prevents excessive scarring.

Key words: antiglaucoma surgery, scarring of the outflow pathways of the intraocular fluid, antimetabolites, cyclosporine A.

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 local Ethics Committee at Siberian State Medical University (Protocol No. 4346 of 16.11.2015).

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steroid-induced glaucoma. *Bulletin of Siberian Medicine.* 2021; 20 (2): 36–43. https://doi.org/10.20538/1682-0363-2021-2-36-43.

Закономерности регенерации конъюнктивы и склеры после интраоперационной аппликации раствора циклоспорина А у кроликов со стероидной моделью глаукомы

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РЕЗЮМЕ

Цель. В эксперименте *in vivo* изучить влияние местной интраоперационной аппликации 0,05%-го раствора (р-ра) циклоспорина А на регенерацию конъюнктивы и склеры после операции на глазах кроликов со стероидной глаукомой.

Материалы и методы. На I этапе эксперимента 29 самцам кроликов калифорнийской породы моделировали стероидную глаукому путем введения под конъюнктиву обоих глаз 0,5 мл 0,4%-го р-ра дексаметазона 1 раз в нед в течение 3 мес (12 инъекций). На II этапе эксперимента, после развития стероидной глаукомы, кроликов разделили на основную группу, состоящую из подгруппы «a» (n = 8) и подгруппы «b» (n = 8), и группу сравнения (n = 8). Всем животным выполняли сквозной разрез конъюнктивы и непроникающий надрез склеры одного из глаз. На область вмешательства в основной группе накладывали гемостатическую губку, пропитанную 0,05%-м р-ром циклоспорина А, в подгруппе «a» на 3 мин, в подгруппе «b» – на 6 мин. В группе сравнения цитостатик не использовали.

Результаты и обсуждение. Применение 0,05%-го р-ра циклоспорина А привело к уменьшению инфильтрации зоны хирургической травмы воспалительными клетками и фибробластами. На 4-е сут после операции клеточная плотность в области вмешательства в подгруппе «a» основной группы с трехминутной аппликацией р-ра цитостатика-антиметаболита была в 2,7 раза меньше (p = 0,043), чем в группе сравнения, превышая при этом показатели подгруппы «b» в 3,2 раза (p = 0,036). Численность фибробластов в подгруппах «a» и «b» была в 3,6 (p = 0,043) и 12,8 раза (p = 0,031) ниже, чем в группе сравнения. При этом сдвиг клеточного состава инфильтрата в сторону фибробластической популяции произошел только на 14-е сут после операции.

Заключение. Интраоперационная аппликация 0,05%-го р-ра циклоспорина А существенно замедляет течение воспалительно-репаративной регенерации, уменьшая инфильтративное воспаление в зоне вмешательства, что предотвращает излишнее рубцевание.

Ключевые слова: антиглаукомная операция, рубцевание путей оттока внутриглазной жидкости, антиметаболиты, циклоспорин А.

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INTRODUCTION

According to modern concepts, glaucoma is a group of diseases characterized by intraocular pressure (IOP) above the tolerant level with development of optic neuropathy and a typical decline in visual functions [1]. Despite a wide choice of antihypertensive drugs and the use of various laser interventions, surgical treatment of this pathology remains the most effective method to compensate for increased IOP [1]. The success of filtration surgeries is determined by the duration of functioning of surgically created intraocular fluid outflow pathways. Scarring in the area of surgery leads to a significant decrease in the hypotensive effect [1, 2].

To provide a stable and prolonged hypotensive effect after antiglaucoma surgery, antimetabolites (5-fluorouracil, mitomycin C) are often used to prevent tissue proliferation in the intervention area with scarring of artificially created intraocular fluid outflow pathways [2–6]. However, widespread use of antimetabolites is limited by the risk of complications, such as secondary maculopathy, keratopathy, cataract progression, and hypotension, in the postoperative period [2].

Therefore, the search for a drug that has an antiproliferative effect with minimal toxic properties and prevents scarring of surgically created intraocular fluid outflow pathways after antiglaucoma surgery is one of the urgent problems of modern ophthalmic surgery.

The aim of the work was to study the effect of local intraoperative application of a 0.05% cyclosporin A solution on conjunctival and scleral regeneration after surgery on the rabbit eyes with induced glaucoma in an *in vivo* experiment.

MATERIALS AND METHODS

The study was carried out at the SSMU Laboratory of Biological Models (supervisor – Vladimir V. Ivanov, Cand. Sci. (Biology)). The protocol of the experiment was approved by the local Ethics Committee at SSMU (No. 4346 of 16.11.2015). The *in vivo* experiment was performed on 29 Californian rabbits (male), weighing 3.5–4.0 kg, which were preliminarily quarantined in the vivarium for 1 week on a standard diet. At the first stage of the experiment, the model of steroid-induced glaucoma was reproduced for all rabbits by injecting 0.5 ml of a 0.4% solution of dexamethasone (Belmedpreparaty, the Republic of Belarus) subconjunctivally in both eyes once a week for 3 months (a total of 12 subconjunctival injections for each rabbit) [7]. The IOP level in the rabbits was measured weekly using a Maklakov's tonometer with the plunger weighing 7.5 g. 2 weeks after the 12th injection of the steroid solution, 5 randomly selected animals were removed from the experiment, followed by enucleation of their eyes. The obtained material was fixed for light microscopy.

The second stage of the *in vivo* experiment was performed on 24 rabbits with steroid-induced glaucoma. 4 weeks after the last subconjunctival injection of 0.4% dexamethasone solution, in the upper part of the right eyeball of all the animals, a penetrating incision of the scleral conjunctiva and a non-penetrating incision of the surface layers of the sclera were performed under general anesthesia with sevoflurane (Baxter Healthcare SA, Puerto-Rico, USA) in the operating room according to aseptic and antiseptic rules. The paired eye of the animals remained intact.

Depending on the course of the surgery, the rabbits were divided into 2 groups: the main group (16 animals) and the comparison group (8 animals). During the surgery, after performing the incisions of the conjunctiva and the superficial layers of the sclera, a hemostatic sponge soaked in 0.05% cyclosporin A solution (Allergan, USA) was placed on the surgical area in all rabbits of the main group. Depending on the antimetabolite application time, the animals of the main group were divided into 2 subgroups: subgroup "a" (8 rabbits) – the application time of the drug was 3 minutes; subgroup "b" (8 rabbits) – the application time of the drug was 6 minutes.

A hemostatic sponge without cyclosporin A solution was placed on the surgical area for 3 minutes in the rabbits of the comparison group (8 animals). At the end of the application, the hemostatic sponge was removed in rabbits of all groups. The scleral conjunctiva incision was stitched with 2 interrupted sutures, and tobrex solution was instilled. In the postoperative period, all animals received one intramuscular injection of gentamicin sulfate solution (MICROGEN, Russia) at the rate of 5 mg / kg of the animal's weight, and tobrex solution was instilled into the conjunctival cavity of the operated eye 3 times a day. On days 4, 10, 14, and 21 after the surgery, 2 rabbits from each group were removed from the experiment, followed by enucleation of their eyes.

At all stages of the experiment, the animals were euthanized in compliance with the rules and norms of the European Society (86/609EEC), the Declaration of Helsinki, and orders of the Ministry of Health of the USSR (No. 742 of 13.11.1984 and No. 48 of 23.01.1985). The rabbits were removed from the experiment by carbon dioxide inhalation. The material

was fixed and then stained with hematoxylin and eosin and according to the Mallory technique for light microscopy at 100- and 200-fold magnification. Using Canon Power Shot G10 digital camera (Japan), 10 random fields of view were photographed for each slice. Digital photographs were subjected to morphometry using the ImageJ 1.46 program (http://rsbweb.nih.gov/ij/) and the Cell Counter plug-in, which were used to calculate the absolute and relative content of cells in the conjunctiva and sclera of the rabbits in the surgical area.

Statistical processing of the obtained data was carried out using the IBM SPSS Statistics 20 software. The Kolmogorov – Smirnov test was used to assess the normal distribution of the results. If the distribution of the indicators did not correspond to normal distribution, the Mann – Whitney U-test was used. The results were presented as $M \pm SD$, where M is the sample mean, and SD is the standard deviation. The differences were considered statistically significant at $p < 0.05$.

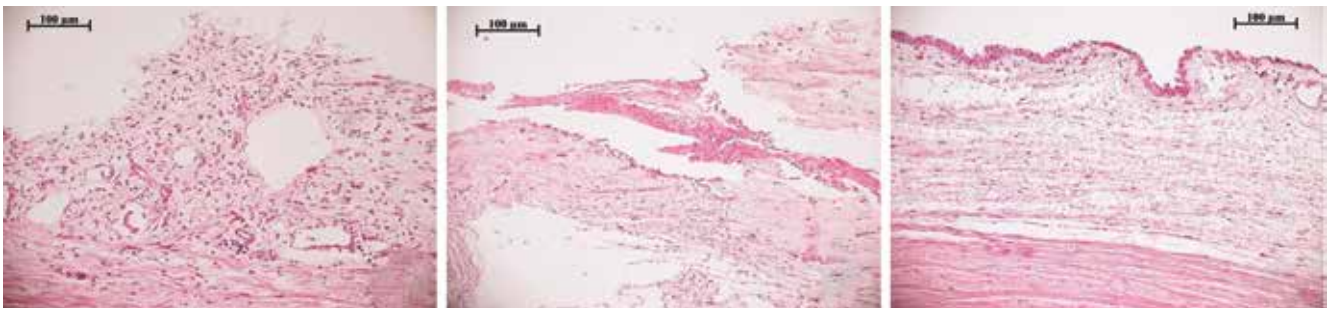
RESULTS

All rabbits (100%) had the initial IOP level within the normal range for this kind of animals, amounting to 13.7 ± 4 mm Hg [8]. After modeling steroidinduced glaucoma, all experimental animals showed an increase in the IOP level in both eyes by 3.5 times compared to the initial value − up to 47 ± 6 mm Hg (p = 0.041). Examination of the ocular fundus in all rabbits showed gradual expansion and deepening of the physiological excavation of the optic disc with the formation of glaucomatous excavation by the end of the 3rd month of the experiment, which was confirmed by the light microscopy findings. According to light microscopy, at the anterior chamber angle of the experimental animals, pathomorphological changes specific to glaucoma were revealed, such as partial lysis of the trabecular plates of the rabbit eye drainage system with the formation of dense conglomerates between the plates and obliteration of cracks between them. Areas of local ectasia of the Schlemm's canal were found, alternating with areas of its complete obliteration.

After instrumental and morphological confirmation of steroid-induced glaucoma development in the rabbits, the analysis of the second stage of the *in vivo* experiment started – the study of the effect of intraoperative application of 0.05% cyclosporin A solution on conjunctival and scleral regeneration of rabbits with induced glaucoma.

According to light microscopy, on the 4th day after the surgery, all rabbits from the subgroup "a" of the main group with intraoperative application of 0.05% cyclosporin A solution for 3 minutes had local conjunctival epithelium destruction in the surgical area. In the stroma, there were edema and fragmentation of collagen fiber bundles, between which multiple spaces were formed. In the sclera in the area of the surgical wound, the bundles of collagen fibers were located in mutually perpendicular directions, but the fibers were homogeneous and quite tightly adjoined each other, there were practically no gaps between them. Single vessels of the sclera were dilated and sanguineous (Fig. 1, *a*). Mononuclear leukocytes (MNL) prevailed in the cellular composition in the intervention area – 819 ± 54 cells (53.9%) in the field of view, the number of polymorphonuclear leukocytes (PML) and fibroblasts was 182 ± 24 (12.2%) and 516 ± 57 cells (33.9%) in the field of view, respectively.

In animals from the subgroup "b" of the main group, on the 4th day after thesurgery in the intervention area with the application of the antimetabolite solution for 6 minutes, pronounced damage to the conjunctival epithelium was detected. In the stroma, significant edema, fragmentation of collagen fibers, and multiple extravasal conglomerates of erythrocytes were detected. In the sclera, edema, hemorrhages, and fibrin clots were identified (Fig. 1, *b*).



a *b* *c*

Fig. 1. The state of the conjunctiva and sclera of the experimental animals in the area of intervention on the 4th day after the surgery: *a* – after a 3-minute application of 0.05% cyclosporin A solution, *b* – after a 6-minute application of 0.05% cyclosporin A solution, *c* – without application of the antimetabolite solution. Staining with hematoxylin and eosin, × 100

In the cellular composition, MNL prevailed – 283 ± 51 cells (59.1%). The number of PML was 53 ± 4 cells (33.9%) in the field of view. The number of fibroblasts was 143 ± 22 cells (29.8%), which is 3.6 times less than the level in the rabbits from the subgroup "a" of the main group ($p = 0.038$).

In the rabbits of the comparison group, on the 4th day after the surgery, in the intervention area without application of the antimetabolite solution, thinning and flattening of the conjunctival epithelium were found. In the sclera, edema and stratification of collagen fibrils were detected (Fig. 1, *c*). In the cellular composition, MNL and fibroblasts dominated with 1,961 ± 236 cells (47.0%) and 1,836 ± 218 cells (44.1%) in the field of view, respectively. The number of fibroblasts exceeded that in the rabbits of the subgroups "a" and "b" by 3.6 times ($p = 0.043$) and 12.8 times ($p = 0.031$), respectively. The PML density was 375 ± 15 cells (8.9%) in the field of view.

On the 10th day, in the animals from the subgroup "a" of the main group, in the intervention area with the application of 0.05% cyclosporin A solution for 3 minutes, local conjunctival defects were completely epithelialized due to stratified squamous epithelium. The stroma of the conjunctiva of the eye was loose and thickened due to diffuse edema; closer to the sclera, longitudinally located cavities were found in it, lined with a single layer of squamous epithelium. In the sclera in the area of intervention, the bundles of collagen fibers were stratified. However, these bundles looked structured. Fibroblasts predominated in the cellular composition – 948 ± 49 cells (68.4%) in the field of view, while their number increased by 1.8 times compared to the level on the 4th day after the surgery ($p = 0.038$). The number of PML was 21 ± 4 cells (1.5%) in the field of view, which is 8.9 times ($p = 0.046$) less than the level on the 4th day after the surgery. The density of MNL decreased by 2.0 times ($p = 0.037$) compared to the value on the 4th day after the surgery, amounting to 417 ± 36 cells (30.1%) in the field of view.

In the rabbits from the subgroup "b" of the main group, on the 10th day after the surgery with the application of 0.05% cyclosporin A solution for 6 minutes, the areas of the conjunctival epithelium destruction decreased, and the stroma of the membrane was extremely thinned. The conjunctival vessels of the operated eye were moderately dilated. In the sclera, the collagen fiber bundles were oriented parallel to each other, but had different thickness. Fibroblasts predominated in the cellular composition — 779 ± 35 cells (68.2%) in the field of view, which was 5.4 times ($p = 0.045$) higher than the level on the 4th day after the surgery. The cell density of the fibroblastic population was 1.2 times lower than in the rabbits from the subgroup "a" of the main group on the 10th day after the intervention ($p = 0.035$). The number of PML decreased by 2.2 times ($p = 0.042$) compared to the value in the animals of the subgroup "b" on the 4th day after the surgery, amounting to 24 ± 3 cells (2.1%) in the field of view. The number of MNL increased to 340 ± 17 cells (29.7%) in the field of view, which was 1.2 times higher ($p = 0.039$) than the level in the rabbits from the subgroup "b" on the 4th day after the operation.

In the rabbits of the comparison group, on the 10th day after the operation without application of 0.05% cyclosporin A solution, the conjunctival structure in the area of surgical injury appeared normal, collagen bundles in the sclera were thick and tightly adjoined each other. Fibroblasts predominated in the cellular composition – 3,174 ± 149 cells (80.0%) in the field of view, which was 1.7 times ($p = 0.034$) higher than their level on the 4th day after the intervention, as well as 3.3 times ($p = 0.031$) and 4.1 times ($p = 0.027$) higher than the values in the rabbits of the subgroups "a" and "b" on the 10th day after the surgery, respectively. The PML density decreased by 2.4 times ($p = 0.031$) compared to the level on the 4th day after the surgery, amounting to 159 ± 8 cells (4%) in the field of view. The number of MNL was 634 ± 31 cells (16%) in the field of view, decreasing by 3.1 times ($p = 0.043$) compared to their level on the 4th day.

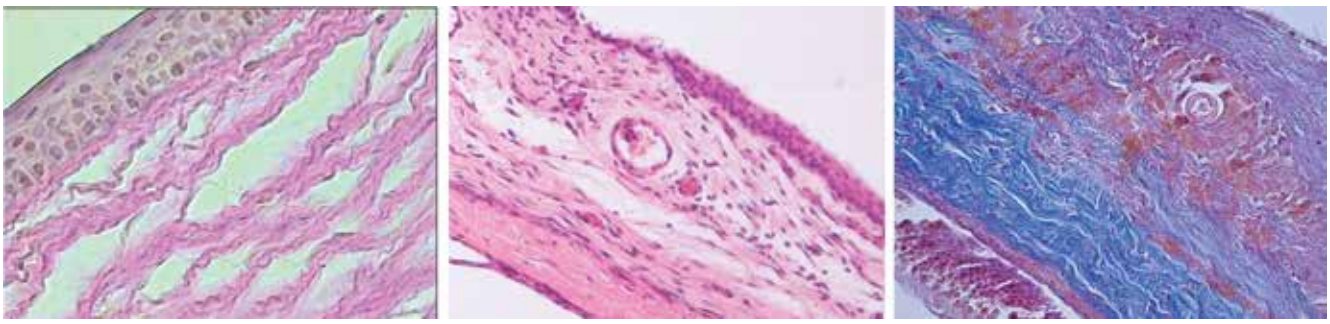
On the 14th day after the surgery, the animals of the subgroup "a" of the main group with the application of the antimetabolite solution for 3 minutes had a normal structure of the conjunctival epithelium in the intervention zone. However, in some regions, areas of surface layer thinning and thickening of the stroma due to residual edema were found. Closer to the sclera, in the conjunctival stroma, longitudinally located channels lined with a single layer of squamous epithelium were revealed. In the sclera, in the area of the surgical injury, stratification of collagen fiber bundles was observed. In the cellular composition, fibroblasts dominated – 1,311 ± 124 cells (69.5%) in the field of view, and their number increased by 1.4 times ($p = 0.035$) compared to the level in the rabbits of the subgroup "a" on the 10th day after the surgery. The density of PML was 84 ± 5 cells (4.4%) in the field of view, and their number increased by 4.1 times ($p = 0.039$) compared to the value in the animals of the subgroup "a" on the 10th day after the surgery.

The MNL level decreased to 492 ± 20 cells (26.1%) in the field of view, while there were no statistically significant differences from the level in the subgroup "a" of the main group on the 10th day after the surgery ($p = 0.062$).

In the rabbits of the subgroup "b", on the 14th day after the intervention with the application of the antimetabolite solution for 6 minutes, the conjunctiva in the area of the surgical injury was covered with stratified squamous epithelium, and slight subepithelial edema was found. The conjunctival stroma was loose. The collagen fiber bundles of the sclera in the area of intervention were loose. Fibroblasts predominated in the cellular composition with 867 ± 44 cells (65%) in the field of view, and there was no statistically significant difference ($p = 0.053$) from their density in the animals of the subgroup «b» on the 10th day after the surgical manipulation. The MNL level was 413 ± 32 cells (31%) in the field of view; there was no statistically significant difference ($p = 0.057$) compared to the value in the rabbits of the subgroup "b" on the 10th day after the surgery. The number of PML increased to 53 ± 2 cells (4%) in the field of view, exceeding by 2.2 times ($p = 0.029$) the value in the rabbits of the subgroup "b" on the 10th day.

In the rabbits of the comparison group, on the 14th day after the surgery without application of the antimetabolite solution, the conjunctiva of the eye in the intervention zone had a normal structure and tightly adjoined the superficial layers of the sclera. Scleral fibers were located tightly to one other. Among the cells in the area of the surgical injury, fibroblasts predominated with 1,972 ± 112 cells (84%) in the field of view, which exceeded the value in the subgroups "a" and "b" on the 14th day by 1.5 times ($p = 0.047$) and 2.3 times ($p = 0.038$), respectively. The density of PML decreased to 117 ± 5 cells (5%) in the field of view, which was 1.4 times (p = 0.047) lower than that on the 10th day. The MNL level was 258 ± 14 cells (11%) in the field of view, decreasing by 2.5 times ($p = 0.038$) compared to the indicator on the 10th day.

On the 21st day, in the animals of the subgroup "a" after application of 0.05% cyclosporin A solution for 3 minutes, the conjunctiva of the eye in the area of the surgical injury had a normal structure and was separated from the underlying sclera by a narrow slit-like space. The collagen fibers of the sclera were structured (Fig. 2, *a*). Fibroblasts predominated in the cellular composition with 839 ± 44 cells (69.7%) in the field of view, which was 1.6 times ($p = 0.036$) less than on the level on the 14th day after the surgery. The number of PML and MNL was 20 ± 2 cells (1.7%) and 344 ± 11 cells (28.6%) in the field of view, respectively. Moreover, the PML level decreased by 4.3 times ($p = 0.042$), and the MNL level – by 1.4 times ($p = 0.031$), compared to those on the 14th day.



a *b* *c*

Fig. 2. The state of the conjunctiva and sclera of the experimental animals in the area of intervention on the 21st day after the surgery: *a* – after a 3-minute application of 0.05% cyclosporin A solution, *b* – after a 6-minute application of 0.05% cyclosporin A solution, *c* – without application of the antimetabolite solution. Staining: *a, b* – with hematoxylin and eosin, × 100; *c* – according to the Mallory method, × 100

In the animals of the subgroup "b" of the main group on the 21st day after the surgery with the application of 0.05% cyclosporin A solution for 6 minutes, the conjunctiva of the eye in the area of the surgical injury had a normal structure and was separated from the underlying sclera by a narrow-slit space. In the conjunctival stroma, structured channels lined with a single layer of squamous epithelium were revealed. In the sclera, in the area of the surgical intervention, the collagen fiber bundles were loose, but they were oriented parallel to one another (Fig. 2, *b*). Fibroblasts predominated in the cellular composition with 799 ± 13 cells (69.3%) in the field of view, with no statistically significant differences ($p = 0.058$) compared to this indicator on the 14th day. The number of MNL decreased by 1.3 times compared to the 14th day, amounting to 320 ± 14 cells

(27.7%) in the field of view ($p = 0.044$). The number of PML decreased by 1.5 times ($p = 0.042$) to 35 ± 3 cells (3%) in the field of view.

In the rabbits of the comparison group, on the 21st day after the surgery without local application of 0.05% cyclosporin A solution, the conjunctiva of the eye in the area of the surgical injury had a normal structure and tightly adjoined the underlying sclera (Fig. 2, *c*). Fibroblasts predominated in the cellular composition – 1,209 ± 132 cells (83%) in the field of view. The cell density of this population decreased by 1.6 times compared to the value on the 14th day ($p = 0.045$), however, it exceeded this indicator by 1.4 times ($p = 0.048$) in the subgroup "a" and by 1.5 times ($p = 0.046$) in the subgroup "b" of the main group. The number of PML was 116 ± 5 cells (8%) in the field of view, with no statistically significant difference from the value on 21th day ($p = 0.059$). The number of MNL decreased by 2.0 times ($p = 0.037$) compared to the level on the 14th day, amounting to 131 ± 7 cells (9%) in the field of view.

DISCUSSION

According to the results of the experiment, the course of the inflammatory-reparative response in the conjunctiva and sclera in the rabbits of the comparison group with steroid-induced glaucoma after the eye surgery was characterized by a consistent change of cell phases in the surgical area with scarring of the conjunctiva and sclera on the 21st day. Local application of 0.05% cyclosporin A solution during the intervention had a significant effect on the postoperative period depending on the application time.

For example, a decrease in the migration of cells responsible for the inflammation intensity to the area of the surgical injury was revealed. On the 4th day after the surgery, the cell density in the intervention area in the subgroup "a" of the main group was 2.7 times lower ($p = 0.043$) than in the comparison group, while exceeding the value in the subgroup "b" by 3.2 times ($p = 0.036$) (Table). Similar dynamics was observed up to the 14th day after the surgery (Table).

Table

Dynamics of the cell density of the infiltrate in 1 mm² section in rabbits of the experimental groups with steroid-induced glaucoma, *M* ± *SD*

Observation period, days	Experimental groups		
	Main group		Comparison group
	Subgroup "a"	Subgroup "b"	
4	1,518 ± 51*/**	479 ± 22*	4,172 ± 181
10	1,386 ± 43*	1,143 ± 47*	3,967 ± 172
14	1,887 ± 84	1,333 ± 78*	2,347 ± 124
21	1,203 ± 54	1,154 ± 56	1,456 ± 97

* $p < 0.05$ when comparing to the values in the rabbits operated without local application of 0.05% cyclosporin A solution (comparison group); ** $p < 0.05$ when comparing the values in the subgroup "a" (application of the antimetabolite for 3 minutes) with the values in the subgroup "b" (application of the antimetabolite for 6 minutes) of the main group.

Intraoperative application of the antimetabolite had a pronounced inhibitory effect on the migration of fibroblasts to the area of the surgical injury. On the 4th day after the surgery, the density of fibroblasts in the intervention area in the subgroup "a" was 3.6 times lower ($p = 0.043$) than in the comparison group, while in the subgroup "b", it was 12.8 times lower ($p = 0.031$) than in the comparison group. On the 10th day after the surgery, the number of fibroblasts in the area of the surgical injury in the comparison group increased by 1.7 times ($p < 0.05$). In the subgroups "a" and "b" of the main group, a shift in the cellular composition in the intervention area towards the fibroblastic population occurred only on the 14th day after the surgery.

As a result, on the 21st day after the surgery, in the rabbits of the subgroup "a" of the main group, in the area of the surgical injury, only partial adhesion between conjunctiva and sclera was formed, in contrast to the animals of the comparison group. In the rabbits of the subgroup "b" of the main group, even on the 21st day after the surgery, a slit-like space between the conjunctiva of the eye and the underlying sclera remained in the intervention zone. Probably, the suppression of T-lymphocyte activation by cyclosporin A following the formation of bonds with cyclophilins in the cell cytoplasm and blocking of the catalytic and regulatory subunits of calcineurin leads to a decrease in the synthesis of proinflammatory cytokines. There-

fore, this slows down the course of the inflammatory-reparative response in the intervention zone [9].

CONCLUSION

Local application of 0.05% cyclosporin A solution during surgery on the conjunctiva and sclera of the eyes of rabbits with steroid-induced glaucoma significantly slows down the course of the inflammatory-reparative response in the intervention area, reducing the cell density by 2.9–3.5 times and preventing tissue scarring. The severity of the antiproliferative effect of the drug depends on the application time: when applied for 3 minutes, the density of fibroblast distribution in the surgical area is 3.6 times lower than in the rabbits of the comparison group, and when applied for 6 minutes, it is 12.8 times lower than in the comparison group. The obtained results are of interest for development of methods to prevent scarring after antiglaucoma surgeries.

REFERENCES

1. National Guidelines for Glaucoma: For Practitioners; ed. E.A. Egorova, Yu.S. Astakhova, V.P. Yericheva; 3rd ed. M .: GEOTAR-Media, 2015: 456 (in Russ.).
2. Belousova N.Yu., Poltanova T.I. Capabilities of cytostatic agents usage in ophthalmology. *Kazan Medical Journal.* 2019; 100 (4): 673–679 (in Russ.). DOI: 10.17816/KMJ2019-673.
3. Lindemann F., Plange N., Kuerten D. et al. Three-year follow-up of trabeculectomy with 5-fluorouracil. *Ophthalmic. Res.* 2017; 58 (2): 74–80. DOI: 10.1159/000464446.
4. Fiscella R., Leiter C.W., Massoomi F., Noecker R., Sheybani A. Adjunctive antifibrotic therapy with mitomycin c and 5-fluorouracil: a review of their use in glaucoma surgery and considerations around ophthalmic compounding. *Current Trends in Ophthalmology.* 2019; 2 (1): 154–169. DOI: 10.18314/ctoy.v2i1.1670.
5. Amoozgar B., Lin S. C., Han Y. A role for antimetabolites in glaucoma tube surgery: current evidence and future directions. *Current Opinion in Ophthalmology*. 2016; 27 (2): 164–169. DOI: 10.1097/ ICU.0000000000000244.
6. Bikbov M.M., Khusnitdinov I.I., Mannanova R.F. Using digel and xenocollagen drainages in glaucoma surgery. Literature review. Practical Medicine. 2017; 1 (110): 131–134 (in Russ.).
7. Gazizova I.R., Alekseev V.N., Nikitin D.N. Experimental reproduction of the glaucomatous process. *Ophthalmic Statements*. 2013; 6 (3): 43–50 (in Russ.).
8. Porfilyev I.A., Gonchar O.P. Intraocular pressure in rabbits. Determination methods. Normal performance. *Bulletin of the Peoples' Friendship University of Russia. Series: Agricultural Sciences. Livestock.* 2005; 12: 73–75 (in Russ.).
9. Matsuda S., Koyasu S. Mechanisms of action of cyclosporine. *Immunopharmacology.* 2000; 47 (2-3): 119–125. DOI: 10.1016/s0162-3109(00)00192-2.

Authors contribution

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Dissimilar populations of EpCam-positive cells in ascitic fluid of ovarian cancer patients: a relationship with the degree of carcinomatosis

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ABSTRACT

Background. Peritoneal carcinomatosis (PC) is one of the most unfavorable factors of ovarian cancer progression. It is often accompanied by accumulation of fluid in the abdominal cavity, which is called ascites. However, prognostic factors associated with malignant ascites are not well understood.

The aim of this study was to evaluate dissimilar populations of EpCAM-positive cells in ascitic fluid and their relationship with the presence of invasive peritoneal implants and the prevalence of carcinomatosis on the Predictive Index Value (PIV) scale in ovarian cancer patients.

Materials and methods. The prospective study included 22 patients aged 36–76 years with newly diagnosed FIGO stage Ic–IV ovarian cancer, who were admitted for treatment to Cancer Research Institute of Tomsk NRMC. The study material included EDTA-stabilized ascitic fluid sampled during laparoscopy.

Various populations of ascites tumor cells were identified by laser multicolor flow cytometry. The degree of carcinomatosis was determined according to the PIV scale.

Results. The study identified twelve populations of EpCAM-positive cells in the ascitic fluid of ovarian cancer patients. Epcam+CD45-CD44-CD24+CD133−Ncadherin+ cells (r = 0.58; p = 0.004) and atypical (hybrid) EpCam+CD45+CD44+CD24+/-CD133+/-Ncadherin+/- cells (r = = 0.51; p = 0.01) had a positive correlation with the PIV index.

Conclusion. The obtained results show a high degree of heterogeneity of tumor cells in the ascitic fluid of ovarian cancer patients. Identified atypical (hybrid) forms of EpCam-positive cells are of great interest for cell biology and require further investigation.

Key words: heterogeneity of tumor cells, carcinomatosis, Predictive Index Value (PIV), ascitic fluid, ovarian cancer, liquid biopsy.

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Различные популяции EpCam-положительных клеток в асцитической жидкости у больных раком яичников: связь со степенью канцероматоза

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РЕЗЮМЕ

Введение. Канцероматоз брюшины является одним из наиболее неблагоприятных факторов прогрессирования рака яичников. Он часто сопровождается накоплением жидкости в брюшной полости – асцитом. Прогностические факторы, связанные со злокачественным асцитом, изучены недостаточно.

Цель. Оценка различных популяций EpCam-положительных опухолевых клеток в асцитической жидкости, их связь с наличием перитонеальных «инвазивных» имплантатов и степенью распространенности канцероматоза по шкале Predictive Index Value (PIV) у больных раком яичников.

Материалы и методы. В проспективное исследование включены 22 больные с впервые диагностированным раком яичников, с Ic–IV стадиями по системе FIGO, в возрасте 36–76 лет, поступившие на лечение в НИИ онкологии Томского НИМЦ. Материалом для исследования служила асцитическая жидкость, стабилизированная ЭДТА, взятая во время лапароскопии.

Количество различных популяций опухолевых клеток в асцитической жидкости определяли методом многоцветной проточной цитометрии и проточной цитометрии с визуализацией. Степень канцероматоза определяли по шкале PIV.

Результаты. Показано, что клеточный состав асцитической жидкости больных раком яичников представляет собой гетерогенную популяцию. Положительная корреляционная связь с PIV, характеризующим степень распространенности канцероматоза, наблюдалась у асцитических клеток с фенотипом EpCam+CD45-CD44-CD24+CD133-Ncadherin+ ($r = 0,58$; $p = 0,004$) и у атипичных / гибридных форм клеток EpCam+CD45+CD44+CD24+/-CD133+/-Ncadherin+/- ($r = = 0,51$; $p = 0,01$).

Заключение. Полученные результаты показывают большую гетерогенность опухолевых клеток в асцитической жидкости больных раком яичников. Обнаруженный факт наличия атипичных (гибридных форм) EpCam-положительных клеток представляет интерес для клеточной биологии и требует дальнейших исследований.

Ключевые слова: гетерогенность опухолевых клеток, канцероматоз, Predictive Index Value, асцитическая жидкость, рак яичников, жидкостная биопсия.

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

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INTRODUCTION

Ovarian cancer is characterized by a unique metastatic process. The earliest and most common route of metastasis is implantation. It is often accompanied by accumulation of fluid in the abdominal cavity, which is called ascites. Ovarian cancer accounts for up to 38% of ascites cases associated with malignancies in females. Ascitic fluid is a promising biological mate-

rial to get information about the nature of the tumor. Unlike serum, ascitic fluid is more informative, especially at early stages of the malignant process [1].

In general, ascites is a multicomponent, dynamic system where all elements combined facilitate the formation of a proinflammatory and immunosuppressive environment. Ascites consists of a complex mixture of cell populations and a rich cytokine profile [2]. The variety of cells is related to several factors. The first factor is phenotypic plasticity arising from the influence of soluble factors and microenvironment signals from immune and stromal cells. Secondly, heterogeneity is associated with hydrodynamic forces that significantly change cell morphology [3]. Thirdly, the source of cancer cells in ascitic fluid is the tumor, and in ovarian cancer, in particular, it is a heterogeneous cell population [4].

Currently, the heterogeneity of tumor cells is evaluated based on their antigenic properties, spectrum of various cell surface markers, and activity of the key signaling pathways. If circulating tumor cells (CTCs) are detected, the epithelial cell adhesion molecule (EpCAM) is widely used as a specific biomarker, because it is overexpressed in more than 70% of ovarian cancer cases, and its level is closely associated with malignant ascites, chemoresistance, and decreased survival in patients. The role of EpCAM is not limited to cell adhesion; there is abundant evidence of its involvement in the epithelial–mesenchymal transition (EMT) [5]. The EMT is known to enable cells to separate, lose their apical–basal polarity typical of epithelial cells, demonstrate enhanced resistance to apoptosis, and return to a more mobile mesenchymal phenotype that promotes peritoneal dissemination. This molecule is also used as a marker of cancer stem cells (CSCs) [6].

Along with EpCAM, the CD44 receptor, widely present on the surface of tumor cells, is used to identify CTCs. It mediates attachment of ovarian cancer cells to the peritoneal mesothelium by binding to hyaluronic acid (HA). CD44, as a biomarker, has several advantages. Firstly, normal cells have a low level of CD44 expression and poor adhesion to hyaluronic acid. Secondly, HA is one of the main components of the tumor extracellular matrix that, along with binding to the CTCs, supports cell integrity [7].

Another CTC marker in ascitic fluid is CD24, which is expressed in 70.1% of ovarian cancer cases and is an independent predictor of survival. CD24 increases the metastatic potential of tumor cells, because it is a ligand of P-selectin, an adhesion receptor on activated endothelial cells. In addition, CD24 induces EMT, which leads to the formation of a highly proliferative phenotype and resistance to chemotherapy via activation of PI3K/Akt, NF-κB, and ERK signaling cascades [8].

A common EMT feature is reduced expression of epithelial cadherin (E-cadherin) and a concomitant increase in or *de novo* expression of neural cadherin (N-cadherin). This so-called "cadherin switch" is associated with increased migratory and invasive behavior of tumor cells. Increased expression of N-cadherin in solid tumors promotes collective cell migration, enhances transmission of fibroblast growth factor(FGF) signals, and modulates the canonical Wnt signaling pathway, which leads to the formation of an aggressive phenotype [9].

CD133 is the most commonly used cell surface antigen for detection and isolation of CSCs in various malignant diseases, including ovarian cancer. High expression of CD133 in tumors is considered a prognostic marker of disease progression. Despite the fact that the functional role of CD133 in malignancies is not fully understood, most studies suggest that CD133 has a significant prognostic value for assessing overall and progression-free survival in various cancer types [10].

Peritoneal dissemination caused by ascites is one of the most unfavorable factors for progression of malignancies. In 2006, Italian authors proposed a Predictive Index Value (PIV) scale for assessing the prevalence of carcinomatosis, taking into account the condition of the parietal peritoneum, diaphragmatic peritoneum, mesentery of the intestine, omentum, intestinal wall, stomach, and liver. The authors showed that with PIV ≥ 8, the probability of R0 resection is practically equal to 0, therefore, neoadjuvant chemotherapy (NACT) is recommended [11].

In connection with the foregoing, the aim of this study was to investigate various populations of tumor cells in the ascitic fluid of ovarian cancer patients and their relationship with the presence of invasive peritoneal implants and the prevalence of carcinomatosis on the PIV scale.

MATERIALS AND METHODS

The study was approved by the local Ethics Committee at Cancer Research Institute of Tomsk NRMC. The prospective study included 22 patients aged 36–76 years with newly diagnosed FIGO stage Ic–IV ovarian cancer, who were admitted for treatment to Cancer Research Institute of Tomsk NRMC. For the purpose of surgical staging, all patients included in

the study underwent laparoscopy using a Karl Storz laparoscopic unit (Germany), followed by morphological examination of biopsy specimens and ascitic fluid. PIV prevalence was also evaluated. The study material included 5 mL EDTA-stabilized ascitic fluid sampled during laparoscopy.

DETERMINING THE PIV INDEX

The PIV index in patients with ovarian cancer was calculated using video materials obtained during laparoscopy with the Karl Storz unit. The PIV scale (Fagotti score, range 0–14) allowed to assess the prevalence of carcinomatosis, taking into account the condition of the parietal peritoneum, diaphragmatic peritoneum, mesentery of the intestine, omentum, intestinal wall, stomach, and liver (Table 1) [11].

Assessing populations of tumor cells in ascitic fluid by multicolor flow cytometry. Different populations of ascites tumor cells (with stemness traits, with EMT traits, without stemness and EMT traits, with a combination of these traits, as well as atypical (hybrid) cell populations) were identified by laser multicolor flow cytometry on a BDFACSCanto apparatus (USA) using a molecular panel of EpCam, CD45, CD44, CD24, CD133, and N-cadherin markers and BD FACSDiva software.

Table 1

Determination of the PIV for assessing the prevalence of carcinomatosis

Parameter	Score = 2	Score = 0
Peritoneal carcinomatosis	Unresectable massive peritoneal damage	Carcinomatosis of isolated regions that can be surgically removed during peritoneoectomy
Diaphragmatic peritoneum	Widespread infiltrating carcinomatosis or confluent nodules in most of the diaphragmatic peritoneum	Focal damage to the peritoneum
Mesentery of the intestine	Big infiltrating nodules or involvement of the mesenteric root	Small nodules that are potentially resectable using argon plasma coagulation
Omentum	Tumor diffusion up to the large curvature of the stomach	Focal damage to the omentum
Infiltration of the bowel	Bowel resection is assumed to be required or miliary carcinomatosis	–
Infiltration of the stomach	Neoplastic damage to the stomach wall	–
Liver damage	Any surface lesions	–

For this purpose, ascitic fluid was incubated with fluorochrome-labeled monoclonal antibodies to CD45: clone HI30 (APC/Cy7) (Biolegend, USA), EpCAM clone 9C4 (PE) (Biolegend, USA), CD44 clone BJ18 (FITC) (Biolegend, USA), CD24 clone ML5 (PE/Cy7) (Biolegend, USA), N-cadherin clone 8C11 (PerCP/Cy5.5) (Biolegend, USA), and CD133 clone AC-133 (APC) (Miltenyi Biotec, USA). Then, erythrocytes in the sample were lysed with a BD Facs lysing solution and washed twice with CellWash buffer. Next, 1 ml of BD Flow was added to the cell pellet. All samples were stored in the dark at 4 °C and analyzed on a flow cytometer within an hour. The cell level was expressed as the number of cells per 1 ml of ascitic fluid.

High-resolution imaging flow cytometry. Cells were incubated with fluorochrome-labeled monoclonal antibodies to CD45: clone HI30 (APC/Cy7) (Biolegend, USA), EpCAM clone 9C4 (PE) (Biolegend, USA), CD44 clone BJ18 (FITC) (Biolegend, USA), CD24 clone ML5 (PE/Cy7) (Biolegend, USA), N-cadherin clone 8C11 (PerCP/Cy5.5) (Biolegend, USA), CD133 clone AC-133 (APC) (Miltenyi Biotec, USA), and DAPI (ZytoVision, Germany). The aliquots were analyzed with an ImageStream Mk II Imaging Flow Cytometer (Luminex, Poland). All data were saved as raw image files for analysis in IDEAS software (version 6.2).

Statistical analysis. The obtained data were processed by variance statistical methods. All the statistical analyses were performed using Statistica 10.0 software package (StatSoft, Inc., USA). Assessment of the normal distribution of the results was performed using the Kolmogorov – Smirnov test. The significance of differences was assessed using the nonparametric Mann – Whitney test (for independent samples). Spearman's correlation analysis (*r)* was also used. The data were presented as the median and the interquartile range *Me* (*Q1–Q3*). The differences were considered statistically significant at $p < 0.05$.

RESULTS

Multicolor flow cytometry of ascitic fluid of ovarian cancer patients revealed 12 popu-

lations of Epcam-positive cells. These included ascites tumor cells without stemness and EMT traits – Epcam+CD45–CD44–CD24–CD133–Ncadherin– (AC-1); ascites tumor cells without stemness traits and with EMT traits – Epcam+CD45–CD44–CD24–CD133–Ncadherin+ (AC-2); ascites tumor cells with stemness traits, without EMT traits, and with phenotypes Epcam+CD45–CD44+CD24–CD133+/-Ncadherin– (AC-3); Epcam+CD45–CD44+CD24+CD133+/–Ncadherin– (AC-6); Epcam+CD45–CD44–CD24+CD133+/-Ncadherin– (AC-8); ascites tumor cells with stemness and EMT traits – Epcam+CD45–CD44+CD24–CD133+/-Ncadherin+ (AC-4); Epcam+CD45–CD44+CD24+CD133+Ncadherin+ (AC-5); Epcam+CD45–CD44–CD24+CD133+Ncadherin+ (AC-7); Epcam+CD45–CD44+CD24+CD133−Ncadherin+ (AC-9); Epcam+CD45–CD44–CD24+CD133–Ncadherin+ (AC-10); atypical (hybrid) cells without stemness traits – Epcam+CD45+CD44–CD24–CD133–Ncadh+/− (AC-11); and atypical (hybrid) cells with stemness traits: Epcam+CD45+CD44+CD24+/−CD133+/−Ncadh+/−(AC-12).

In patients with invasive peritoneal implants, the number of cancer stem cells with phenotypes Epcam+CD45-CD44+CD24+CD133−Ncadherin+ and Epcam+CD45-CD44-CD24+CD133−Ncadherin+ in ascitic fluid significantly exceeded the one in ascitic fluid of ovarian cancer patients with non-invasive peritoneal implants (Table 2).

Table 2

The level of various populations of EpCAM-positive cells in the ascitic fluid of ovarian cancer patients with non-invasive or invasive implants, cells/ml, *Me* (Q_1–Q_3)

Phenotype of ascites tumor cells	Invasive peritoneal implants, $n = 13$	Non-invasive peritoneal implants, $n = 9$
AC-1 EPCam+CD45-CD44-CD24-CD133-Ncadherin-	290 (110–4,270)	55 (0–420) $p_{1-2} = 0.082$
AC-2 EpCam+CD45-CD44-CD24-CD133-Ncadherin+	40 (0–1,220)	170 (0–320) $p_{1-2} = 0.84$
AC-3 EpCam+CD45-CD44+CD24-CD133+/-Ncadherin-	10 (0–530)	0 (0–80) $p_{1-2} = 0.35$
AC-4 EpCam+CD45-CD44+CD24-CD133+/-Ncadherin+	300 (30–720)	40 (0–90) $p_{1-2} = 0.126$
AC-5 EpCam+CD45-CD44+CD24+CD133+Ncadherin+	360 (160–740)	110 (0–283) $p_{1-2} = 0.160$
AC-6 EpCam+CD45-CD44+CD24+CD133+/-Ncadherin-	50 (0,00–1,100)	0 (0–140) $p_{1-2} = 0.640$
AC-7 EpCam+CD45-CD44-CD24+CD133+Ncadherin+	312 (20–940)	0 (0–10) $p_{1-2} = 0.064$
AC-8 EpCam+CD45-CD44-CD24+CD133+/-Ncadherin-	170 (0–1,030)	0 (0–30) $p_{1-2} = 0.194$
AC-9 EpCam+CD45-CD44+CD24+CD133-Ncadherin+	2,030 (520–11,420)	150 (0–540) $p_{1-2} = 0.025$
AC-10 EpCam+CD45-CD44-CD24+CD133-Ncadherin+	1,640 (260–3,870)	10 (0–50) $p_{1-2} = 0.003$
AC-11 EpCam+CD45+CD44-CD24-CD133-Ncadherin+/-	185 (29–716)	219 (18–436) $p_{1-2} = 0.940$
AC-12 EpCam+CD45+CD44+CD24+/-CD133+/-Ncadherin+/-	2,476 (813–3,835)	907 (216–1,032) $p_{1-2} = 0.016$

Figures 1 and 2 present a part of the multicolor flow cytometry protocol in assessing different populations of Epcam+ cells in ascitic fluid of the ovarian cancer patient (Fig. 1) and photographs of the abdominal cavity of this patient obtained during laparoscopy (Fig. 2). The photos show diffuse infiltrating carcinomatosis, confluent nodules in different regions of the diaphragmatic peritoneum, and big infiltrating nodules.

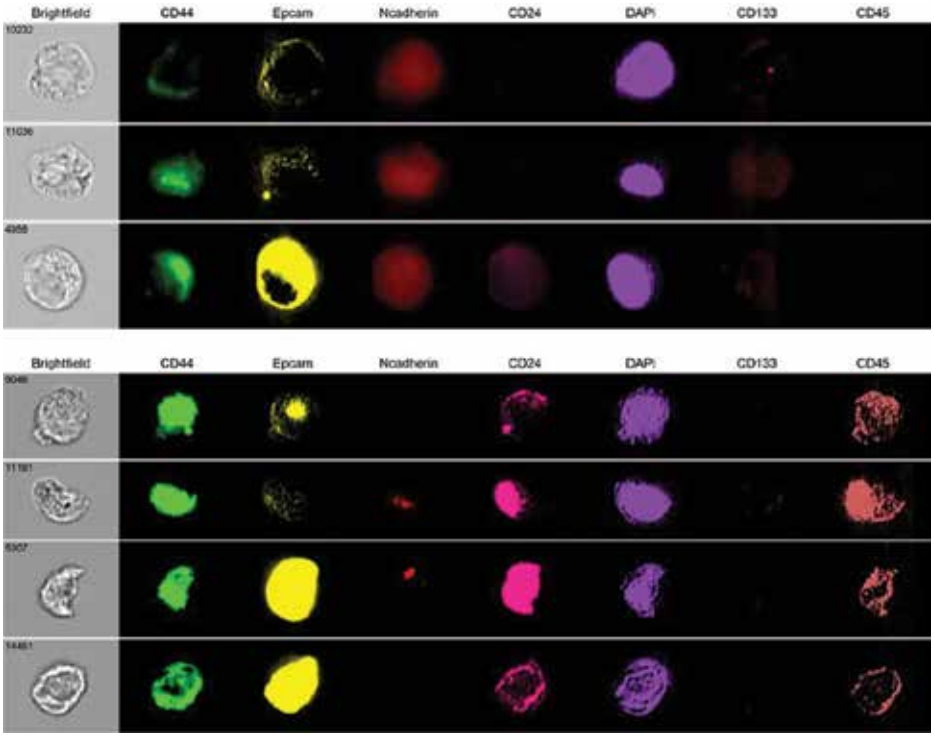

Fig. 1. Different populations of EpCam-positive cells in the ascitic fluid of the ovary cancer patient, high-resolution imaging flow cytometry (Amnis ImageStreamX)

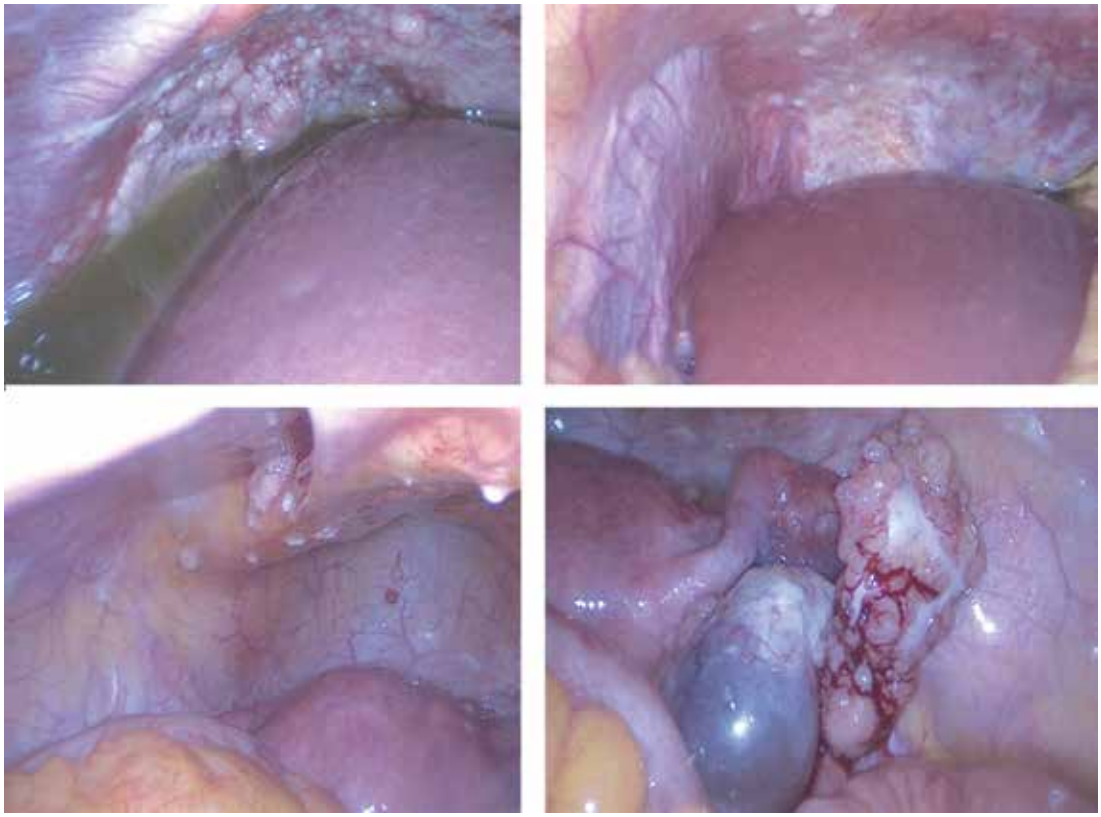

Fig. 2. Photographs of the abdominal cavity of the ovary cancer patient V, 50 years old, T3c Nx M0, obtained during laparoscopy using a Karl Storz laparoscopic unit. PIV index = 8

We assessed the extent of carcinomatosis using the PIV scale in our study. The range of the PIV index in patients included in the study ranged from 0 to 11. The correlation analysis showed that a statistically significant, positive relationship with the PIV index was observed in AC-9 cells with the Epcam+CD45-CD44-CD24+CD133− Ncadherin+ phenotype (r = –0.58; p = 0.004) and in atypical (hybrid) cell forms with stemness features Epcam+CD45+CD44+CD24+/−CD133+/− Ncadherin+/− (r = –0.51; p = 0.01) (Table 3).

Table 3

Correlation between the number of dissimilar populations of Epcam-positive cells in the ascitic fluid of ovarian cancer patients and the PIV index

A pair of parameters	*r*	*p*
AC1 – PIV	0.32	0.137
AC2 – PIV	−0.02	0.92
AC3 – PIV	0.20	0.35
AC4 – PIV	0.19	0.38
AC5 – PIV	0.29	0.27
AC6 – PIV	0.12	0.64
AC7 – PIV	0.46	0.07
AC8 – PIV	0.21	0.42
AC9 – PIV	0.34	0.11
AC10 – PIV	0.58	0.004
AC11 – PIV	0.15	0.49
AC12 – PIV	0.51	0.01

DISCUSSION

Metastasis of ovarian cancer occurs mainly due to detachment of cells from the primary tumor and their invasion of the abdominal cavity filled with malignant ascites. The cells spread widely with fluid flow and cause secondary tumor growth. At all stages of this unique process, tumor cells change their phenotype and co-evolve together with surrounding fibroblasts, macrophages, adipocytes, endothelial, and other cells.

This study revealed different tumor cell populations in ascitic fluid: atypical forms (hybrid cells) both with and without stemness traits, with EMT traits, and with a combination of these traits and stromal and immune cell populations, identification and characterization of which may be a useful tool in predicting the disease course and response to chemotherapy.

According to the literature data, out of 150 different marker combinations, the most common panel includes three markers: CD44, CD24, and Epcam. Expression of these molecules in OVCAR-5, SKOV-3, and IGROV-1 lines corresponded to cells with greater colony-forming ability. These cells demonstrated a short *in vivo* relapse-free interval after xenotransplantation and a greater migratory capacity in an *in vitro* invasion study compared to CD44-CD24-Epcam cells. In addition, doxorubicin, cisplatin, and paclitaxel promoted an increase in this population, which indicates drug resistance, but Müllerian inhibiting substance (MIS) effectively suppressed its growth [12].

In our study, we showed that the concentration of tumor cells with phenotypes Epcam+CD45-CD44+CD24+CD133−Ncadherin+ and Epcam+CD45-CD44-CD24+CD133−Ncadherin+ was higher in the ascitic fluid of ovarian cancer patients with invasive peritoneal implants compared to the level of these cells in the ascitic fluid of ovarian cancer patients with non-invasive peritoneal implants. We also showed the presence of atypical (hybrid) cells in the ascitic fluid of ovarian cancer patients. The number of atypical (hybrid) cells with stemness features (Epcam+CD45+CD44+CD24+/− CD133+/− Ncadherin+/−) was also significantly higher in the ascitic fluid of patients with invasive peritoneal implants compared to the level of these cells in the ascitic fluid of ovarian cancer patients with non-invasive peritoneal implants. It should be noted that these cell populations are positive for the CD24 marker. In a study aimed at investigating the mechanisms of acquired drug resistance, it was shown that the CD24+ fraction obtained from samples of tumor tissue from the ovarian cancer patient was relatively resistant to cisplatin *in vitro* compared to its CD24− cells. In addition, the tumorigenicity of CD24+ also surpasses that of CD24− cells, as evidenced by the shorter period before the appearance of tumors in mice (Nude mice) injected with an equal number of CD24+ and CD24− cells. It was also found that CD24+ cells express higher mRNA levels of several stemness-related genes (including Nestin, β-catenin, Bmi-1, Oct4, Oct3/4, Notch1, and Notch4, which are involved in modulating many stem cell functions) and a lower E-cadherin mRNA level than CD24− cells [13].

Our clinical trial results are consistent with the literature. Numerous studies have shown that the Epcam+CD44+CD24+CD133+CD117+ population has an increased ability to initiate cancer and/or stimulate metastasis *in vivo* [13, 14].

In a mouse model (NOD/Shi-scid/IL-2Rγ null mice), CD24+ and CD133+ cells were demonstrated to be more capable of forming spheres, spreading, and initiating tumors *in vivo*. In addition, CD24+ cells showed a more mesenchymal phenotype with higher expression of Twist1, Snail, and Vimentin, which relates the CD24 marker to the EMT phenotype. Interestingly, CD24- cells are also capable of initiating tumor growth, albeit to a lesser extent than CD24+.

This is probably determined by the fact that a subset of CD24- cells with stemness traits has a lower proliferation rate than CD24+ cells [15].

The hybrid cells and multicellular aggregates which role in cancer has long been examined [16–18], were also discovered in our study. Other researchers revealed fusion of blood cells and epithelial cells in Lewis lung carcinoma and metastatic epithelial ovarian carcinoma [19]. Similar results were obtained by A.E. Powell et al. for colorectal cancer [20]. Another study claimed that their formation and form depend on the cadherin expression profile. For instance, Ncad+ cells formed stable and dense spherical structures, and Ecad+ cells – clusters with lower adhesion (compared to Ncad+) [21].

In our study, we showed that the number of Epcam+CD45-CD44-CD24+CD133− Ncadherin+ cells and atypical (hybrid) Epcam+CD45+CD44+CD24+/− CD133+/-Ncadherin+/− cells has a direct correlation with the PIV index, characterizing the prevalence of carcinomatosis. This index takes into account the condition of the parietal peritoneum, diaphragmatic peritoneum, mesentery of the intestine, omentum, intestinal wall, stomach, and liver [11, 22–24]. A. Fagotti et al. (2006) showed that with PIV ≥ 8, the probability of R0 resection is practically equal to 0, and NACT is recommended [11]. The effectiveness of this approach was subsequently confirmed by a series of randomized trials.

In 2017, data from a Dutch multicenter randomized trial were published, including treatment analysis for 201 patients with advanced ovarian cancer [24]. A total of 102 patients underwent laparoscopy with PIV determination to assess the possibility of performing primary optimal cytoreductive surgery (treatment group), and 99 patients underwent primary cytoreductive surgery without laparoscopic assessment (control group). The authors considered a decrease in the number of unjustified laparotomies (diagnostic operations, suboptimal cytoreductions), that reduce the effectiveness of treatment in this category of patients, as one of the main advantages of the proposed technique [24].

The results obtained in our study showed a direct correlation between the PIV index and the level of ascites tumor cells with EMT traits (AC-10) and atypical (hybrid) cells with stemness features (AC-12). It can be assumed that determining Epcam+CD45-CD44-CD24+CD133−Ncadherin+ and Epcam+CD45+CD44+CD24+/−CD133+/−Ncadherin+/− cells in ascitic fluid will be useful for specifying the treatment strategy for ovarian cancer patients.

CONCLUSION

The results of the study show high heterogeneity of tumor cells in the ascitic fluid of patients with ovarian cancer. The presence of atypical (hybrid) forms of Epcam-positive cells is of interest for cell biology and requires further research. The cell populations identified in our study (Epcam+CD45-CD44-CD24+CD133− Ncadherin+ and Epcam+CD45+CD44+CD24+/− CD133+/−Ncadherin+/−) and their detection in ascitic fluid can be useful for determining the treatment strategy for patients with ovarian cancer.

Further study of various populations of tumor cells in ascitic fluid and their relationship with the clinical course of the disease and effectiveness of chemotherapy for patients with ovarian cancer is reasonable and opens up great prospects for practical developments in the field of targeted therapy and liquid biopsy.

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REFERENCES

1. Villert A.B., Kolomiets L.A.,Yunusova N.V., Ivanova A.A. Ascites as an object of studies in ovarian cancer. *Siberian Journal of Oncology*. 2019; 18 (1): 116–123 (in Russ.). DOI: 10.21294/1814-4861-2019-18-1-116-123.
2. Weinberg R.A. Coevolution in the tumor microenvironment. *Nat. Genet.* 2008; 40: 494–495. DOI: 10.1038/ng0508-494.
3. Hyler A.R., Baudoin N.C., Brown M.S., Stremler M.A., Cimini D., Davalos R.V., Schmelz, E.M. Fluid shear stress impacts ovarian cancer cell viability, subcellular organization, and promotes genomic instability. *PLoS One.* 2018; 13 (3): e0194170. DOI: 10.1371/journal.pone.
4. Kaigorodova E.V., Fedulova N.V., Ochirov M.O., Dyakov D.A., Molchanov S.V., Chasovskikh N.Yu. Dissimilar populations of tumor cells in ascitic fluid of ovarian cancer patients. *Bulletin of Siberian Medicine.* 2020; 19 (1): 50–59 (in Russ.). DOI: 10.20538/1682-0363-2020-1-50-58.
5. Kaigorodova E.V., Savelieva O.E., Tashireva L.A., Tarabanovskaya N.A., Simolina E.I., Denisov E.V., Slonimskaya E.M., Choynzonov E.L., Perelmuter V.M. Heterogeneity of circulating tumor cells in neoadjuvant chemotherapy

of breast cancer. *Molecules.* 2018; 23 (4): 727–737. DOI: 10.3390/molecules23040727.
6. Tayama S., Motohara T., Narantuya D., Li C., Fujimoto K., Sakaguchi I., Tashiro H., Saya H., Nagano O., Katabuchi H. The impact of EpCAM expression on response to chemotherapy and clinical outcomes in patients with epithelial ovarian cancer. *Oncotarget.* 2017; 8 (27): 44312–44325. DOI: 10.18632/oncotarget.17871.
7. Zheng J., Zhao S., Yu X., Huang S., Liu H.Y. Simultaneous targeting of CD44 and EpCAM with a bispecific aptamer effectively inhibits intraperitoneal ovarian cancer growth. *Theranostics.* 2017; 7 (5): 373–1388. DOI: 10.7150/thno.17826.
8. Nakamura K., Terai Y., Tanabe A., Ono Y.J., Hayashi M., Maeda K., Fujiwara S., Ashihara K., Nakamura M., Tanaka Y. et al. CD24 expression is a marker for predicting clinical outcome and regulates the epithelial-mesenchymal transition in ovarian cancer via both the Akt and ERK pathways. *Oncol. Rep.* 2017; 37 (6): 3189–3200. DOI: 10.3892/or.2017.5583.
9. Mrozik K.M., Blaschuk O.W., Cheong C.M., Zannettino A.C., Vandyke W.K. N-cadherin in cancer metastasis, its emerging role in haematological malignancies and potential as a therapeutic target in cancer. *BMC Cancer.* 2018; 18 (1): 939. DOI: 10.1186/s12885-018-4845-0.
10. Glumac P.M., LeBeau A.M. The role of CD133 in cancer: A concise review. *Clin. Transl. Med.* 2018; 7 (1): 18. DOI: 10.1186/s40169-018-0198-1.
11. Fagotti A., Ferrandina G., Fanfani F., Ercoli A., Lorusso D., Rossi M., Scambia G. A laparoscopy-based score to predict surgical outcome in patients with advanced ovarian carcinoma: A pilot study. *Ann. Surg. Oncol.* 2006; 13 (8): 1156–1161.
12. Wei X., Dombkowski D., Meirelles K., Pieretti-Vanmarcke R., Szotek P.P., Chang H.L., Preffer F.I., Mueller P.R., Teixeira J., MacLaughlin D.T. et al. Mullerian inhibiting substance preferentially inhibits stem/progenitors in human ovarian cancer cell lines compared with chemotherapeutics. *Proc. Natl. Acad. Sci. USA.* 2010; 107 (44): 18874–18879. DOI: 10.1073/pnas.1012667107.
13. Gao M.Q., Choi Y.P., Kang S., Youn J.H., Cho N.H. CD24+ cells from hierarchically organized ovarian cancer are enriched in cancer stem cells. *Oncogene.* 2010; 29 (18): 2672–2680. DOI: 10.1038/onc.2010.35.
14. Burgos-Ojeda D., Rueda B.R., Buckanovich R.J. Ovarian cancer stem cell markers: Prognostic and therapeutic implications. *Cancer Lett.* 2012; 322 (1): 1–7. DOI: 10.1016/j.canlet.2012.02.002.
15. Burgos-Ojeda D., Wu R., McLean K., Chen Y.C., Talpaz M., Yoon E., Cho K.R., Buckanovich R.J. CD24+ Ovarian cancer cells are enriched for cancer-initiating cells and dependent on jak2 signaling for growth and metastasis. *Mol. Cancer Ther.* 2015; 14 (7): 1717–1727. DOI: 10.1158/1535-7163.MCT-14-0607.
16. Gast C.E., Silk A.D., Zarour L. et al. Cell fusion potentiates tumor heterogeneity and reveals circulating hybrid cells that correlate with stage and survival. *Sci. Adv.* 2018; 4 (9): e7828. Published 2018 Sept. 12. DOI: 10.1126/sciadv.aat7828.
17. Carter A. Cell fusion theory: can it explain what triggers metastasis? *J. Natl. Cancer Inst.* 2008; 100 (18): 1279–1281. DOI: 10.1093/jnci/djn336.
18. Larizza L., Schirrmacher V., Pflüger E. Acquisition of high metastatic capacity after *in vitro* fusion of a nonmetastatic tumor line with a bone marrow-derived macrophage. *J. Exp. Med.* 1984; 160 (5): 1579–1584. DOI: 10.1084/jem.160.5.1579.
19. Ramakrishnan M., Mathur S.R., Mukhopadhyay A. Fusion-derived epithelial cancer cells express hematopoietic markers and contribute to stem cell and migratory phenotype in ovarian carcinoma. *Cancer Res.* 2013; 73 (17): 5360–5370. DOI: 10.1158/0008-5472.CAN-13-0896.
20. Powell A.E., Anderson E.C., Davies P.S. et al. Fusion between Intestinal epithelial cells and macrophages in a cancer context results in nuclear reprogramming. *Cancer Res.* 2011; 71 (4): 1497–1505. DOI: 10.1158/0008-5472.CAN-10-3223.
21. Klymenko Y., Johnson J., Bos B., Lombard R., Campbell L., Loughran E., Stack M.S. Heterogeneous cadherin expression and multicellular aggregate dynamics in ovarian cancer dissemination. *Neoplasia.* 2017; 19 (7): 549–563. DOI: 10.1016/j.neo.2017.04.002.
22. Vizzielli G., Costantini B., Tortorella L., Pitruzzella I., Gallotta V., Fanfani F., Gueli Alletti S., Cosentino F., Nero C., Scambia G. et al. A laparoscopic risk-adjusted model to predict major complications after primary debulking surgery in ovarian cancer: A single-institution assessment. *Gynecol. Oncol.* 2016; 142 (1): 19–24. DOI: 10.1016/j.ygyno.2016.04.020.
23. Feng Z., Wen H., Jiang Z., Liu S., Ju X., Chen X., Xia L., Xu J., Bi R., Wu X. A triage strategy in advanced ovarian cancer management based on multiple predictive models for R0 resection: A prospective cohort study. *J. Gynecol. Oncol.* 2018; 29 (5): e65. DOI: 10.3802/jgo.2018.29.e65.
2. Rutten M.J., Van Meurs H.S., Van De Vrie R., Naaktgeboren C.A., Fons G., Opmeer B.C., Spijkerboer A., Bossuyt P.M., Kenter G.G., Buist M.R. et al. Laparoscopy to predict the result of primary cytoreductive surgery in patients with advanced ovarian cancer: a randomized controlled trial. *J. Clin. Oncol.* 2017; 35 (6): 613–621. DOI: 10.1200/JCO.2016.69.2962.

Authors contribution

Kaigorodova E.V., Ochirov M.O., Molchanov S.V., Dyakov D.A., Rogachev R.R., Kovalev O.V., Vtorushin S.V. – carrying out of research, analysis and interpretation of data. Ochirov M.O., Molchanov S.V, Shpileva O.V., Chernyshova A.L. – diagnosis and treatment of patients with ovarian cancer. Kaigorodova E.V. – conception and design, drafting of the manuscript.

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Retrospective analysis of the effectiveness of local corticosteroid therapy in children with oligoarticular juvenile idiopathic arthritis

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ABSTRACT

Background. Despite the progress in diagnosis and treatment of chronic rheumatic diseases in children, the choice of anti-inflammatory drugs in case of the onset of oligoarticular juvenile idiopathic arthritis (JIA) still remains relevant. Till present, pediatric rheumatologists have not reached a consensus on this issue yet.

The aim of this study was to search for predictors of early failure of local steroid therapy and assess its feasibility in patients with oligoarticular JIA.

Materials and methods. In a retrospective study, 92 children aged 11 months–9 years with chronic oligoarticular JIA without extra-articular manifestations were monitored. The features of the clinical, instrumental, and laboratory diagnosis during the disease onset were studied, along with the dynamics of the articular syndrome and the effectiveness of intra-articular administration of corticosteroid drugs.

Results and discussion. The data on 92 children with 164 active joints who received 218 local intra-articular injections of triamcinolone acetonide at the onset of the disease were analyzed. Intra-articular injections of triamcinolone acetonide at a dose of 20–40 mg were performed with an interval of 3, 6, and 12 months, depending on the intensity of the disease. In about one third of children with oligoarticular JIA, arthritis became inactive on average after two intra-articular injections of triamcinolone acetonide. The study did not reveal the predictors of early ineffective topical corticosteroid monotherapy in children. No clinical, instrumental, and laboratory signs were identified that would directly indicate the need for early therapy with methotrexate.

Conclusion. Triamcinolone acetonide is an effective and safe drug for children with oligoarticular JIA. Despite the widespread use of biological, gene, and other innovative therapies, application of local corticosteroids as the first-line therapy in children with oligoarticular JIA should not be neglected.

Key words: oligoarticular juvenile idiopathic arthritis, local steroid therapy, triamcinolone acetonide.

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Conformity with the principles of ethics. All patients (their representatives) signed an informed consent to participate in the study. The study was approved by the local Ethics Committee at H. Turner National Medical Research Center for Children's Orthopedics and Trauma Surgery (Protocol No. 1 of 20.01.2014).

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Оценка эффективности локальной стероидной терапии у детей с олигоартикулярным вариантом ювенильного артрита: результаты ретроспективного исследования

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РЕЗЮМЕ

Актуальность. *Несмотря* на большой *прогресс* в диагностике и лечении ревматических заболеваний у детей, все еще остается актуальным вопрос выбора противовоспалительной терапии в случае дебюта хронического олигоартрита. Единого мнения на этот счет у детских ревматологов нет и по настоящий день.

Цель. Поиск предикторов ранней неэффективности и оценка целесообразности локальной стероидной монотерапии у пациентов с дебютом олигоартикулярного варианта ювенильного артрита.

Материалы и методы. Основу ретроспективного исследования составили 92 ребенка в возрасте от 11 мес до 9 лет с хроническим олигоартритом без экстраартикулярных проявлений (олиго-ЮА). Были изучены особенности клинико-инструментальной и лабораторной диагностики в дебюте заболевания, динамика суставного синдрома и эффективность внутрисуставного введения глюкокортикостероидного препарата.

Результаты и обсуждение. Проанализированы данные 92 детей со 164 «активными» суставами, которые получили 218 изолированных внутрисуставных манипуляций по введению стероидного препарата (триамцинолон ацетонид). Триамцинолон ацетонид вводился внутрисуставно в дозе 20–40 мг с интервалом 3, 6, 12 мес в зависимости от активности заболевания. Около одной трети детей с олиго-ЮА достигли неактивной стадии болезни в среднем после двукратного введения данного препарата. Исследование не позволило выявить предикторов ранней неэффективности монотерапии локальными стероидными препаратами у детей. Не выявлено достоверных клинико-инструментальных и лабораторных признаков, которые напрямую указывали бы на необходимость начала ранней терапии препаратом «Метотрексат».

Заключение. Триамцинолон ацетонид является эффективным и безопасным препаратом у детей с олигоартикулярным вариантом ювенильного артрита. Несмотря на популяризацию генно-инженерной биологической терапии, не следует пренебрегать лечением локальными стероидными препаратами как первой линией противоревматической терапии у детей.

Ключевые слова: ювенильный артрит, хронический олигоартрит, триамцинолон ацетонид.

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

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

Соответствие принципам этики. Все пациенты и их представители подписали добровольное информированное согласие на участие в исследовании. Исследование одобрено локальным этическим комитетом Научно-исследовательского детского ортопедического института им. Г.И. Турнера (протокол № 1 от 20.01.2014).

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INTRODUCTION

Juvenile idiopathic arthritis (JIA) is a chronic childhood inflammatory musculoskeletal disease which progressive course leads to joint contractures and loss of their function. Increased incidence of JIA worldwide at the end of the 20th century, joint contractures at the early stage of the disease, and high disability rate determine the relevance of improving the methods of diagnosis and treatment of the rheumatic pathology. JIA is an autoimmune disorder characterized by chronic inflammation of the synovium in one or more joints inevitably resulting in arthrosis–arthritis [1, 2]. Therefore, rapid achievement of the inactive stage of arthritis is considered as a priority goal of drug therapy in JIA. Pediatric rheumatologists have not reached a consensus on the best method of treatment of oligoarticular JIA to this day.

JIA encompasses a group of heterogeneous forms of arthritis characterized by persistent joint inflammation of unknown origin lasting longer than 6 weeks. This heterogeneity is determined by different clinical forms of arthropathies, including the ones with extra-articular manifestations. Chronic oligoarthritis with simultaneous damage to no more than 4 joints prevails in children with JIA [3].

Oligoarticular JIA can be persistent (this type affects fewer than four joints throughout the course of the disease), extended (asymmetric arthritis that developed from oligoarthritis 6 months after the onset of the disease) or have a short-term (abortive) course. The most common manifestation of oligoarthritis is asymmetric lesion of the joints of the lower extremities (except for the hip joint).

This subtype mostly affects pre-school girls with the onset at the age of 6–8 years (peak at the age of 2–4 years). Besides the knee and ankle joints, the elbow, wrist, or one or two small joints of the hand and (or) foot can be remotely involved. More rarely, the clinical course of oligoarticular JIA may resemble symmetric arthritis or monoarthritis. Besides high risk of eye lesions, features of oligoarticular JIA include low laboratory disease activity and variability of clinical symptoms in the joint [4, 5].

Currently, the majority of pediatric rheumatologists adhere to a stepwise scheme of treatment in oligoarticular JIA. This method consists in gradual need for amplification of anti-inflammatory therapy, which is determined by the persistence of "active arthritis", progressing articular syndrome, or uveitis (the exception is systemic arthritis).

This approach to JIA treatment allows to provide fast inactivation of arthritis and control over the disease course [6, 7]. The treatment strategy for JIA without systemic manifestations can be represented by three main directions: proactive therapy, actual treatment of clinical symptoms of arthritis, and orthopedic and surgical elimination of disease consequences.

Despite the evidence of highly aggressive nature of JIA, a number of unresolved issues regarding the treatment strategy for oligoarticular JIA remain. It is well known that some children with uveitis-negative chronic oligoarthritis have experience in controlling the course of the disease without systemic disease modifying antirheumatic drugs (DMARDs). The opinions of pediatric rheumatologists about treatment of this subtype of oligoarticular JIA in most cases differ.

The aim of this study was to assess the feasibility and efficacy of local steroid therapy for the onset of oligoarticular JIA in children in the Russian Federation.

MATERIALS AND METHODS

The study included 92 children (85% were girls) with 164 active joints who were treated at the Orthopedics and Rheumatology Department No. 7 of H. Turner National Medical Research Center for Children's Orthopedics and Trauma Surgery from 2012 to 2018. All children met Edmonton ILAR criteria for oligoarticular JIA and did not have extra-articular manifestations (ILAR 1997; 2001; the Edmonton revision 2004). All patients (their representatives) signed an informed consent to take part in the study.

As of the time of inclusion in the study, none of the patients had received therapy with DMARDs (Fig. 1). The age of the children ranged from 11 months to 9 years (average age (4.2 ± 2.6) years). Children under 2 years accounted for 32.7% of cases (30 / 92; all girls), children from 2 to 6 years accounted for 54.3% (50 / 92; 40 girls, 10 boys), and a group of older children amounted to 13% (12 / 92; all girls).

Triamcinolone acetonide at a dose of 20–40 mg / joint was administered intra-articularly without ultrasound guidance in no more than 3 joints

at a time. Extra-articular administration of the drug was prohibited. The maximum allowable number of consecutive isolated intra-articular injections (is-IAI) was 4. The interval between injections varied from 2 to 12 months.

All children were divided into two groups depending on the intensity of the disease. The first group contained children with inactive arthritis resulting from effective local corticosteroid therapy. The second group included children who were prescribed therapy with DMARDs due to low effectiveness of local steroid injections. Joining of eye lesions and (or) subsequent increase in the active joint count by 2 or more at this stage of therapy, and 3 or 4 ineffective consecutive intra-articular injections with corticosteroids in one joint were considered a reason for the start of parenteral methotrexate therapy (at the rate of 15 mg / m² / week).

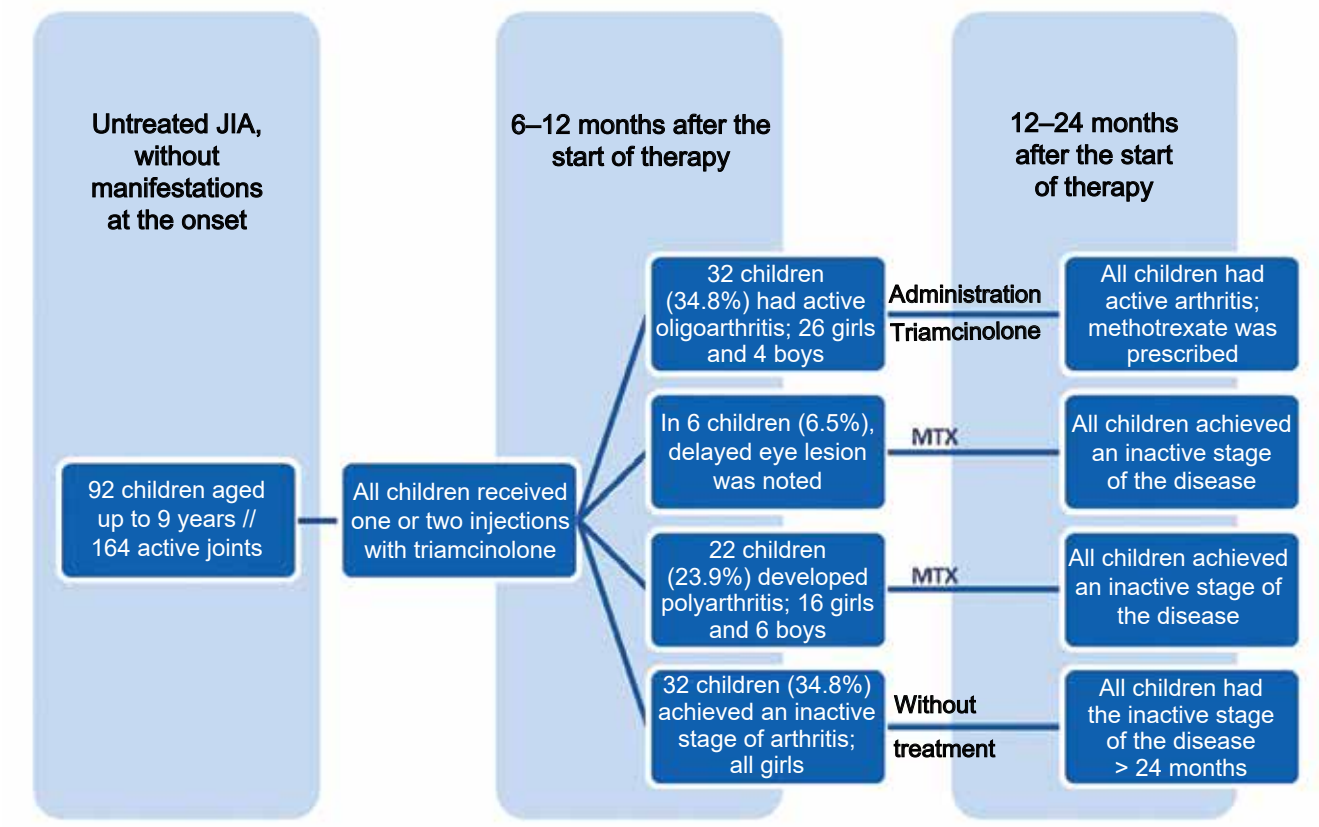

Fig. 1. Study design. MTX – treatment with methotrexate

The degree of disease intensity and the dynamics in the active joint count against the background of therapy, markers of active inflammation in the blood serum and synovial fluid, and the nature of the joint lesion according to instrumental data as of the moment of the first visit were assessed. Achievement of inactive disease and clinical remission of JIA were evaluated according to the criteria proposed by C. Wallace et al. in 2011 (the disease phase that lasted no less than 24 months was considered statistically significant). Assessment of X-ray changes in juvenile arthritis was performed using a modified Steinbrocker scoring method (2000).

The disease intensity and the effectiveness of treatment of oligoarticular JIA were evaluated by the clinical Juvenile Arthritis Disease Activity Score (cJADAS10) adapted for pediatric rheumatology practice [7]. Digital data were statistically processed using the Microsoft Excel and Statistica 6.0 software package (Microsoft, USA). A comparative analysis of empirical data was presented in tables. The results were represented as the median and the interquartile range *Me* [25; 75].

Immunological research was carried out at the laboratory of H.Turner National Medical Research Center for Children's Orthopedics and Trauma

Surgery (head – Bogdanova S.L.) and the laboratory of autoimmune diseases of Saint-Petersburg State Medical University (head – Lapin S.V.). The concentration of tumor necrosis factor alpha (TNF-α) in the serum and synovial fluid was determined using enzyme-linked immunosorbent assay (TNF-α-ELISA-BEST kit; Vector-Best, Russia). The serum and synovial fluid concentration of interleukin 6 (IL-6) was determined by the electrochemiluminescence immunoassay (ECLIA) method on the Cobas E411 analyzer (Roche, Switzerland). Antinuclear factor (ANF) in the blood serum was detected by the indirect immunofluorescence technique using HEp-2 human epithelial cells derived from a larynx carcinoma as the substrate (laboratory of Saint-Petersburg State Medical University).

RESULTS

The efficacy of therapy for oligoarticular JIA in 92 children was studied. The duration of the disease as of the moment the diagnosis of JIA was established was 4–5 [3;8] months on average. The average patient follow-up was 48 [38;62] months, the maximum follow-up period was 98 months. A total of 218 active joints were injected with triamcinolone acetonide: knees – 156 injections, ankles – 62 injections. Simultaneous administration of the corticosteroid in 2 or more joints was performed in almost one third of children (simultaneous injection in 2 or more joints is one manipulation). Single or double is-IAI of triamcinolone was performed in 65.2% (60 / 92) of children, the injection was performed three or more times in 34.8% (32 / 92) of children. Only one joint was treated in 43.5% (40 / 92) of children, in 39.1% (36 / 92) of cases – two different joints, and in 17.4% (16 / 92) of children – three or more joints were treated.

34.8% of children (32 / 92; all girls) achieved the inactive stage of the disease after the intra-articular administration of the corticosteroid; of them 21.7% (20 / 92) of children had monoarthritis, 8.7% (8 / 92) – asymmetric oligoarthritis, and 4.4% (4 / 92) – symmetric oligoarthritis of the lower extremities (Table 1). The average number of intra-articular injections in this group of children was 2 [1.75; 2], The average duration of the inactive phase of the disease between two consecutive injections was 7 [5.25; 10] months.

Table 1

Clinical manifestation of the onset of oligoarticular JIA in children, abs. (%)

Parameter	Group 1, *n* = 32	Group 2, *n* = 60	*p*
Monoarthritis	20♀ (62.5%) 0♂ (0%)	20♀ (33.3%) 4♂ (6.7%)	<0.01 >0.05
Asymmetric oligoarthritis	8♀ (25%)	16♀ (26.7%)	>0.05
Symmetric oligoarthritis	4♀(12.5%) ♂ (0%)	12♀ (20%) 4♂ (6.7%)	>0.05 >0.05
Psoriatic oligoarthritis	0♀ (0%) 0♂ (0%)	2♀ (3.3%) 2♂(3.3%)	>0.05 >0.05

Note. ♀ – girls, ♂ – boys

Table 2

Clinical and laboratory features at the onset of oligoarticular JIA in children

Parameter	Group 1, *n* = 32	Group 2, *n* = 60	*p*
Girls, abs. (%)	20 (83.3%)	50 (92.6%)	< 0.01
Age of JIA onset, years, *Me* [25;75]	2 [2; 3]	4 [3; 7]	> 0.05
Active joint count, abs., *Me* [25;75]	1 [1; 2]	1 [1; 2]	> 0.05
JADAS, *Me* [25;75]	10 [8; 12]	11 [8.5; 14]	> 0.05
ESR, mm / h, *Me* [25;75]	14 [6; 28]	17 [10; 25]	> 0.05
CRP, mg /l, *Me* [25;75]	2.7 [1.3; 4.5]	2.2 [1.8; 9.3]	> 0.05
Hemoglobin, g / l, *Me* [25;75]	114 [110; 128]	112 [108; 124]	> 0.05
Leukocytes, 10^9 / l, *Me* [25;75]	8.0 [6.8; 10.6]	7.4 [6.2; 10.8]	> 0.05
Platelets, 10^9 / l, *Me* [25;75]	442 [416; 476]	438 [408; 466]	> 0.05
Gamma globulins, %, *Me* [25;75]	21.6 [19.6; 22.3]	22.3 [20.4; 23.5]	> 0.05
IL-6, pg /ml, *Me* [25;75]	4.1 [2.5; 6.75]	5.8 [3.4; 10.5]	> 0.05
TNFα serum, pg /ml, *Me* [25;75]	0.65 [0.2; 0.85]	0.6 [0.1; 0.9]	> 0.05
ANF ≥ 1/160, abs. (%)	26 (81.25%)	45 (75%)	> 0.05
ANF ≥ 1/1,280, abs. (%)	12 (37.5%)	14 (23.3%)	> 0.05

Note. JADAS – Juvenile Arthritis Disease Activity Score; ESR – erythrocyte sedimentation rate; CRP – C-reactive protein; TNFα serum – tumor necrosis factor alpha in the serum; ANF – antinuclear factor (here and in Table 5).

The remaining 65.2% of children (60 /92) did not achieve the inactive phase of the disease after is-IAI of triamcinolone, which required application of conventional anti-rheumatic therapy. In this group, 23.9% of children (22 / 92; 16 girls and 6 boys) developed polyarthritis after two is-IAI; in 6.5% of cases (6 / 92; all girls), delayed onset of uveitis was registered, and in 34.8% of children (32 / 92; 26 girls and 4 boys), active disease persisted after more than three is-IAI. The average number of intra-articular injections in this group of children was 3 [2; 4].

The average duration of the inactive phase of the disease between the first two consecutive injections was 5.5 [4.25; 7] months and between subsequent injections – 2 [2; 3] months.

The main treatment-related complications were post-injection reversible (transient) manifestations of local atrophy and hypopigmentation of the skin. Complication rates in the knee and ankle joints did not exceed 10%. No other complications were noted. The comparative analysis of the active joint count and cJADAS-10 score in children of both groups did not reveal statistically significant differences in the disease onset.

The main laboratory inflammatory markers (erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and the platelet and leukocyte levels) were the same in both groups of children with oligoarticular JIA. The concentrations of TNFα and IL-6 in the blood serum and the titer of antinuclear antibodies (ANA) were comparable (Table 2).

The average IL-6 and TNFα levels in the inflamed synovial fluid at the onset of inactive and active oligoarticular JIA did not significantly differ (Table 3). Moreover, the nature of the articular lesion and the frequency of early erosion did not differ significantly, despite different outcomes of the disease. Dry synovitis, which is considered a rare form of JIA, had almost the same incidence in both groups (Table 4).

An attempt was made to search for models of predicting the efficacy of non-systemic steroid therapy in children. It was revealed that girls with monoarthritis predominated in the group of children with inactive oligoarticular JIA (20 / 62.5% – achieved remission vs. 20 /33.3% – active JIA; $p = 0.0087$, $\chi^2 = 2.8$). However, the statistical analysis did not reveal a correlation between monoarthritis and the inactive stage against the background of local steroid therapy (R^2 (coefficient of determination) = 0.158, T = 1.23, odds radio (OR) = 4.01, 95% confidence interval (CI) 0.57–27.69, $p = 0.227$).

Table 3

Cytological analysis and immunological features of synovial fluid at the onset of oligoarticular JIA in children

Parameter	Group 1, $n = 32$	Group 2, $n = 60$
Cytosis, 10^9 / l	4.3 [3.5; 7]	4.35 [3.7; 6.35]
Lymphocytes, %	54 [31.75; 66.5]	32 [26.5; 53.5]
Neutrophils, %	18 [12.7; 37.5]	40 [6; 57.3]
Monocytes, %	16 [10; 21.75]	14 [9.2; 19.3]
Synoviocytes, %	1 [0; 7]	1 [0; 4]
Ragocytes, %	3 [2; 4]	3 [2; 7]
IL-6 synovial (sIL-6), pg /ml	2,208 [710; 4,564]	3,234 [1,265; 16,902]
TNFα synovial (sTNFα), pg /ml	3.3 [2.5; 3.8]	1.1 [0.6; 3.7]

Note. Ragocytes – cells (macrophages, neutrophils) containing large granules – phagolysosomes, including immune complexes, various immunoglobulins, and rheumatoid factor; $p > 0.05$.

A significant positive correlation was not found in the multiple linear regression analysis between the efficacy of steroid therapy and different inflammatory laboratory parameters (Table 5). The linear regression analysis revealed a direct relationship between the ESR (mm / h) and interleukin 6 in the synovial fluid ($p < 0.001$; Fig. 2).

Table 4

Clinical and instrumental features of synovitis at the onset of oligoarticular JIA in children, abs. (%)

Parameter	Group 1, $n = 32$	Group 2, $n = 60$
Exudative synovitis	16 (50%)	28 (46.7%)
Exudative and proliferative non-erosive synovitis	9 (28.125%)	18 (30.0%)
Exudative and proliferative erosive synovitis	1 (3.125%)	2 (3.3%)
Dry non-erosive synovitis	4 (12.5%)	8 (13.3%)
Dry erosive synovitis	2 (6.25%)	4 (6.7%)

Note. $p > 0.05$.

The analysis revealed a direct correlation between a short period of disease inactivity after consecutive intra-articular injections of triamcinolone acetonide and a risk of active arthritis development (with an inactive phase of arthritis lasting less than 3 months, OR = 2.09, $p < 0.001$; with an inactive phase lasting less than 2 months – OR = 8.9, $p < 0.001$; Table 6).

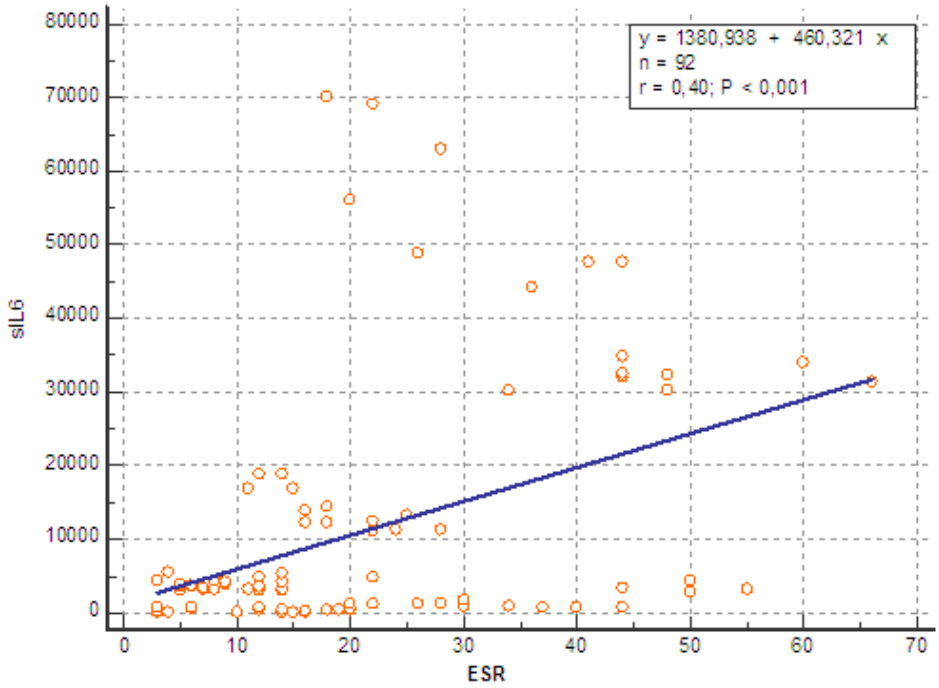

Fig. 2. Linear regression analysis of the direct relationship between ESR (mm/h) and IL-6 in the synovial fluid (pg / ml): ESR – erythrocyte sedimentation rate, sIL6 – interleukin 6 in the synovial fluid

Table 5

The results of constructing the model of "inactive" arthritis by the method of logistic and multiple linear regression in children with JIA based on laboratory tests

Parameter	R^2	Standard error	T-test	OR	95% CI	*p*
	–0.003841	0.009596	–0.400	0.9913	0.8934– 1.0998	0.8638
CRP, mg /l	–0.01390	0.02447	–0.568	0.9443	0.7508– 1.1878	0.6246
IL-6 serum, pg /ml	–0.03193	0.02386	–1.338	0.8366	0.6151–1.1379	0.2557
TNFα serum, pg /ml	0.1144	0.06898	1.659	2.2208	0.7480– 6.5938	0.1507
I IL-6 synovial, pg /ml	–0.000009239	0.00000764	–1.209	0.999	0.9997– 1.0001	0.2913
TNFα synovial, pg /ml	0.02611	0.01474	–1.772	1.1221	0.6138– 1.1007	0.1882

Table 6

The results of constructing the model of "active" arthritis by the method of logistic and multiple linear regression in children with JIA based on the duration of the inactive stage between intra-articular injections of triamcinolone acetonide

Time, months	R^2	Standard error	T-test	OR	95% CI	*p*
More than 6	–0.7745	0.04702	–16.470	5.09	0.956– 1.000	< 0.001
3–5	0.1311	0.09810	1.337	2.03	0.393–4.643	0.1312
Less than 3	0.5849	0.1249	4.683	2.09	0.534–0.749	< 0.001
Less than 2	0.5849	0.1151	5.081	8.9	0.680–0.868	< 0.001

DISCUSSION

Our study analyzed the effectiveness of intra-articular injections of long-acting corticosteroids (triamcinolone acetonide) in children with oligoarticular-onset JIA. The main aim of the research was to find possible predictors (clinical, instrumental or laboratory) of early ineffectiveness of is-IAI in children with chronic oligoarticular JIA. Sample homogeneity, the volume of laboratory and instrumental diagnosis, and management of the participants in one medical center allowed to study the nature of the disease onset, the dynamics of the articular syndrome, and the effectiveness of monotherapy with corticosteroids.

Moreover, the parents had a great interest in the course of the study. It is known that parents do not agree to early aggressive therapy with methotrexate. This opinion is based on the amount of information available on the Internet about side effects of methotrexate, conclusions of specialists not related to rheumatology, and a fear of lifelong treatment. In addition, a small number of affected joints and seemingly low activity of the disease leave hope for a favorable outcome or misdiagnosed JIA (in the opinion of most parents). In this situation, parents of the child would rather agree to the next intra-articular injection of triamcinolone into the inflamed joint than to methotrexate therapy.

Therefore, this study was aimed at searching for the clinical, instrumental, and laboratory predictors that could help to forecast early ineffectiveness of local steroid therapy. However, it is worth noting that our conclusions do not reflect the degree of intensity of the disease course, possible risks of articular syndrome progression and eye lesion development, and unfavorable outcomes of the disease.

In the Russian literature, the need for application of local steroid therapy in children with JIA is mostly described in works published earlier than 2010, despite the fact that the national clinical rheumatology guidelines contain this treatment option [8, 9]. I.M. Vorontsov and N.N. Kuzmina in their studies indicated subtypes of JIA with lowly aggressive course and sometimes complete recovery [10, 11]. A meta-analysis of data from various trials showed the effectiveness of local intra-articular corticosteroid injections in JIA.

These results of retrospective studies on JIA treatment are available only in English-language publications [12–14]. Currently, in Europe and the USA, the long-acting steroid which is approved and most commonly used in JIA for intra-articular treatment is triamcinolone hexacetonide (TH). In the Russian Federation, the drug that is certified and approved for intra-articular injection in JIA is triamcinolone acetonide (TA).

Triamcinolone acetonide (TA) is a synthetic corticosteroid with anti-inflammatory properties. The drug in the form of a sustained-release suspension is poorly soluble and deposits in the articular cavity, providing a long-term effect on the inflamed synovium. Triamcinolone decreases the expression of proinflammatory cytokines (TNFα, IL-1β, IL-6), chemokines, and growth factors (VEGF, IGF, PDGF, CSF-1), blocks proteolytic activity of matrix metalloproteinases (MMP-2, MMP-3, MMP-9), and reduces migration of inflammatory cells in the synovial cavity. Moreover, the drug decreases the proliferative capacity of synoviocytes associated with reduction of NF-κB transcriptional activity [15–17].

Most multicenter studies have demonstrated high effectiveness, good tolerance, and safety of intra-articular injections with triamcinolone hexacetonide. Triamcinolone acetonide was viewed as an alternative form acceptable for intra-articular injections, that demonstrated good clinical effects [18–20]. Long-term effectiveness of early local therapy with triamcinolone hexacetonide / acetonide in JIA was also described in more recent studies, however, triamcinolone hexacetonide was preferred [21–23].

In the present study, effectiveness of non-systemic corticosteroid therapy of JIA was related to a number of determining factors: the first injection of triamcinolone at an earlier stage of oligoarticular JIA, solely intra-articular administration of triamcinolone with preliminary dilution of the drug, and special post-injection regimen. The optimal timing for is-IAI in children with JIA should be strictly limited to 6–12 months after the onset of the disease. The following injections can be performed in the inflamed joints without signs of disease progression [24].

Preliminary dilution of triamcinolone and active / passive joint movements improve the penetration of

the corticosteroid into the inflamed synovial membrane. Reduced axial loads on the lower extremity decrease risks of aseptic necrosis and contribute to prolonged therapeutic effect [25]. Long-term effects of intra-articular administration of triamcinolone is related to anti-inflammatory, anti-proliferative, immunosuppressive, and vasoconstrictive effects on the synovial membrane. Triamcinolone has the slowest joint clearance and is the most potent in producing synovial atrophy. The "scalding" effect of triamcinolone on the inflamed synovial tissue can be associated with vasoconstriction of blood vessels in the subsynovial layers and return of cell sensitivity to pro-apoptotic factors [26].

The present study demonstrated the effectiveness and safety of non-systemic corticosteroid therapy with triamcinolone acetonide in children with oligoarthicular-onset JIA. The research did not identify predictors of oligoarticular JIA which could directly indicate possible ineffectiveness of steroid therapy beforehand. However, the study revealed that the degree of aggressiveness of JIA was inversely proportional to the duration of the inactive phase of arthritis between two consecutive is-IAIs. Moreover, the study revealed a direct relationship between ESR and the concentration of IL-6 in the synovial fluid. Summarizing the data of the 7-year follow-up of children with oligoarticular JIA, we can state that about one third of the children achieve stable remission against the background of local steroid therapy.

CONCLUSION

Local corticosteroids are effective and safe in children with oligoarticular-onset JIA. Rational use of isolated intra-articular injections in children with JIA contributes to assessment of the degree of disease aggressiveness. Application of treat-to-target principles and early disease-modifying anti-rheumatic drugs in patients with oligoarticular-onset JIA artificially reduce the effectiveness of steroid therapy in pediatric rheumatology.

REFERENCES

1. Alekseeva E.I., Bzarova T.M. Algorithm of diagnosis and treatment of juvenile arthritis. *Issues of Modern Pediatrics*. 2010; 9 (6): 78–104 (in Russ.).
2. Kozhevnikov A.N., Pozdeeva N.A., Konev M.A., Maricheva O.N., Afonichev K.A., Novik G.A. X-ray diagnosis of chronic oligoarthritis in children. *Bulletin of Siberian Medicine*. 2017; 16 (3): 224–234 (in Russ.). DOI: 10.20538/1682-0363-2017-3-224–234.
3. Young Dae Kim, Alan V Job, Woojin Cho. Differential diagnosis of juvenile idiopathic arthritis. *J. Rheum. Dis.* 2017; 24 (3): 131–137. DOI: 10.4078/jrd.2017.24.3.131.
4. Petty R.E., Southwood T.R., Manners P., Baum J., Glass D.N., Goldenberg J., He X., Maldonado-Cocco J., Orozco-Alcala J., Prieur A.M., Suarez-Almazor M.E., Woo P. International League of Associations for R. International league ofassociations for rheumatology classification of juvenile idiopathic arthritis: second revision, Edmonton. 2001. *J. Rheumatol*. 2004; 31 (2): 390–392.
5. Tsurikova N.A., Ligostaeva E.A., Zholobova E.S. Oligoarticular juvenile idiopathic arthritis at early stages of the disease: a path to diagnosis verification. *Issues of Practical Pediatrics.* 2017; 2: 33–38 (in Russ.). DOI: 10.20953/1817-7646-2017-2-33-38.
6. Ringold S., Angeles-Han Sh.T., Beukelman T., Lovell D., Cuello C.A., Becker M.L., Colbert R.A., Feldman B.M., Ferguson P.J., Gewanter H., Guzman J., Horonjeff J., Nigrovic P.A., Ombrello M.J., Passo M.H., Stoll M.L., Rabinovich C.E., Schneider R., Halyabar O., Hays K., Shah A.A., Sullivan N., Szymanski A.M., Turgunbaev M., Turner A., Reston J. 2019 American College of Rheumatology/Arthritis Foundation Guideline for the treatment of juvenile idiopathic arthritis: therapeutic approaches for non-systemic polyarthritis, sacroiliitis, and enthesitis. *Arthritis & Rheumatology*. 2019; 71 (6): 846–863. DOI: 10.1002/art.40884doi:10.1002/art.40884.
7. Beukelman T., Patkar N.M., Saag K.G., Tolleson-Rinehart .S, Cron R.Q., DeWitt E.M., Ilowite N.T., Kimura Y., Laxer R.M., Lovell D.J., Martini A., Rabinovich C.E., Ruperto N. 2011 American college of rheumatology recommendations for the treatment of juvenile idiopathic arthritis: initiation and safety monitoring of therapeutic agents for the treatment of arthritis and systemic features. *Arthritis Care & Research*. 2011; 63 (4): 465–482. DOI: 10.1002/acr.20460.
8. Kaleda M.I., Nikishina I.P. Modern view on the intra-articular use of corticosteroids in comprehensive therapy of juvenile arthritis. *Pediatrics*. 2016; 95 (3): 55–64 (in Russ.).
9. Baranov A.A., Alekseeva E.I., Bzarova T.M., Valieva S.I., Denisova R.V., Isaeva K.B., Karagulyan N.A., Litvitskiy P.F., Mitenko E.V., Sleptsova T.V., Fetisova A.N., Chistyakova E.G., Taybulatov N.I., Morev S.Yu. Protocol of managing patients with juvenile arthritis. *Issues of Modern Pediatrics*. 2013; 12 (1): 37–56 (in Russ.). DOI: 10.15690/vsp.v12i1.557.

10. Kuzmina N.N., Shelepina T.A. Rehabilitation and drug therapy of juvenile idiopathic arthritis. *Medical Council*. 2007; 3: 36–42 (in Russ.).
11. Novik G.A., Abakumova L.N., Letenkova N.M., Slizovskiy N.V., Slizovskaya N.N. Juvenile arthritis: experience in diagnosis and treatment. *Treating Physician.* 2008; 4: 23–27 (in Russ.).
12. Ravelli A., Davì S., Bracciolini G., Pistorio A., Consolaro A., van Dijkhuizen E.H.P., Lattanzi B., Filocamo G., Verazza S., Gerloni V., Gattinara M., Pontikaki I., Insalaco A., De Benedetti F., Civino A., Presta G., Breda L., Marzetta V., Pastore S., Magni-Manzoni S., Maggio M.C., Garofalo F., Rigante D., Gattorno M., Malattia C., Picco P., Viola S., Lanni S., Ruperto N., Martini A., Italian Pediatric Rheumatology Study Group. Intra-articular corticosteroids versus intra-articular corticosteroids plus methotrexate in oligoarticular juvenile idiopathic arthritis: a multicentre, prospective, randomised, open-label trial. *Lancet* 2017; 389 (10072): 909–916. DOI: 10.1016/S0140-6736(17)30065-X.
13. Nieto-González J.C., Monteagudo I. Intra-articular joint injections in juvenile idiopathic arthritis: state of the art. *Reumatol. Clin*. 2019; 15 (2): 69–72. DOI: 10.1016/j.reuma.2018.07.015.
14. Jennings H., Hennessy K., Hendry G.J. The clinical effectiveness of intra-articular corticosteroids for arthritis of the lower limb in juvenile idiopathic arthritis: a systematic review. *Pediatr. Rheumatol. Online J*. 2014; 12: 23. DOI: 10.1186/1546-0096-12-23.
15. Olyunin Yu.A. Intra-articular injections of drugs in the comprehensive therapy of rheumatic disorders. *Modern Rheumatology*. 2015; 9 (1): 78–83 (in Russ.). DOI: 10.14412/1996-7012-2015-1-78-83.
16. Reshetnyak T.M., Seredavkina N.V., Dydykina I.S., Nasonov E.L. Corticosteroids in the therapy of systemic lupus erythematosus. *Clinician*. 2013; 7 (3-4): 14–26 (in Russ.). DOI: 10.17650/1818-8338-2013-3-4-14-26.
17. Konai M.S., Vilar Furtado R.N., dos Santos M.F., Natour J. Monoarticular corticosteroid injection versus systemic administration in the treatment of rheumatoid arthritis patients: a randomized double-blind controlled study. *Clin. Exp. Rheumatol*. 2009; 27 (2): 214–221.
18. Cleary A.G., Murphy H.D., Davidson J.E. Intra-articular corticosteroid injections in juvenile idiopathic arthritis. *Arch. Dis. Child*. 2003; 88 (3): 192–196. DOI: 10.1136/adc.88.3.192.
19. Zulian F., Martini G., Gobber D., Plebani M., Zacchello F., Manners P. Triamcinolone acetonide and hexacetonide intra-articular treatment of symmetrical joints in juvenile idiopathic arthritis: a double-blind trial. *Rheumatology.* 2004; 43 (10): 1288–1291. DOI: 10.1093/rheumatology/keh313/
20. Marti P., Molinari L., Bolt I.B., Seger R., Saurenmann R.K. Factors Influencing the efficacy of intra-articular steroid injections in patients with juvenile idiopathic arthritis. *Eur. J. Pediatr*. 2008; 167 (4): 425–430. DOI: 10.1007/s00431-007-0525-9.
21. Cunha A.L., Miotto E.S.V.B., Osaku F.M., Niemxeski L.B., Furtado R.N., Natour J., Sande M.T., Terreri L.R. Intra-articular injection in patients with juvenile idiopathic arthritis: factors associated with a good response. *Rev. Bras. Reumatol. Engl. Ed.* 2016; 56 (6): 490–496. DOI: 10.1016/j.rbre.2016.01.001.
22. Leow O.M.Y., Lim L.K., Ooi P.L., Shek L.P.C., Ang E.Y.N., Son M.B. Intra-articular glucocorticoid injections in patients with juvenile idiopathic arthritis in a Singapore hospital. *Singapore Med. J*. 2014; 55 (5): 248–252. DOI: 10.11622/smedj.2014066.
23. Papadopoulou C., Kostik M., Gonzalez-Fernandez M.I., Bohm M., Nieto Gonzalez J.C., Pistorio A., Lanni S., Consolaro A., Martini A., Ravelli A. Delineating the role of multiple intraarticular corticosteroid injections in the management of juvenile idiopathic arthritis in the biologic era. *Arthritis Care Res*. 2013; 65 (7): 1112–1120. DOI: 10.1002/acr.21947.
24. Gotte A.C. Intra-articular corticosteroids in the treatment of juvenile idiopathic arthritis: Safety, efficacy, and features affecting outcome. A comprehensive review of the literature. *Open Access. Rheumatol*. 2009; 1: 37–49. DOI: 10.2147/oarrr.s5103.
25. Lanni S., Bertamino M., Consolaro A., Pistorio A., Magni-Manzoni S., Galasso R., Lattanzi B., Calvo-Aranda B., Martini A., Ravelli A. Outcome and predicting factors of single and multiple intra-articular corticosteroid injections in children with juvenile idiopathic arthritis. *Rheumatology*. 2011; 50 (9): 1627–1634. DOI: 10.1093/rheumatology/ker165.
26. Furtado R.N.V., Machado F.S., Luz K.R.D., Santos M.F.D., Konai M.S., Lopes R.V., Natour J. Intra-articular injection with triamcinolone hexacetonide in patients with rheumatoid arthritis: prospective assessment of goniometry and joint inflammation parameters. *Rev. Bras. Reumatol. Engl. Ed.* 2017; 57 (2): 115–121. DOI: 10.1016/j.rbre.2016.08.001.

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Possibilities of detecting and correcting decreased heart rate variability in patients with coronary artery disease in combination with depressive disorders in a cardiology department

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ABSTRACT

Aim. To identify the presence of decreased heart rate variability (HRV) and a method for correcting it in patients with chronic coronary artery disease (CAD) and comorbid depressive disorders (DD).

Materials and methods. 79 patients with CAD (with class II–III angina pectoris and myocardial infarction that occurred more than 6 months ago) were divided into two groups. The first group included 50 CAD patients with depression, and the second – 29 CAD patients without depression. 17 patients received agomelatine (1st subgroup), 12 patients received fluvoxamine and fluoxetine (2nd subgroup), and 21 patients refused to take antidepressants (3rd subgroup). Initially and after 6 months, the HRV parameters were evaluated using the SCHILLER MT-200 Holter-ECG apparatus (Switzerland).

Results. A significant decrease in HRV was revealed in the patients with depression compared to the patients without it: SDNN (96 [83; 117] ms vs. 110 [98; 127] ms; $p = 0.02$), SDANN (80.5 [67; 94] ms vs. 91 [79; 102] ms; $p = 0.03$), SDNNindex (46.5 [38; 56] ms vs. 55 [48; 66] ms; $p = 0.006$), rMSSD (29 [23; 38] ms vs. 33 [29; 45] ms; $p = 0.04$), pNN50% (3.9 [2.4; 5,7] vs. 5.7 [2.9; 12.6]; $p = 0.03$). Initially, the 1st, 2nd, and 3rd subgroups did not differ in all HRV parameters. Against the background of antidepressant therapy, there were significant differences between the 2nd and 3rd subgroups in SDNN (110 [96; 140] ms vs. 85.5 [75; 103] ms; $p = 0.008$), SDANN (93.7 ± 22.9 ms vs. 72.7 ± 21.4 ms; $p = 0.02$), SDNNindex (55.8 ± 16.4 ms vs. 42.4 ± 10.8 ms; $p = 0.01$) and pNN50% (7.8 ± 6.7 vs. 3.6 ± 1.8; $p = 0.02$), as well as between the 1st and 3rd subgroups (SDANN (93.6 ± 28.5 ms vs. 72.7 ± 21.4 ms; $p = 0.03$), rMSSD (36.5 [28.5; 51] ms vs. 26.5 [25; 32] ms; $p = 0.02$)).

Conclusion. In patients with CAD with comorbid DD, significant impairment of heart rhythm regulation occurs due to a pronounced decrease in HRV, which can seriously affect the course and prognosis of CAD. Prescribing modern antidepressants can be used as a method of correcting autonomic dysfunction in patients with CAD with comorbid depression.

Key words: coronary artery disease, depressive disorders, myocardial infarction, heart rate variability, antidepressants.

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Возможности выявления и коррекции сниженной вариабельности ритма сердца у больных ишемической болезнью сердца в сочетании с депрессиями в условиях кардиологического отделения

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РЕЗЮМЕ

Цель. Выявить наличие и способ коррекции сниженной вариабельности ритма сердца (ВРС) у больных с хронической ишемической болезнью сердца (ИБС) в сочетании с депрессивными расстройствами (ДР).

Материалы и методы. Больные с ИБС (n = 79, со стенокардией напряжения II–III функциональных классов и перенесенным инфарктом миокарда давностью более 6 мес), распределены на две группы: 50 больных ИБС с депрессивными расстройствами (первая группа) и 29 больных ИБС без депрессивной симптоматики (вторая группа). Антидепрессант агомелатин получали 17 больных (1-я подгруппа), 12 больных – флувоксамин, флуоксетин (2-я подгруппа). От приема антидепрессантов отказался 21 больной (3-я подгруппа). Исходно и через 6 мес проведена оценка параметров ВРС с помощью аппарата SCHILLER MT-200 Holter-ECG (Швейцария).

Результаты. У пациентов с депрессией в сравнении с пациентами без нее выявлено значимое снижение ВРС: SDNN (96 [83; 117] мс vs 110 [98; 127] мс, p = 0,02), SDANN (80,5 [67; 94] мс vs 91 [79; 102] мс, p = 0,03), SDNNindx (46,5 [38; 56] мс vs 55 [48; 66] мс, p = 0,006), rMSSD (29 [23; 38] мс vs 33 [29; 45] мс, p = 0,04), pNN50% (3,9 [2,4; 5,7] vs 5,7 [2,9; 12,6], p = 0,03). Исходно 1-, 2-, 3-я подгруппы по всем параметрам ВРС не различались. На фоне терапии антидепрессантами между 2-й и 3-й подгруппами появились существенные отличия по SDNN (110 [96; 140] мс vs 85,5 [75; 103] мс, p = 0,008), SDANN (93,7 ± 22,9 мс vs 72,7 ± 21,4 мс, p = 0,02), SDNNindx (55,8 ± 16,4 мс vs 42,4 ± 10,8 мс, p = 0,01) и pNN50% (7,8 ± 6,7 vs 3,6 ± 1,8, p = 0,02), а также между 1-й и 3-й подгруппами (SDANN 93,6 ± 28,5 мс vs 72,7 ± 21,4 мс, p = 0,03), rMSSD (36,5 [28,5; 51] мс vs 26,5 [25; 32] мс, p = 0,02)).

Заключение. У больных ИБС при наличии ДР возникает серьезное нарушение регуляции сердечного ритма вследствие выраженного снижения показателей ВРС, что может серьезно повлиять на течение и прогноз коронарной болезни. Назначение современных антидепрессантов можно использовать в качестве способа коррекции вегетативной дисфункции у больных ИБС в сочетании с депрессией.

Ключевые слова: ишемическая болезнь сердца, депрессивные расстройства, инфаркт миокарда, вариабельность ритма сердца, антидепрессанты.

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

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

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

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INTRODUCTION

Currently, very high comorbidity of two such common pathologies as cardiovascular disease (CVD) and depressive disorders (DD) takes place. According to the World Health Organization (WHO) forecasts, by 2030, coronary artery disease (CAD) and depression will become the leading causes of disability in high-income countries around the world [1, 2].

On the one hand, depression is known to increase the risk of CAD. On the other hand, it is a strong predictor of poor prognosis in patients with cardiovascular diseases. Severe depressive symptoms are associated with the risk of death in patients with cardiovascular pathology [3–5]. Mortality in patients with myocardial infarction and depression is 3–6 times higher than in patients without mental disorders, while DD is detected in 35–40% of patients after myocardial infarction [4].

There has been significant deterioration in the clinical presentation of CAD against the background of DD, which is manifested by aggravation of angina pectoris, and a significant decrease in exercise tolerance; patients have a lower quality of life [6]. It should be taken into account, that only 30% of patients have complaints of psychological nature, leading to development of depressive disorders in the future, which contributes to underdiagnosis of DD and untimely administration of appropriate therapy [7, 8]. Therefore, patients more often seek medical help at a polyclinic, call an ambulance, and are more often hospitalized [9].

The most important mechanism in the effect of depression on the prognosis of CAD is activation of the sympathetic-adrenal medullary system. This causes dysfunction in the regulation of the heart rate, reducing its variability, which increases the risk of developing rhythm disturbances [10, 11]. The presented data dictate the need for timely recognition of depression, correction of psychoemotional factors [12], and administration of modern antidepressants in addition to the first-line therapy for chronic CAD. The beneficial effect of the antidepressant therapy on the clinical course of CAD has been proved [4, 5, 11]. However, there is little experience in studying the effect of antidepressants on autonomic dysfunction in patients with CAD after myocardial infarction in combination with DD. In turn, it is autonomic dysfunction that plays an important role in the prognosis of CAD.

Therefore, the aim of the study was to identify the presence of the decreased HRV and a method for its correction in patients with chronic CAD with comorbid DD.

MATERIALS AND METHODS

At the Department of Cardiology, 79 patients with chronic coronary artery disease (class II-III angina pectoris) who had had acute myocardial infarction more than 6 months prior to the experiment were examined and included in the study. All patients signed an informed consent to participate in the study. The patients were divided into two groups: 50 patients with CAD with depressive disorders (the first group) and 29 patients with CAD without depressive symptoms (the second group). The average age in the groups was (57.5 ± 6.4) years and (57.5 ± 7.4) years, respectively.

During the examination of the underlying pathology (CAD), all patients were offered to be tested with special scales for detecting depression (Hospital Anxiety and Depression Scale (HADS) and Beck Depression Inventory (BDI)). When an increased level of depression was detected (more than 8 points on the HADS or more than 19 points on the BDI), the patients were referred to a psychiatrist. The diagnosis of DD was confirmed by the psychiatrist, and follow-up of the patients was also conducted by a psychiatrist as a member of a multidisciplinary team. When the diagnosis of DD was confirmed, the patients were prescribed antidepressants.

Therefore, 29 patients with DD were treated with antidepressants: 17 patients received agomelatine, an agonist of MT_1, MT_2 melatonergic receptors (1st subgroup), and 12 patients received drugs from the group of selective serotonin reuptake inhibitors (fluvoxamine, fluoxetine) (2nd subgroup) for 6 months. The dose was selected by the psychiatrist individually for each patient. For various reasons, 21 patients refused to take antidepressants (3rd subgroup). During the examination, all patients, initially and after 6 months, underwent 24-hour Holter monitoring using the SCHILLER MT-200 Holter-ECG apparatus (Switzerland).

We assessed the following HRV parameters through time domain variables: the percentage of consecutive intervals differing by more than 50 ms (pNN50, %); standard deviation of the RR interval (SDNN, ms); square root of the average sum of squares of the differences between adjacent normal RR-intervals (r-MSSD, ms); standard deviation of the average values of RR intervals for all 5-minute fragments (SDANN, ms); and mean value of standard deviations over all 5-minute sections (SDNN index, ms). HRV was determined only in sinus rhythm. For normal HRV parameters, time domain variables for healthy individuals were used (J.T. Bigger et al.,

1995): pNN50 – 9 ± 7%, SDNN – 141 ± 38 ms, SDNN index – 54 ± 15 ms, rMSSD – 27 ± 12 ms, SDANN – 127 ± 35 ms.

Statistica for Windows v.10.0 software (StatSoft Inc., USA) was used for statistical processing of the results. The obtained data were presented in the form of $M \pm SD$; n (%); the median and the interquartile range *Me* [25%; 75%]. The level of statistical significance of the differences was $p < 0.05$. The Shapiro – Wilk test was used to check the normal distribution of the actual data. With normal distribution of the sample, the significance of differences was assessed using the Student's *t*-test. With nonparametric distribution and the number of groups equal to two, the Mann – Whitney test was used. When the number of samples was more than two, in order to avoid the effect of multiple comparisons, the nonparametric Kruskal – Wallis *H*-test was used. To compare two dependent samples for any criterion, the Student's *t*-test (with normal distribution of the sample) and the Wilcoxon test (with abnormal distribution of the sample) were used. To check the significance of differences in the qualitative variables, analysis of contingency tables and the Pearson's $\chi 2$ test (at low frequencies – with Yates' correction for continuity) were used.

RESULTS

According to the main clinical and demographic characteristics, the functional class of angina pectoris, hemodynamic parameters, and cholesterol and triglyceride levels, the groups were comparable (Table 1).

All patients achieved the target values of blood pressure and heart rate. The patients received the main groups of drugs for treatment of stable angina pectoris without significant differences between the first and second groups (Table 2).

In the analysis of HRV parameters, significant differences were initially revealed between the first and second groups for all parameters (Table 3). In addition, in both groups in comparison with healthy individuals, a decrease in SDNN and SDANN was registered.

Initially, CAD patients with comorbid DD (1st, 2nd, 3rd subgroups) did not differ in all HRV parameters ($p > 0.05$). For 6 months, the patients were taking the selected basic therapy for CAD. In patients of the 2nd subgroup, in comparison with patients of the 3rd subgroup, a significant increase in HRV parameters (SDNN, SDANN, SDNN index and pNN50%) was observed after 6 months (Table 4).

Table 1

Clinical and demographic characteristics of the groups

Parameters	First group $n = 50$	Second group $n = 29$	p
Average age, years, $M \pm SD$	57.5 ± 6.4	57.5 ± 7.4	0.4
Men, abs. (%)	45 (90)	26 (90)	1
Women, abs. (%)	5 (10)	3 (10)	1
Anterior myocardial infarction, abs. (%)	26 (52)	18 (62)	1
Posterior myocardial infarction, abs. (%)	24 (48)	16 (55)	0.9
Coronary artery disease, months, *Me* [25%; 75%]	48.0 [20.5; 96]	30.0 [12; 84]	0.2
Recentness of myocardial infarction, months, *Me* [25%; 75%]	20.5 [7; 96]	24 [7; 72]	0.7
Hypertensive disease, abs. (%)	50 (100)	29 (100)	1.0
Hypertension, months, *Me* [25%; 75%]	90 [24; 162]	90 [36; 132]	0.8
Smoking, abs. (%)	25 (50)	15 (52)	0.9
Body mass index, (BMI), kg/m^2, M ± *SD*	28.4 ± 4.3	28.5 ± 3.5	1.0
Obesity (BMI > 29.9), abs. (%)	17 (34)	11 (38)	0.7
History of PCI, abs. (%)	40 (80)	27 (93)	0.1
Single-vessel lesion of the coronary bed, abs. (%)	15 (30)	9 (31)	0.9
Double-vessel lesion of the coronary bed, abs. (%)	20 (40)	12 (41)	0.9
Triple-vessel lesion of the coronary bed, abs. (%)	15 (30)	8 (28)	0.9
FC of angina pectoris, abs. (%): – FC II – FC III	 38 (76) 12 (24)	 24 (83) 5 (17)	 0.5 0.5
Total cholesterol, mmol / l, *Me* [25%; 75%]	5.3 [4.4; 6.4]	5.3 [4.6; 6.3]	1.0
Triglycerides, mmol / l, *Me* [25%; 75%]	1.9 [1.3; 2.3]	1.6 [1.4; 2.0]	0.3
EF, B-mode, %, M ± *SD*	60.9 ± 10.4	61.2 ± 7.7	0.9
EDS / ESS, mm M ± *SD*, *Me* [25%; 75%]	48.7 ± 6.2/ 31 [28; 37]	49.4 ± 4.4/ 32.7 [29; 37]	0.1/0.4
EDV / ESV, ml, *Me* [25%; 75%]	105 [97; 137]/ 40 [33; 55]	116 [100;135]/ 44 [35; 56]	0.4/0.5
Average DBP, mm Hg, M ± *SD*	121 ± 12.4	120.5 ± 8.43	1
Average SBP, mm Hg *Me* [25%; 75%]	76 [70; 82]	75 [73; 78]	0.7
Average daily HR, min, *Me* [25%; 75%]	65 [61; 71]	64 [61; 67]	0.3

Note. PCI – percutaneous coronary intervention, FC – functional class, EF – ejection fraction, EDS – end diastolic size, ESS – end systolic size, EDV – end diastolic volume, ESV – end systolic volume, SBP – systolic blood pressure, DBP – diastolic blood pressure, HR – heart rate.

Table 2

Comparative characteristics of the treatment in the studied groups

Parameter	First group $n = 50$	Second group $n = 29$	p
Beta-adrenergic blockers, abs. (%)	47 (94)	27 (93)	0.9
ACE inhibitors, abs. (%)	40 (80)	26 (89)	0.3
Disaggregating agents, abs. (%)	50 (100)	29 (100)	1.0
Statins, abs (%)	50 (100)	29 (100)	1.0
Calcium antagonists, abs. (%)	15 (30)	7 (24)	0.6
Nitrates, abs. (%)	1 (2)	1 (3.4)	0.2
Diuretics, abs. (%)	14 (28)	5 (17)	0.3

Note. ACE – angiotensin-converting enzyme.

Table 3

Comparison of initial HRV parameters in patients of the first and second groups

Parameter	First group $n = 50$	Second group $n = 29$	p
SDNN, ms, *Me* [25%; 75%]	96 [83; 117]	110 [98; 127]	0.02
SDANN, ms, *Me* [25%; 75%]	80.5 [67; 94]	91 [79; 102]	0.03
SDNNindex, ms, *Me* [25%; 75%]	46.5 [38; 56]	55 [48; 66]	0.006
rMSSD, ms, *Me* [25%; 75%]	29 [23; 38]	33 [29; 45]	0.04
pNN, 50%, ms, *Me* [25%; 75%]	3.9 [2.4; 5.7]	5.7 [2.9; 12.6]	0.03

Note. Here and in Table 4: pNN50% – the percentage of consecutive intervals differing by more than 50 ms, r-MSSD – the square root of the mean sum of squares of the differences between adjacent normal RR intervals, SDANN – standard deviation of the mean values of RR intervals for all 5-minute fragments, SDNN – standard deviation of the RR interval, SDNN index – the mean of the standard deviations over all 5-minute sections.

Table 4

Comparison of HRV parameters in patients of the 2nd and 3rd subgroups after 6 months

Parameter	Second subgroup, $n = 12$	Third subgroup, $n = 21$	p
SDNN, ms, *Me* [25%; 75%]	110 [96; 140]	85.5 [75; 103]	0.008
SDANN, ms, $M \pm SD$	93.7 ± 22.9	72.7 ± 21.4	0.02
SDNNindex, ms, $M \pm SD$	55.8 ± 16.4	42.4 ± 10.8	0.01
rMSSD, ms, *Me* [25%; 75%]	31 [24; 51]	26.5 [25; 32]	0.4
pNN50%, ms, $M \pm SD$	7.8 ± 6.7	3.6 ± 1.8	0.02

A significant increase in two HRV parameters was noted in patients of the 1st subgroup in comparison with patients of the 3rd subgroup who did not receive antidepressants (SDANN (93.6 ± 28.5 ms vs. 72.7 ± 21.4 ms, $p = 0.03$); rMSSD (36.5 [28.5; 51] ms vs. 26.5 [25; 32] ms, $p = 0.02$).

DISCUSSION

We have studied one of the major mechanisms of the influence of affective disorders on the course of CAD. The study confirmed that patients with CAD and diagnosed depression have low HRV [10, 11]. The CAD patients, both with and without depression, had a decrease in SDNN and SDANN parameters in comparison with healthy people, which indicates rhythm rigidity in patients with postinfarction cardiosclerosis. A decrease in SDNN indicates a decrease in the overall HRV activity, and a decrease in SDANN reflects the activation of sympathetic tone and suppression of parasympathetic influences.

The CAD patients with depression and without a mental disorder did not differ in the main clinical and demographic parameters and concomitant therapy, which excluded their influence on the HRV parameters. When CAD was accompanied by DD, there was an increase in sympathoadrenal activity, which affected heart rate regulation and led to an even greater decrease in the main HRV parameters (SDNN, SDANN SDNNindex, rMSSD, pNN50%). Thus, the patients suffering from both CAD and DD have a worse prognosis, which was repeatedly proved in many studies [3–5].

This is determined by the fact that low HRV is a predictor of sudden death due to possible development of life-threatening arrhythmias. Of course, such patients require more attention and observation, as well as timely administration of modern antidepressants. The patients of this study received antidepressants of two groups – selective serotonin reuptake inhibitors (fluvoxamine, fluoxetine) and an agonist of $MT_{1,}$ MT_2 melatonergic receptors (agomelatine). The choice of such antidepressants is associated with their efficacy and safety in cardiac patients according to the literature [4–6, 11].

To assess autonomic dysfunction, a temporary method of HRV analysis was used, which has high prognostic value and reproducibility of indicators. In this study, selective serotonin reuptake inhibitors had the greatest beneficial effect on autonomic dysfunction; an increase in most of the analyzed HRV parameters (SDNN, SDANN, SDNNindex and pNN50%) was observed against the background of a six-month course. The six-month course of agomelatine resulted in an increase in HRV time domain variables, such as SDANN and rMSSD. Therefore, the administration of antidepressants of both groups in addition to the first-line therapy for CAD can increase the overall autonomic tone and parasympathetic activity and suppress

sympathetic activity, which can significantly reduce the risk of heart rhythm disturbances and improve the clinical course of CAD.

CONCLUSION

The clinical presentation of CAD is accompanied by an unfavorable prognostic disorder: a decrease in HRV parameters in the presence of comorbid DD. The results of this study showed that prescription of modern antidepressants can be used as a way to correct autonomic dysfunction in patients with CAD with comorbid depression.

REFERENCES

1. Khandaker G.M., Zuber V., Rees J.M.B., Carvalho L., Mason A.M., Foley C.N., Gkatzionis A., Jones P.B., Burgess S. Shared mechanisms between coronary heart disease and depression: findings from a large UK general population-based cohort. *Molecular. Psychiatr.* 2020: 25 (7): 1477–1486. DOI: 10.1038/s41380-019-0395-3.
2. Patel H., Mazur W., Williams K.A. Sr, Kalra D.K. Myocardial viability-state of the art: is it still relevant and how to best assess it with imaging? *Trends Cardiovasc. Med.* 2018; 28 (1): 24–37. DOI: 10.1016/j. tcm.2017.07.001.
3. Pushkarev G.S., Kuznetsov V.A., Fisher Y.A. et al. Effect of depressive symptoms on the risk of death from all causes in patients with chronic heart failure who underwent cardiac resynchronization therapy. *Cardiology.* 2019; 59 (1): 5–11 (in Russ.). DOI: 10.18087/cardio.2019.1.10211.
4. Lebedeva E.V., Nonka T.G., Repin A.N., Shishneva E.V., Schastny E.D., Simutkin G.G., Levchuk L.A. Modern psychocardiology. Tomsk: Publishing house LLC Integral Binding, 2019: 160 (in Russ.).
5. Schastnyy E.D., Simutkin G.G., Lebedeva E.V., Yakovleva A.L., Losenkov I.S., Repin A.N., Nonka T.G. Clinical-dynamic and biological aspects of polymorphism and the efficacy of therapy of mood disorders. *Siberian Medical Journal.* 2018; 33 (3): 86–92 (in Russ.). DOI: 10.29001/2073-8552-2018-33-3-86-92.
6. Lebedeva E.V., Schastnyy E.D., Simutkin G.G., Repin A.N., Nonka T.G. Clinical characteristics of affective disorders and the effectiveness of antidepressant therapy in patients with chronic coronary heart disease. *Bulletin of Siberian Medicine.* 2018; 17 (4): 85–93 (in Russ.). DOI: 10.20538/1682-0363-2018-4-85-93.
7. Spurgeon L., James G., Sackley C. The Hospital Anxiety and Depression Scale: a pilot study to examine its latent structure and the link between psychological state and symptom severity in transient ischaemic attack patients. *Psychol. Health Med.* 2016; 21 (5): 632–638. DOI: 10.1080/13548506.2015.1074711.
8. Arat S., de Cock D., Moons P., Vandenberghe J., Westhovens R. Modifiable correlates of illness perceptions in adults with chronic somatic conditions: A systematic review. *Res. Nurs. Health*. 2018; 41 (2): 173–184. DOI: 10.1002/nur.21852.
9. Palacios J., Khondoker M., Mann A., Tylee A., Hotopf M. Depression and anxiety symptom trajectories in coronary heart disease: Associations with measures of disability and impact on 3-year health care costs. *J. Psychosom. Res.* 2018; 104: 1–8. DOI: 10.1016/j.jpsy-chores.2017.10.015.
10. Vershuta V.A., Halilova M.A., Gognieva D.G., Bogdanova R.S., Syrkina E.A., Mesitskaja D.F., Kopylov F.Ju., Syrkin A.L. Anxiety and depressive disorders in patients with cardiac arrhythmias. *Cardiology and Cardiovascular Surgery.* 2019; 12 (4): 314–320 (in Russ.). DOI: 10.17116/kardio201912041314.
11. Baranov A.P., Strutynsky A.V., Oynotkinova O.Sh., Baranova A.A., Trishina V.V., Golubev Yu.Yu., Kruzhalov A.N. Treatment options for anxiety and depressive disorders in patients with chronic heart failure. *Russian Journal of Cardiology.* 2017; 22 (1): 128–135 (in Russ.). DOI: 10.15829/1560-4071-2017-1-128-135.
12. Kubareva M.I., Ibatov A.D. Psychosocial factors and coronary heart disease. *Therapeutic Archive.* 2019; 91 (12): 70–74 (in Russ.). DOI: 10.15829/1560-4071-2017-1-128-135.

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Exacerbation of asthma and neutrophil-dominated airway inflammation in patients with cold-induced airway hyperresponsiveness

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ABSTRACT

Background. Neutrophils can play a significant role in the formation of bronchial inflammation in asthma exacerbation in patients with cold-induced airway hyperresponsiveness (CIAHR).

Aim. To evaluate the role of neutrophils in the dynamics of the inflammatory pattern of bronchi in the exacerbation of asthma in patients with CIAHR.

Materials and methods. In 31 patients (average age (37.2 ± 2.7) years) with persistent bronchial asthma (BA) with moderate exacerbation and previously established CIAHR during cold air isocapnic hyperventilation (CAIH) (– 20 ºC, 3 min), the level of asthma control (Asthma Control Test (ACT), score) and external respiration (forced expiratory volume in the first second (FEV_1), forced expiratory flow between 25% and 75% of the vital capacity ($FEF_{25–75}$)) were assessed; induced sputum (IS) was examined initially and after 24 weeks of follow-up. At the time of the examination, the patients were additionally prescribed prednisone orally (at a maximum dose of 30 mg) for the first 10 days in order to stop the exacerbation, and then they continued treatment with a combination of budesonide / formoterol (640 / 18 μg per day) for 24 weeks.

Results. At the time of the initial examination, the ACT score was 17.0 (13.0; 19.5), FEV_1 was 89.1 ± 3.9%, and the number of neutrophils in the sputum was 55.9 ± 5.6%. At the end of treatment, the ACT score was 22.0 (17.0; 24.5) (p = 0.037), FEV_1 was 96.2 ± 2.9% (p = 0.038), the number of neutrophils in IS decreased, but remained high enough (40.0 ± 5.5%; p = 0.048); and the number of eosinophils did not change. A linear regression equation was made reflecting the relationship between the initially high number of neutrophils in the sputum, other cellular elements in the sputum, the level of asthma control, and the degree of severity of the bronchial response after a bronchoprovocation test with CAIH.

Conclusion. Asthma exacerbation in patients with CIAHR is associated with an increase in the neutrophil pool of the bronchial inflammatory infiltrate and correlates with the degree of severity of the airway response to bronchoprovocation with cold and the level of asthma control.

Key words: bronchial asthma, cold-induced airway hyperresponsiveness, exacerbation, neutrophil-dominated airway inflammation, pattern of bronchial inflammation, asthma control.

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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 Committee on Biomedical Ethics of the Far Eastern Scientific Center of Physiology and Pathology of Respiration (Protocol No. 120/1 of 25.10.2017).

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Обострение астмы и нейтрофильный сегмент воспаления бронхов у пациентов с холодовой гиперреактивностью дыхательных путей

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РЕЗЮМЕ

Введение. Нейтрофилы могут играть значительную роль в формировании бронхиального воспаления при обострении бронхиальной астмы (БА) у больных с холодовой гиперреактивностью дыхательных путей (ХГДП).

Цель. Оценить роль нейтрофилов в динамике воспалительного паттерна бронхов при обострении БА у пациентов с ХГДП.

Материалы и методы. У 31 больного (средний возраст (37,2 ± 2,7) лет) персистирующей БА со среднетяжелым обострением и ранее установленной холодовой гиперреактивностью дыхательных путей при проведении стандартной изокапнической гипервентиляции холодным (–20 ºС, 3 мин) воздухом (ИГХВ) оценивали уровень контроля БА (Asthma Control Test, ACT, баллы), функцию внешнего дыхания (объем форсированного выдоха за первую секунду ($ОФВ_1$), максимальная объемная скорость выдоха на уровне 25–75% форсированной жизненной емкости легких ($МОС_{25–75}$)), исследовали индуцированную мокроту (ИМ) исходно и через 24 нед наблюдения. На момент обследования больным с целью купирования обострения в течение первых 10 дней дополнительно назначался преднизолон перорально (в максимальной дозе 30 мг), затем 24 нед они продолжали лечение комбинацией будесонид/формотерол (640/18 мкг/сут).

Результаты. На момент первичного обследования ACT составил 17,0 (13,0; 19,5) баллов, $ОФВ_1$ 89,1 ± 3,9%, число нейтрофилов в мокроте 55,9 ± 5,6%. В конце лечения уровень контроля над астмой составил 22,0 (17,0; 24,5) (p = 0,037), $ОФВ_1$ 96,2 ± 2,9% (p = 0,038), количество нейтрофилов в ИМ снижалось, но оставалось достаточно высоким (40,0 ± 5,5%; p = 0,048); число эозинофилов не изменялось. Построено уравнение линейной регрессии, показавшее зависимость между исходно высоким количеством нейтрофилов в мокроте, другими клеточными элементами мокроты, уровнем контроля над болезнью и степенью выраженности реакции бронхов после проведения острой бронхопровокационной пробы ИГХВ.

Заключение. Обострение у больных бронхиальной астмой с холодовой гиперреактивностью дыхательных путей ассоциируется с увеличением нейтрофильного пула бронхиального воспалительного инфильтрата и коррелирует со степенью выраженности реакции на холодовую бронхопровокацию и уровнем контроля над заболеванием.

Ключевые слова: бронхиальная астма, холодовая гиперреактивность дыхательных путей, фаза обострения, нейтрофильный сегмент воспаления, паттерн воспаления бронхов, контроль астмы.

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

Источник финансирования. *Исследование выполнено при финансовой поддержке Российского фонда фундаментальных исследований (грант № 18-54-53019).*

Соответствие принципам этики. Все пациенты подписали информированное согласие на участие в исследовании. Исследование одобрено локальным комитетом по биомедицинской этике ДНЦ ФПД (протокол № 120/1 от 25.10.2017).

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INTRODUCTION

The clinical syndrome of cold-induced airway hyperresponsiveness (CIAHR), associated with constant exposure to such an ecologically conditioned trigger as low ambient air temperature, is diagnosed in the majority (60–80%) of patients with bronchial asthma (BA) of any severity [1]. In addition to other disturbing factors, exposure to low temperatures, associated with cold-induced bronchospasm, is an inducer of airway oxidative stress, which is interpreted as a typical pathological process that initiates the development and progression of various lung diseases [2].

Taking into account the fact that clinical manifestations of BA are modulated by chronic inflammation [3], a priority role in studying the mechanisms of inactive control and exacerbations of the disease can be assigned to the leading cellular effectors of inflammation – eosinophils and neutrophils [4], generating reactive oxygen species (ROS) and other mediators of oxidative stress, which are presented as signaling molecules that regulate the expression of proinflammatory cytokines [2]. An increased level of proinflammatory cytokines is potentially capable of activating a cascade of inflammatory reactions that determine the severe clinical course of BA [5].

It is known that with steroid-resistant asthma in patients receiving high doses of systemic corticosteroids, a high level of neutrophils in the bronchoalveolar lavage is identified, whereas in patients not receiving systemic corticosteroids, eosinophilia predominates [6]. During acute exacerbations and fatal BA attacks, neutrophil-dominated inflammation proceeds in the airways [6]. Neutrophilia of the bronchial infiltrate contributes to aggravation of clinical manifestations of BA, limits the possibility of achieving the disease control, and is accompanied by a decrease in airway patency and an increase in the frequency of airway response to a cold stimulus [4, 7].

Based on the study of bronchial biopsy specimens obtained from patients with severe exacerbations of BA and endotracheal intubation for respiratory failure, it was found that at the stage of exacerbation the bronchial mucosa is intensively infiltrated by eosinophils and, to a greater extent, by neutrophils [8]. This does not exclude the fact that such patients have airway neutrophilia before the exacerbation – due to the previous severe course of BA against the background of treatment with high doses of inhaled corticosteroids (ICS) [9].

The role of the morphological and functional status of neutrophils in the manifestation of the inflammatory pattern in the bronchi during BA exacerbation in patients with CIAHR has not been clarified. Since the response of the bronchi to cold exposure is clearly associated with a low level of asthma control and difficulties in reducing a cold-induced bronchospasm [10], the possibility of activation of neutrophil-initiated oxidative stress during the exacerbation could result in loss of control and an increase in BA severity. The solution to this problem is associated with the search for methods of appropriate pharmacotherapy for the neutrophil component of inflammation.

The aim of this work was to assess the dynamics of changes in the neutrophil component of the inflammatory pattern in the bronchi during exacerbation and without exacerbation of BA in patients with CIAHR.

MATERIALS AND METHODS

The study included patients (n = 31, average age 37.2 ± 2.7 years) of both sexes (14 men, 17 women) with an established diagnosis of persistent asthma (disease duration ≥ 2 years) and clinical symptoms of exacerbation, according to the criteria [3], and previously identified CIAHR during a bronchoprovocation test with cold air isocapnic hyperventilation (CAIH) (3 min; – 20 °C) [1].

Before inclusion in the study, the patients received anti-inflammatory therapy with a combined medication ICS / long-acting beta(2)-agonists (LABA) at a daily dose of < 1000 μg equivalent to beclomethasone for at least 3 months. The therapy was inadequate and irregular for various reasons, mainly economic ones. From the start of the study (1st visit) for the entire follow-up period (24 weeks), treatment of patients with the first-line anti-inflammatory drug budesonide/formoterol (Symbicort® Turbuhaler®) was planned in a stable dosing regimen, at an increased dose of 640 /18 μg per day. To relieve exacerbation symptoms, oral prednisolone therapy (at a maximum dose of 30 mg) was used for 5–10 days [3], and then the treatment with budesonide/formoterol at a stable dose continued for 24 weeks.

The study design included assessment of BA severity and determination of the pulmonary function with analysis of the indices of the forced expiratory flow-volume curve (forced expiratory volume in the first second (FEV_1), forced expiratory flow between 25% and 75% of the vital capacity ($FEF_{25–75}$), forced vital capacity (FVC), the FEV_1/FVC ratio, peak expiratory flow (PEF), maximal expiratory flow at 50% of vital flow capacity (MEF_{50}), maximal expiratory flow at 75% of vital flow capacity (MEF_{75})) by spirometry on an Easy on-PC (NDD Medizintechnik AG, Switzerland) with subsequent registration of parameters after inhalation of a β_2-agonist (salbutamol, 400 μg) initially (1st visit), 10 days after the start of the treatment (2nd visit), and at the end of 24 weeks of the therapy (3rd visit). At the 1st and 3rd visits, asthma control was assessed using the Asthma Control Test (ACT, Quality Metric Inc., 2002). The criteria for complete, good, and insufficient control of the disease were 25, 24–20 and less than 20 points, respectively.

At the 1st and 3rd visits, samples of induced sputum (IS) were collected and studied [11]. The cytosis level was determined by the number of cells contained in 1 μl of sputum. In order to determine the cellular composition, the sputum smears were examined according to the standard technique using optical microscopy, with calculation of at least 400 cells in 100 fields of view in the central and peripheral parts of the specimen. The number of neutrophils, eosinophils, macrophages, lymphocytes, and bronchial epithelial cells counted in the cytological smears was expressed as a percentage; according to the results, cytograms were formed.

Statistical analysis of the obtained results was carried out using the Statistica 10.0 software package (StatSoft, Inc., USA) and the Automated System for Scientific Research program [12]. Compliance of the trait with the law of normal distribution was evaluated using the Kolmogorov – Smirnov and Pearson – von Mises tests. With normal distribution, Student's *t*-test was used. With the distribution of data other than normal, the Wilcoxon test was used. Descriptive statistics of quantitative variables was presented as the mean, a standard error of the mean ($M \pm m$), as well as the median and the interquartile range Me (Q_1; Q_3). In order to establish the type of dependence and build a mathematical model between a random variable and values of several independent variables, stepwise multiple regression analysis was used with creation of a regression equation. For all values, the level of p less than 0.05 was considered statistically significant.

RESULTS

In accordance with the criteria [3], clinical symptoms of moderate exacerbation at the time of the initial examination were present in all patients with BA included in the study and were characterized by increased respiratory discomfort, shortness of breath (92%), an increase in the number of daytime episodes of shortness of breath and their appearance at night (64%), suffocation (56%), cough varying in intensity and nature (88%), wheezing (60%), chest congestion (50%), and an increase in the need for short-acting bronchodilators (94%).

Interviewing patients with subsequent assessment of the severity of the disease using a validated ACT questionnaire showed a low level of asthma control and significant improvement in the bronchial patency (ΔFEV_1) during the bronchodilation test (Table). The clinical success of the treatment, which was greatly determined by patients' adherence to therapy (it was 85% at the final stage of observation), was reflected in the dynamics of ACT (Table). When assessing ACT values in the group as a whole at the 3rd visit, a statistically significant increase in the level of asthma control was observed (Table). At the final stage of monitoring (3rd visit), 62% of patients reported signs of achieving asthma control (ACT score > 20), 22% of patients receiving regular ICS therapy assessed their condition as the one without changes (ACT score < 19), in 16% of patients, a decrease in ACT compared to the initial testing (score less than 15) and clinical signs of repeated exacerbation were observed. 38% of patients continued to experience the need for the use of short-acting bronchodilators.

At the 2nd visit, after 10 days of oral prednisolone (at a dose of 30 mg), there was no change in bronchial patency parameters (Table). A statistically significant improvement in the ventilation function of the lungs with a decrease in the response during the bronchodilation test occurred by the 3rd visit, with long-term regular use of a stable dose of ICS/LABA.

Calculation of the main cellular elements in induced sputum was performed at baseline, before prescribing corticosteroid therapy, and 6 months after ICS treatment. In all patients included in the clinical study, sputum induction was carried out increasingly by means of 7-minute inhalations with 3-, 4- and 5% sodium chloride solution. To perform the cytological study, a sample was selected that had a minimum level of contamination with squamous epithelial cells (less than 20% of squamous epithelial cells from the total number of cells).

Table

Pulmonary ventilation function and the level of asthma control in patients with BA at runtime, *n* = 31

Parameter	1st visit	2nd visit	3rd visit	*p*	p_1
FEV_1, l, *M* ± *m*	3.17 ± 0.22	3.10 ± 0.16	3.43 ± 0.23	0.024	0.022
MEF_{25-75}, l/s, *M* ± *m*	2.66 ± 0.24	2.61 ± 0.24	3.21 ± 0.26	0.013	0.006
FEV_1/FVC, %, *M* ± *m*	72.7 ± 1.7	74.8 ± 1.5	78.0 ± 1.5	0.005	0.008
FEV_1, % of pred., *M* ± *m*	89.1 ± 3.9	88.3 ± 2.3	96.2 ± 2.9	0.038	0.016
MEF_{25-75}, % of pred., *M* ± *m*	64.6 ± 4.3	64.8 ± 3.1	76.0 ± 3.8	0.015	0.006
ΔFEV_1, %, *M* ± *m*	12.3 (6.0; 20.0)	11.0 (5.0; 15.0)	4.5 (2.0; 13.5)	0.018	> 0.05
ACT, points, *M* ± *m*	17.0 (13.0; 19.5)	–	22.0 (17.0; 24.5)	0.037	–

Note. *p* – level of significance of the differences between the 1st and 2nd visits; p_1 – between the 2nd and 3rd visits.

Relevant sputum samples, which allowed to assess changes in the cellular pattern of bronchial inflammation, were obtained in only 26 people. As the results of the data analysis showed, the pattern of bronchial inflammation underwent transformation: from predominantly mixed during exacerbation of BA, with a high number of eosinophils (>2%) and neutrophils (> 1%) in the sputum, to eosinophilic, with a pronounced neutrophil component, without exacerbation of the disease, at the end of the examination period (Figure).

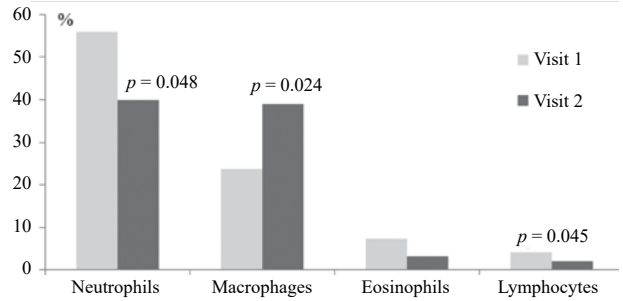

Figure. Cellular composition of induced sputum (%) in BA patients at runtime

If the percentage of eosinophils in the sputum at the 1st and 3rd visits did not differ significantly (2.2 (1.5; 7.8) and 1.6 (0.8; 3.6) %, respectively, $p > 0.05$), the number of neutrophils dramatically decreased from 55.9 ± 5.6% during exacerbation to 40.0 ± 5.5%; ($p = 0.048$) without exacerbation, remaining sufficiently high. Additionally, without exacerbation, the level of lymphocytes decreased (from 4.1 ± 0.79 to 2.1 ± 0.41%; $p = 0.045$) and the level of macrophages increased (from 23.8 ± 2.9 to 39.0 ± 6.1%; $p = 0.024$).

Linear regression analysis was used to build a model that would best show the relationship between the studied indices. When assessing the baseline values of parameters at the time of the initial examination, a relationship was revealed between the initially high number of neutrophils (N) in the sputum, other cellular elements of the sputum (macrophages, M, %), the level of control over the disease (ACT, points), and the severity of the bronchial response (ΔFEV_1) after a bronchoprovocation test with IHCA:

N (%) = 93.0 – 1.4 × M (%) – 0.3 × ACT (points) – 0.25 × ΔFEV1 (%)

The regression is significant with 98.8% probability, explains 71.7% of the dispersion. This dependence disappeared after regular long-term use of ICS/LABA.

DISCUSSION

In our earlier works concerning the relationship between the clinical manifestations of BA and the inflammatory pattern in the bronchi in patients with CIAHR, the negative effect of neutrophils on the achievement of clinical criteria for disease control was repeatedly mentioned [4]. Thus, in patients with persistent mild-to-moderate BA of mixed type, a decrease in the clinical and functional features of BA depended on an increase in the number of neutrophils and a rise in the level of neutrophil peroxidase in the inflammatory pattern of the bronchi [4].

The use of a 24-week treatment regimen with a combination of ICS/LABA in patients with moderate asthma exacerbation did not lead to a controlled decrease in the number of neutrophils in the airways, which was interpreted as a risk factor of the possible loss of the achieved asthma control [7]. The prognostically unfavorable mixed pattern of inflammation in patients with severe uncontrolled asthma in combination with CIAHR was characterized not only by a large pool of neutrophils, but also by a high degree of activity of oxidative enzymes and destruction and cytolysis of bronchial granulocytes, which was clinically manifested through a more severe course of the disease and a more complex problem of asthma control [13].

According to a number of authors, a mixed pattern of inflammation, determined in approximately 10–15% of patients with BA, is combined with more severe symptoms and more frequent exacerbations difficult to treat with ICS, compared to the Th2 pattern of bronchial inflammation [14, 15]. Neutrophilia and a mixed pattern of inflammation are associated

with increased expression of non-Th2 cytokines – IL-17 cytokine and proinflammatory IFNγ [14]. The expression of IL-17 is associated with the *in vitro* and *in vivo* identified phenomena of neutrophil NETosis, which often develops in BA patients according to the so-called non-lytic pathway with the formation of enucleated cytoplasts that induce differentiation of naive $CD4^+$ T-helper lymphocytes ($CD4^+$ Th0) into a subpopulation of T-helpers 17 (Th17) – producers of IL-17 [14, 15].

Enucleated cytoplasts, resulting from disintegration of the nuclear envelope into many vesicles and eruption of decondensed chromatin through the rupture in the neutrophil plasma membrane with resealing of the latter, have a set of rather significant functional properties. If the discarded double-stranded DNA can interact with dendritic cells of the airways *via* TLR2 receptors, which leads to the formation of $CD4^+$ Th2, then activation of dendritic cells by cytoplasts, on the contrary, causes differentiation of Th0 into antigen-specific Th17 [14, 15].

Overproduction of Th17-related cytokines, including IL-17A and IL-17F, is considered to be the main driving force for recruitment and activation of neutrophils through induction of cytokines and chemokines, such as CXCL8, IL-6, G-CSF and GM-CSF, IL-8, and CXCL1 and CXCL5, the expression of which correlates with the severity of BA and bronchial neutrophilia [16]. Cytokines and activated enzymes interacting in Th17 and Th1 inflammatory responses modify the structure of the respiratory tract in BA patients and cause remodeling and an increase in bronchial obstruction, which contributes to a fall in FEV*1* [17]. Differentiation of Th2 cells of the respiratory tract into double positive cells Th2 / Th17 is of particular interest. *In vivo* studies showed that the predominance of double positive Th2/Th17 cells in the bronchoalveolar lavage of BA patients is associated with a high degree of airway obstruction and hyperresponsiveness and an increase in the severity and cortical resistance of the disease [18].

Comparing the data on the accumulation of neutrophils, activation of Th17-associated cytokines, and the possibility of development of non-Th2 inflammation during increasing BA severity with identification of a mixed inflammatory phenotype in patients with CIAHR in the exacerbation phase, it can be assumed that deterioration of the clinical characteristics of BA during exacerbation is associated with stimulation of neutrophil-dominated inflammation. Without exacerbation, despite the decrease, the number of neutrophils in IS in 71% of patients remained quite high (more than 41%), as a result of which a pronounced neutrophil component in the inflammatory pattern was isolated.

Maintenance of relative neutrophilia in the bronchi of BA patients with CIAHR without exacerbation can be explained by the role of budesonide / formoterol therapy in the increase in the functional activity of neutrophils, namely, the antiapoptotic effect of budesonide on neutrophils. The ability of ICS to suppress the cytotoxicity of pulmonary NK cells, leading to a decrease in the intensity of NK-mediated apoptosis in granulocytes, which promotes efferocytosis in macrophages, was proven [13]. Researchers argue that neutrophilia in patients with BA can be observed independently of hormonal therapy, and non-eosinophilic asthma is the phenotype of the disease that is characterized by insensitivity to ICS therapy [17].

The mechanisms responsible for the decrease in the number of neutrophils in the airways of patients with CIAHR without exacerbation include classical NETosis – the process of programmed oxygen-dependent cell death, the purpose of which is to form highly active neutrophil extracellular traps (NETs) in response to irritants, which serve as an important tool for elimination of pathogens and inflammation products [19–21]. The formation of NETs begins with priming of neutrophils, triggering of the NADFH-oxidase enzyme complex and respiratory burst, and generation of ROS, inducing neutrophil elastase and PAD-4, which convert arginine and methylarginine residues into citrulline in histones of the nucleus. As a result, chromatin decondensation occurs with a simultaneous disturbance of the structural integrity of the cytoplasmic granule membranes. When decondensed chromatin (DNA strands, histones) is mixed with enzymes of lysosomal granules, net-like NETs are secreted into the extracellular space [19–21].

Exocytosis of myeloperoxidase (MPO) can take place not only as a result of neutrophil NETosis. As it is known, MPO, when interacting with H_2O_2, catalyzes the oxidation of halides (Cl^-, Br^-, I^-), generating the production of hypohalogenites (active forms of halogens (AFH)), hypohalogenite derivatives (HOCl, HOBr, and HOI), and their ionized forms (hypochlorite, hypobromite, and hypoiodite), resulting in a link between oxidative and halogenated stress [22, 23]. Being a product of azurophilic granules of neutrophils, MPO is secreted into the intercellular environment during cell degranulation associated with a respiratory burst.

It was shown that degranulation of neutrophils in IS of BA patients with CIAHR is capable of intensifying to the level of destruction [4, 7, 13]. Total degranulation of cells, which induces destruction and to the fullest discloses the effector capabilities of neutrophils with a maximally pronounced respiratory burst, is preceded by enzymatic activation in the form of enhanced synthesis and intragranular deposition of MPO, proportional to the needs of bronchial inflammation in AFH, involved in the prolongation and maintenance of CIAHR. Enhanced accumulation of peroxidase reserves in neutrophils, stimulated by accelerated utilization of highly reactive halogen-containing compounds in the bronchial matrix, ends with functional depletion of cells, depletion of the peroxidase-positive granule reserve, intensive destruction, and cytolysis with destruction of the cytoplasm and then the nucleus with cell lysis.

Therefore, a slight decrease in the number of neutrophils in the inflammatory pattern of the bronchi of BA patients with CIAHR without exacerbation was a consequence of ROS-stimulated NETosis, as well as destructive and cytolytic processes, the manifestation of which corresponded to the period of exacerbation associated with the activation of non-Th2 inflammatory response, the prevalence of proinflammatory cytokines, and escalating oxidative stress.

CONCLUSION

It can be concluded that first-line anti-inflammatory therapy, accompanied by the elimination of clinical and functional manifestations of exacerbation and the increase in the level of control over the disease, promoted transformation of the mixed inflammatory pattern in the bronchi into the eosinophilic one. At the same time, the neutrophil component remained quite pronounced without exacerbation, which indicated the limited effectiveness of the proposed therapy in relation to the regulation of neutrophil-dominated inflammation. This is an evidence of the preserved difficulty of comprehensive drug control over inflammation in asthma, in particular, over the pool of neutrophils in the granulocyte population, infiltrating the bronchi of patients with CIAHR.

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REFERENCES

1. Prikhodko A.G., Perelman Yu.M., Kolosov V.P. Airway hyperresponsiveness. Vladivostok: Dal'nauka; 2011: 204 (in Russ.).
2. Soodaeva S.K. Free radical mechanisms of injury in respiratory disease. *Russian Pulmonology.* 2012; 22 (1): 5–10 (in Russ.). DOI: 10.18093/0869-0189-2012-0-1-5-10.
3. Global Initiative for Asthma (GINA). Global strategy for asthma management and prevention (2018 update). URL: http://www.ginasthma.com.
4. Pirogov A.B., Prikhodko A.G., Perelman Yu.M., Zinoviev S.V. Influence of neutrophilic component of bronchial inflammation on the level of disease control and lung function in patients with asthma. *Bulletin Physiology and Pathology of Respiration*. 2016; 61: 16–24 (in Russ.). DOI: 10.12737/21434.
5. Kulikov E.S., Ogorodova L.M., Freidin M.B., Deev I.A., Selivanova P.A., Fedosenko S.V., Kirillova N.A. Molecular mechanisms of severe asthma. *Molecular Medicine*. 2013; 2: 24–32 (in Russ.).
6. Ogorodova L.M., Selivanova P.A., Gereng E.A., Bogomyakov V.S., Volkova L.I., Pleshko R.I. Pathomorphological characteristics of unstable bronchial asthma (brittle phenotype). *Therapeutic Archive.* 2008; 3: 39–43 (in Russ.).
7. Pirogov A.B., Prikhodko A.G., Perelman Yu.M., Zinoviev S.V. Dynamics of inflammatory-cellular profile of the induced sputum in patients with bronchial asthma and cold airway hyperresponsiveness under basic anti-inflammatory therapy. *Bulletin Physiology and Pathology of Respiration*. 2016; 60: 15–22 (in Russ.). DOI: 10.12737/19935. https://elibrary.ru/download/elibrary_26298679_48969552.pdf.
8. Qiu Y., Zhu J., Bandi V., Guntupalli K.K., Jeffery P.K. Bronchial mucosal inflammation and upregulation of CXC chemoattractants and receptors in severe exacerbations of asthma. *Thorax*. 2007; 62(6):475–482. DOI: 10.1136/thx.2006.066670.
9. Turato G., Baraldo S., Zuin R., Saetta M. The laws of attraction: chemokines, neutrophils and eosinophils in severe exacerbations of asthma. *Thorax.* 2007; 62 (6): 465–466. DOI: 1136/thx.2006.070656.
10. Kolosov V.P., Pirogov A.B., Perelman Yu.M., Naryshkina S.V., Maltseva T.A. Achievement of asthma control in patients with cold airway hyperresponsiveness at different variants of basic therapy. *Eur. Respir. J.* 2013; 42 (57): 400.
11. Bakakos P., Schleich F., Alchanatis M., Louis R. Induced sputum in asthma: From bench to bedside. *Curr. Med. Chem*. 2011; 18 (10): 1415-1422. DOI: 10.2174/092986711795328337.
12. Ulyanychev N.V. Systematic research in medicine. Saarbrücken: LAP LAMBERT; 2014: 140 (in Russ.).
13. Pirogov A.B., Kolosov V.P., Perelman Yu.M., Prikhodko A.G., Zinoviev S.V., Gassan D.A., Maltseva T.A. Airway inflammation patterns and clinical and functional features in patients with severe uncontrolled asthma and cold-induced airway hyperresponsiveness. *Russian Pulmonology*. 2016; 26 (6): 701–707 (in Russ.). DOI: 10.18093/0869018920162667017 07.
14. Duvall M.G., Krishnamoorthy N., Levy B.D. Non-type 2 inflammation in severe asthma is propelled by neutrophil cytoplasts and maintained by defective resolution. *Allergology International*. 2019; 68 (2): 143–149. DOI: 10.1016/j.alit.2018.11.006.

15. Krishnamoorthy N., Douda D.N., Brüggemann T. R., Ricklefs I., Duvall M.G., Abdulnour R.E., Martinod K., Tavares L., Wang X., Cernadas M., Israel E., Mauger D.T., Bleecker E.R., Castro M., Erzurum S. C., Gaston B.M., Jarjour N.N., Wenzel S., Dunican E., Fahy J.V., Irimia D., Wagner D.D., Levy B.D. Neutrophil cytoplasts induce TH17 differentiation and skew inflammation toward neutrophilia in severe asthma. *Science Immunology.* 2018. 3 (26): eaao4747. DOI: 10.1126/sciimmunol.aao4747.
16. Wisam A.R., Pre´fontaine D., Chouiali F., Martin J. G., Olivenstein R., Lemie`re C., Hamid Q. TH17-associated cytokines (IL-17A and IL-17F) in severe asthma. A*llergy Clin. Immunol.* 2009; 123 (5): 1185–1187. DOI: 10.1016/j.jaci.2009.02.024.
17. Esteban-Gorgojo I., Antolín-Amérigo D., Domínguez-Ortega J., Quirce S. Non-eosinophilic asthma: current perspectives. *Journal of Asthma and Allergy.* 2018; 11: 267–281. DOI: 10.2147/jaa.s153097.
18. Irvin C., Zafar I., Good J., Rollins D., Christianson C., Gorska M.M., Martin R.J., Alam R. Increased frequency of dual-positive TH2/TH17 cells in bronchoalveolar lavage fluid characterizes a population of patients with severe asthma. *J. Allergy Clin. Immunol.* 2014; 134 (5): 1175–1186. DOI: 10.1016/j.jaci.2014.05.038.
19. Korotina O.L., Generalov I.I. Neutrophilic extracellular traps: formation mechanisms, functions. *International Journal of Immunopathogy, Allergology, Infectology.* 2012; 4: 23–32 (in Russ.).
20. Kravtsov A.L. Formation of extracellular traps – the effective mechanism of organism protection from pathogen. *Problems of Particularly Dangerous Infections.* 2012; 112: 69–74 (in Russ.). DOI: 10.21055/0370-1069-2012-2(112)-69-74.
21. Brinkmann V., Zychlinsky A. Beneficial suicide: why neutrophils die to make NETs. *Nat. Rev. Microbiol*. 2007; 5 (8): 577–582. DOI: 10.1038/nrmicro1710.
22. Panasenko O.M., Sergienko V.I. Halogenating stress and biomarkers. *Annals of the Russian Academy of Medical Sciences*. 2010; (1): 27–39 (in Russ.).
23. Senthilmohan R., Kettle A.J. Bromination and chlorination reactions of myeloperoxidase at physiological concentrations of bromide and chloride. *Arch. Biochem. Biophys.* 2006; 445 (2): 235–244. DOI: 10.1016/j.abb.2005.07.005.

Authors contribution

Pirogov A.B. – conception and design, analysis and interpretation of data, drafting of the article. Prikhodko A.G. – analysis and justification of the manuscript, critical revision for important intellectual content. Afanaseva E.Yu., Shvetsova Ya.G., Sheludko E.G. – selection and management of patients in the clinical study, collection and processing of biological material, statistical analysis of the obtained material. Zhou X., Li Q. – analysis and interpretation of data. Perelman Yu.M. – critical revision for important intellectual content, final approval of the manuscript for publication.

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Clinical characteristics and efficiency of antidepressant therapy of mood disorders with comorbid alcohol use disorder

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ABSTRACT

Aim. To determine the nosological and clinical features of mood disorders (MD) with comorbid alcohol use disorder (AUD) and efficiency of antidepressant therapy.

Materials and methods. We examined 88 patients with MD and comorbid AUD – 33 females (37.5%) and 55 males (62.5%). The first group included 31 patients with AUD without comorbid affective symptoms, the second group contained 29 patients with MD without AUD, the third group included 28 patients with AUD and MD. In the study, we applied clinical-psychopathological, clinical-dynamic, and statistical methods with Pearson's χ 2 test, Mann – Whitney *U*-test (for comparison of independent samples), Kruskal – Wallis test (for more than two independent samples), and Wilcoxon test (for comparison of dependent samples). At the level of statistical significance, no differences between the groups according to the gender – age composition were revealed ($p = 0.115$ – according to gender composition, $p = 0.248$ – according to age composition, Pearson's χ 2 test).

Results. The patients with the diagnosis of AUD with comorbid MD showed worse dynamics of the reduction of depressive [from 24.0 (18.3; 33.0) to 9.0 (4.3; 12.0) points according to the Structured Interview Guide for the Hamilton Depression Rating Scale – Seasonal Affective Disorder (SIGH-SAD) ($p = 0.001$, Wilcoxon test)] and anxiety [from 20.5 (12.5; 25.0) to 5.5 (3.3; 8.0) points according to the Hamilton Anxiety Rating Scale (HARS) ($p = 0.001$, Wilcoxon test)] symptoms against the background of the therapy with initially lower indices compared to the group with MD alone [from 27.0 (21.0; 36.0) to 6.0 (5.0; 11.0) points according to SIGH-SAD ($p = 0.001$, Wilcoxon test) (intergroup differences upon admission $p = 0.046$; upon discharge $p = 0.683$, Mann – Whitney *U*-test) and from 21.0 (14.0; 29.0) to 5.0 (3; 10.5) points according to HARS ($p = 0.001$, Wilcoxon test) (intergroup differences upon admission – $p = 0.082$; upon discharge – $p = 0.825$, Mann – Whitney *U*-test)]. The course of AUD is characterized by a larger extent of malignancy in the group with a comorbidity: a decrease in pathological alcohol craving from 31.5 (16.3; 43.5) to 8 (2.3; 14.8) points ($p = 0.001$, Wilcoxon test) in the group with a comorbidity and from 29.5 (21.8; 37.0) to 7 (3.0; 11.3) points with AUD alone ($p = 0.001$, Wilcoxon test) (intergroup differences upon admission – $p = 0.058$; upon discharge – $p = 0.04$, Mann – Whitney *U*-test on the Obsessive Compulsive Drinking Scale (OCDS)).

Conclusion. Clinical-dynamic characteristics of MD with comorbid AUD result in therapeutic difficulties associated with comparatively worse dynamics in reduction of the symptoms of both diseases.

Key words: alcohol addiction, depressive disorders, comorbidity, antidepressant therapy, anti-craving therapy.

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

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Conformity with the principles of ethics. All patients signed an informed consent to participate in the study. The study was approved by the local Ethics Committee of Mental Health Research Institute, Tomsk NRMC (Protocol No. 6 of 21.06.2017).

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Клиническая характеристика и эффективность антидепрессивной терапии аффективных расстройств при коморбидности с алкогольной зависимостью

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РЕЗЮМЕ

Цель исследования – определение нозологической структуры, клинических особенностей аффективных расстройств (АР) при коморбидности с алкогольной зависимостью (АЗ) и эффективности антидепрессивной терапии.

Материалы и методы исследования. Обследованы 88 человек с АР и АЗ – 33 женщины (37,5%) и 55 (62,5%) мужчин. Первая группа – 31 пациент с АЗ без коморбидной аффективной симптоматики, вторая – 29 больных с расстройством настроения без зависимости от алкоголя, третья – 28 пациентов с коморбидным течением АЗ и АР. В исследовании использовался клинико-психопатологический, клинико-динамический и статистический методы с использованием критериев χ^2 Пирсона, Манна – Уитни (для сравнения независимых выборок), Краскела – Уоллиса (для более двух независимых выборок), Вилкоксона (для сравнения зависимых выборок). По уровню статистической значимости различий между группами по половозрастному составу не выявлено ($p = 0{,}115$ – по половому составу, $p = 0{,}248$ – по возрастному составу, критерий χ^2).

Результаты. Пациенты с коморбидным диагнозом АЗ и АР демонстрируют худшую динамику редукции депрессивной (с 24,0 (18,3; 33,0) до 9,0 (4,3; 12,0) баллов по шкале SIGH-SAD ($p = 0{,}001$, критерий Вилкоксона)) и тревожной (с 20,5 (12,5; 25,0) до 5,5 (3,3; 8,0) баллов по шкале HARS ($p = 0{,}001$, критерий Вилкоксона)) симптоматики на фоне лечения, при изначально более низких показателях, в сравнении с группой с «чистыми» АР (с 27,0 (21,0; 36,0) до 6,0 (5,0; 11,0) баллов по SIGH-SAD ($p = 0{,}001$, критерий Вилкоксона) (межгрупповые различия при поступлении $p = 0{,}046$; при выписке $p = 0{,}683$, критерий Манна – Уитни) и с 21,0 (14,0; 29,0) до 5,0 (3; 10,5) баллов по HARS ($p = 0{,}001$, критерий Вилкоксона) (межгрупповые различия при поступлении $p = 0{,}082$; при выписке $p = 0{,}825$, критерий Манна – Уитни). Течение АЗ отличается большей злокачественностью в группе с коморбидностью: снижение патологического влечения к алкоголю с 31,5 (16,3; 43,5) балла до 8 (2,3; 14,8) ($p = 0{,}001$, критерий Вилкоксона) в группе с коморбидностью и с 29,5 (21,8; 37,0) до 7 (3,0; 11,3) баллов при «чистой» АЗ ($p = 0{,}001$, критерий Вилкоксона) (межгрупповые различия при поступлении $p = 0{,}058$; при выписке $p = 0{,}04$, критерий Манна – Уитни по обсессивно-компульсивной шкале употребления алкоголя.

Заключение. Клинико-динамические характеристики коморбидного сочетания АР и АЗ ведут к терапевтическим затруднениям, сопряженным со сравнительно худшей динамикой редуцирования симптомов каждого из заболеваний.

Ключевые слова: алкогольная зависимость, депрессивные расстройства, коморбидность, антидепрессивная терапия, антикрейвинговая терапия.

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов в связи с публикацией данной статьи.

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INTRODUCTION

In the current concept of comorbidity of alcohol use disorder (AUD) and mood disorders (MD), co-occurrence of two pathologies is regarded as a synergetic condition, unfavorable for prognosis of each of them [1, 2]. Taking into account the polymorphism of psychopathological symptoms, a personalized therapeutic approach is needed, focused not only on the correction of emotional disturbances but also on the anti-craving therapy for the dependence syndrome.

Among the main factors influencing formation of alcohol addiction and unfavorable prognosis of its course, comorbid mental disorders, primarily of the schizophrenia and affective spectrum, are distinguished [3–6]. In both cases, comorbidity leads to worsening of the disease prognosis [7, 8]. In clinical practice, the comorbidity of MD and AUD is often unrecognized – this can be associated with clinical pathomorphism, when a combination of severe disturbances in one disease and obliterated manifestations of the other often look like manifestations of the first, and the second disorder is either overlooked or ignored [9, 10].

The choice of only one of the existing psychiatric disorders as a therapeutic target negatively affects the effectiveness of therapy, increases its duration, and reduces the duration and quality of remissions. It is important to determine the clinical features and identify suicidal behavior in patients with MD in comorbid mental and somatic diseases [11–13].

MATERIALS AND METHODS

The study included 88 patients admitted to the clinic of Mental Health Research Institute of Tomsk NRMC with a verified diagnosis of AUD (F10.2) or MD (F31.3, F31.6, F32, F33, F34.1) according to ICD-10. Clinical-psychopathological, psychometric, clinical-dynamic, and statistical research methods were used. To evaluate the clinical dynamics, a structured interview for evaluation of depression severity according to HARS (1959) and SIGH-SAD (2002) was used. A risk of alcohol addiction was evaluated according to the Alcohol Use Disorders Identification Test (AUDIT, 1993). The Obsessive Compulsive Drinking Scale (OCDS, 1995) was applied to assess alcohol craving.

Statistical data processing was performed using IBM SPSS Statistics 25 software with the Pearson's χ 2 test, Mann – Whitney *U*-test (for comparison of independent samples), Kruskal – Wallis test (for more than two independent samples), and Wilcoxon test (for comparison of dependent samples). The samples were previously tested for compliance with the law of normal distribution using the Shapiro – Wilk test, which tests the hypothesis that there are no differences between the observed distribution of a trait and the theoretically expected normal distribution. In case of distribution other than normal, quantitative data were presented as the median and the interquartile range *Me* (*Q1*; *Q3*). When testing the hypothesis, the critical level of significance *p* was equal to 0.05.

RESULTS

According to the results of the examination, the patients were divided into three groups. The first group included AUD patients without comorbid affective symptoms (n = 31), 87.1% of them were males, the average age of patients in this group was 44 (40; 53) years. The second group contained MD patients without alcohol addiction (n = 29), 72.4% of whom were females, the average age of patients was 50 (36.5; 57) years. The third group included patients with AUD and comorbid MD (n = 28), among whom 71.4% were males, the average age was 44.5 (36.5; 48.75) years. We did not reveal statistically significant differences

between the groups according to the gender – age composition ($p > 0.05$, Pearson's χ 2 test). The structure of MD in the group without comorbid dependence syndrome was represented by depressive episodes (DE) of various degrees of severity in 34.5% of patients, DE within a recurrent depressive disorder in 31% of individuals, and DE within bipolar disorder (BD) in 24.1% of cases. The comorbid affective pathology was represented equally by dysthymia and DE within a recurrent depressive disorder (32.1% each). Depressive symptoms within BD were revealed in 21.4% of cases. In 14.3% of cases, the comorbid diagnosis was DE of moderate (10.7%) or mild (3.6%) severity. The duration of MD was 5 (2.5; 11.0) years in the group of patients with affective pathology alone and 7.5 (2.25; 13.0) years in patients with a comorbidity ($p < 0.05$, Mann – Whitney *U*-test). The duration of AUD (since the age of formation of the alcohol withdrawal syndrome (AWS)) in the groups with AUD alone and AUD and a comorbidity was 10 (6; 18.5) and 14 (10; 19.75) years, respectively ($p < 0.05$, Mann – Whitney *U*-test).

Based on these terms for disease duration, it can be concluded that in the group with MD with comorbid AUD, substance dependence, as a rule, preceded the MD manifestation. Alcohol use in both groups had a pseudo-binge-drinking nature in 100% of observations. Besides, it is worth noting that the duration of pseudo-binge-drinking and alcohol tolerance were statistically significantly lower in patients with MD and AUD ($p < 0.05$, Mann – Whitney *U*-test). So, the average duration of pseudo-binge-drinking episodes in patients with MD alone was 7 (4; 17) days, and in patients with MD with a comorbidity – 5.5 (3.5; 9.5) days. The tolerance was 16 (11; 23) and 11 (11; 17.75) standard alcohol servings, respectively.

Despite relatively lower volume and duration of alcohol use by patients with dual diagnosis, the duration of AWS was compatible to that in patients with AUD alone: 3 (2; 4) and 3 (2; 5) days, respectively ($p = 0.785$, Mann – Whitney *U*-test). These data indicate poorer tolerance of ethanol effects in patients with MD with comorbid AUD. In the group of patients with MD with comorbid AUD, attention is drawn to the predominance, along with neurovegetative variant of AWS in 78.6% ($n = 22$) of observations, of the psychopathological variant – 14.3% ($n = 4$), which manifested itself predominantly through affective symptoms (depressive, anxious, dysphoric affect). In the group with AUD alone, the second most prevalent variant after the neurovegetative one (83.9%, $n = 26$) was the cerebral variant of AWS (9.7%, $n = 3$), which manifested itself predominantly through cephalgia, dizziness, and muscle twitching. After AWS management, affective disturbances in patients with MD with comorbid AUD not only remained, but also acquired apparent clinical presentation.

The main motive for alcohol consumption was the desire for pleasure in the AUD group: hedonistic motivation was observed in 45.2% ($n = 14$) of cases, while in patients with a comorbidity, it was present only in 10.7% ($n = 3$) of individuals. Half of the patients with comorbid MD used alcohol with the aim to correct the emotional state – 50% ($n = 14$), and among patients with AUD alone, there were 9.6% of such patients ($n = 3$). The duration of AUD remissions in patients with a comorbidity reached 12 (3; 24) months, while with comorbid MD, it was 6 (1.25; 34.5) months ($p = 0.037$, Mann – Whitney *U*-test). In cases of MD alone and comorbid MD, these values were 5 (3; 21.75) and 4 (1; 12) months, respectively ($p = 0.048$, Mann – Whitney *U*-test). All patients with a comorbidity noted a pronounced relationship between MD remission and AUD, that is, the cessation of alcohol use led to normalization of the emotional state, and stable emotional background reduced alcohol consumption to a minimum. In this cohort, in 46% ($n = 13$) of cases, failure to achieve AUD remission was preceded by an increase in MD symptoms, and in 32% ($n = 9$) of patients – by resumed alcohol use. Symptoms of both disorders developed simultaneously in 22% ($n = 6$) of the respondents.

Based on the complaints presented by the patient at the time of the initial examination (during the 1st week of hospitalization, after AWS management, in case of seeking medical care in AWS), the leading complaints were identified that characterize the patient's subjective assessment of the condition and determine the therapeutic request when seeking medical care (Table 1).

Table 1

Complaints of the examined patients upon admission

Parameter	Patients with AUD	Patients with MD	Patients with dual diagnosis
Alcohol craving	75.8% ($n = 23$)	–	–
Low mood	16.2% ($n = 5$)	51.7% ($n = 15$)	78.6% ($n = 22$)
Emotional lability, irritability, hot temper	–	10.4% ($n = 3$)	10.7% ($n = 3$)
Anxiety, feeling of inner tension	1.6% ($n = 1$)	37.9% ($n = 11$)	7.1% ($n = 2$)
Anergy, fatigue, asthenia	6.4% ($n = 2$)	–	3.6% ($n = 1$)

Probably, depressive symptoms (decreased mood) in the group of AUD patients were revealed as an obligate component of post-withdrawal syndrome as well as an emotional component of pathological alcohol craving [14]. Asthenic symptoms (anergy, fatigue, asthenia) in both groups of patients who used alcohol could be associated with immediate toxic effect of ethanol on the central nervous system (CNS) [15]. It is worth noting that the patients with AUD with comorbid MD had complaints of the affective spectrum, that is they named correction of the emotional state as the reason for seeking medical care, which, in their opinion, led to excessive alcohol use.

In accordance with the clinical presentation and the leading symptoms, the patients received psychopharmacotherapy with antidepressants and mood stabilizers (Table 2).

Table 2

The main group of psychopharmaceuticals			
Parameter	Antidepressants	Mood stabilizers	No therapy
Patients with AUD	22.5% (n = 7)	64.5% (n = 20)	13% (n = 4)
Patients with MD	79.3% (n = 23)	20.7% (n = 6)	–
Patients with dual diagnosis	60.7% (n = 17)	35.7% (n = 10)	3.6% (n = 1)

For the patients hospitalized in the state of withdrawal, the treatment was administered after management of the withdrawal syndrome, on the 3–5th day of hospitalization. In the groups of AUD patients, there were cases with no maintenance psychopharmacotherapy, which was associated with contraindications to its administration due to comorbid physical pathology. In such a situation, the treatment was focused on symptomatic and psychotherapeutic correction. The patients with AUD were treated with escitalopram (15 mg / day) in 71.4% (n = 5) of cases and agomelatine (25 mg / day) in the remaining 28.6% (n = 2) of cases. Treatment with these drugs cured sleep disorders induced by alcohol dependence, did not affect the parameters of cardiovascular therapy, and did not impair sexual functions. As an alternative to antidepressant therapy, 75.0% (n = 15) of patients were prescribed carbamazepine (400 mg / day), and the remaining 25.0% (n = 5) – topiramate (100 mg / day).

Treatment of patients with MD in 47.8% (n = 11) of cases was carried out with vortioxetine (10 mg/ day), in 21.7% (n = 5) of cases – with sertraline (150 mg / day), in 21.7% (n = 5) of cases – with escitalopram (25 mg / day), in 8.8% (n = 2) of cases – with agomelatine (25 mg / day). The preferential treatment with these modern drugs had high potential for relieving the main symptoms of depression with their excellent tolerance. An alternative strategy for correcting affective disorders in the context of bipolar disorder was the administration of valproic acid sodium salts (750 mg / day) in all cases (n = 6).

The patients with a dual diagnosis received vortioxetine (10 mg / day) in 41.2% (n = 7) of cases, agomelatine (50 mg / day) in 23.6% (n = 4) of cases, escitalopram (25 mg / day) in 17.6% (n = 3) of cases, and sertraline (100 mg / day) in 17.6% (n = 3) of cases. The multi-target and highly selective mechanisms of action of these drugs influenced anhedonia, one of the key symptoms of depressive and addictive disorders, which was associated with suicidal behavior in these patients. Another part of the patients received treatment with carbamazepine (400 mg / day) in 70.0% (n = 7) of cases or valproic acid sodium salts (500 mg / day) in 30.0% (n = 3) of cases to correct affective disorders.

According to the follow-up data, after previous visits for medical care, the majority of patients (68.9%, n = 18), suffering only from affective pathology, received maintenance psychopharmacotherapy. In 62% (n = 18) of cases, it was a drug from the group of selective serotonin reuptake inhibitors (SSRIs): sertraline (n = 8, 100 mg / day), escitalopram (n = 5, 20 mg / day), fluvoxamine (n = 3, 150 g / day), fluoxetine (n = 2, 40 mg / day). 6.9% (n = 2) of patients received a mood stabilizer (valproic acid, 500 mg / day). The average duration of drug intake was 6 (3; 12) months (p = 0.04, Pearson's χ 2 test).

Among the patients with depression associated with AUD, only 21.5% (n = 6) of the respondents received maintenance therapy. The drug from the SSRI group was taken by 17.9% (n = 5) of patients: escitalopram (n = 3, 10 mg / day), sertraline (n = 2, 50 mg / day); 3.6% (n = 1) of patients received a mood stabilizer (carbamazepine, 400 mg / day). The period of independent intake of drugs was 3 (2; 11.25) months (p = 0.03; Pearson's χ 2 test). The presented data indicate low adherence of patients suffering from AUD (both alone and in combination with another pathology) to long-term treatment. Patients do not always follow medical recommendations and tend to stop taking medications earlier than the recommended time [16].

The examined individuals from the group of AUD patients in 98.4% of cases did not receive any maintenance therapy: either the drug was not prescribed by the doctor, or the patients themselves refused to take drugs after discharge from the clinic. Despite the fact that administration of antidepressants is considered to

be the therapy of choice for depressive disorders in the structure of the pathological alcohol craving and suppression of the pathological craving for substances is their independent property, regardless of manifestation of the antidepressant effect, addiction specialists rarely resorted to prescribing antidepressant psychopharmacotherapy [17]. Anticonvulsants, actively used by doctors in substance abuse treatment centers, represent an alternative for benzodiazepine tranquilizers for correction of AWS [18]. However, there are no recommendations on their use in anti-craving therapy for alcohol dependence. Most of the requests for drug treatment ended with implementation of one or another type of subject-mediated hypnosuggestion of a ban on alcohol consumption. Such prevalent techniques as aversion therapy, implanting chemicals under patient's skin, and implanting an anti-alcohol placebo-drug are now an officially recognized anachronism prohibited in state institutions and not included in the Standards for the Provision of Primary Health Care and Specialized Narcological Aid [19, 20].

The examinations carried out upon admission (point 1) and discharge (point 2) using the SIGH-SAD and HARS scales made it possible to objectively assess the severity of depressive (typical and atypical) and anxiety symptoms (Kruskal – Wallis test), and their clinical dynamics (Wilcoxon test) (Tables 3–4).

Table 3

Dynamics of the score on the SIGH-SAD scale

Parameter	Typical symptoms		Atypical symptoms		Total score	
	Point 1	Point 2	Point 1	Point 2	Point 1	Point 2
Patients with AUD	7.0 (3.0; 12.3)*	1.0 (0; 4.0)	2.0 (0; 2.3)*	0 (0; 0)	9.0 (4.0; 14.3)**	1.0 (0; 4.0)
Patients with MD	23.0 (19.5; 29.0)	6.0 (3.5; 11.0)	4.0 (1.0; 7.5)	1.0 (0; 2.0)	27.0 (21.0; 36.0)	6.0 (5.0; 11.0)
Patients with a dual diagnosis	20.0 (16.0; 25.8)	7.0 (4.0; 10.0)	4.0 (1.0; 6.0)	2.0 (0.5; 4.2)	24.0 (18.3; 33.0)**	9.0 (4.3; 12.0)

* $p = 0.001$ (Kruskal – Wallis test) for all cases, ** $p = 0.001$ (Wilcoxon test) for all cases.

Table 4

Dynamics of the score on the HARS scale

Parameter	Point 1	Point 2
Patients with AUD	8.0 (3.8; 14.3)* (**)	1.0 (0; 2.0)
Patients with MD	21.0 (14.0; 29.0)**	5.0 (3; 10.5)
Patients with a dual diagnosis	20.5 (12.5; 25.0)**	5.5 (3.3; 8.0)

* $p = 0.001$ (Kruskal – Wallis test) for all cases, ** $p = 0.001$ (Wilcoxon test) for all cases.

At the 1st week of treatment, patients in the group with MD alone noted greater severity of both typical and atypical depressive symptoms on the SIGH-SAD scale, as well as anxiety on the HARS scale, compared to the group of patients with a dual diagnosis ($n = 0.046$; Mann – Whitney U-test for SIGH-SAD and $p = 0.082$ for HARS). The levels of anxiety and depression in patients with AUD alone were initially significantly lower than in the other groups ($p = 0.001$; Kruskal – Wallis test for HARS and SIGH-SAD) and were probably detected within the affective component of AWS.

Against the background of psychopharmacotherapy, by the end of the treatment, there was a decrease in the intensity of affective symptoms in the groups of patients with MD ($p = 0.001$; Wilcoxon test) (with and without a comorbidity) to statistically comparable values ($p = 0.683$; Mann – Whitney U-test for SIGH-SAD and $p = 0.825$; Mann – Whitney U-test for HARS), and there were significant intergroup differences compared to the group of patients with AUD alone ($p = 0.001$; Kruskal – Wallis test). Therefore, patients with a dual diagnosis demonstrated comparatively worse dynamics in the reduction of depressive (both typical and atypical symptoms) and anxiety symptoms during treatment with initially lower rates compared to patients with depression alone.

The AUDIT and OCDS scales allowed to assess the subjective severity of AUD. The AUDIT test was developed by the World Health Organization for screening assessment of alcohol use disorders [21]. The sum of the AUDIT scores in the group of patients with AUD alone was 24 (19; 28.25). In AUD with comorbid MD, this score was higher – 26.5 (20.5; 30.5) ($p = 0.03$; Mann – Whitney U-test). In other words, the patients with AUD with comorbid MD showed a tendency to more active alcohol use, as well as a higher risk of adverse events from alcohol abuse.

The OCDS scale is designed for self-assessment of manifestations of attitudes towards alcohol over the past week. According to OCDS scores, alcohol craving was higher at both points of examination in the group with a dual diagnosis (31.5 (16.3; 43.5) and 8 (2.3; 14.8), respectively) than in the group of patients with AUD alone (29.5 (21.8; 37.0) and 7 (3.0; 11.3), respectively) (upon admission – $p = 0.058$; Mann – Whitney *U*-test, upon discharge – $p = 0.04$; Mann – Whitney *U*-test, intragroup dynamics – $p = 0.001$; Wilcoxon test). The analysis of the results of the study following the OCDS and AUDIT tests showed that alcohol addiction with a comorbid affective pathology was characterized by a more malignant clinical course. Pathological alcohol craving was more pronounced and less responsive to therapy, and alcohol consumption was characterized by a more pronounced risk of developing disorders associated with alcohol abuse.

DISCUSSION

Earlier, clinical polymorphism and features of therapy for MD with comorbid AUD were repeatedly pointed out [22–27]. At the same time, there is no consensus regarding the clinical effect of comorbidity on the course of each disease. According to some Russian researchers, AUD, as a rule, accompanies minor depressive disorders, and with an increase in MD, alcohol abuse may stop altogether [28]. Depressions in AUD are often described as "disharmonious", with a large proportion of asthenic-apathetic or dysphoric symptoms [29]. The results of the study also showed that the clinical presentation of MD with comorbid AUD is characterized by lower clinical severity of MD symptoms (according to the SIGH-SAD and HARS scales) compared to the group of patients with affective pathology alone, but by worse dynamics against the background of psychopharmacotherapy.

The commonality of the neurochemical mechanisms in the pathogenesis of the pathological alcohol craving and depressive disorders determines the dependence of actualization or regression of craving on the severity of affective symptoms [30]. Alcohol craving in comorbid patients is much stronger than in the group with AUD alone (according to OCDS). Probably, this should be considered not only as patient's perception of alcohol as a "therapeutic" means for self-treatment to correct the emotional state or reduce side effects of psychopharmacological drugs [31], but also as interest in the formation of symptoms of a wide range of neurotransmitter systems [32]. Such pathogenetic affinity of MD and craving explains its relative persistence in the group of patients with MD with comorbid AUD (according to OCDS), which leads to the conclusion that it is necessary to intensify anti-craving therapy for this cohort of patients.

CONCLUSION

It was found that patients with a dual diagnosis demonstrate the worst dynamics in terms of reduction of depressive (both typical and atypical symptoms) and anxiety symptoms during treatment, with initially lower values compared to the group of patients suffering from depression alone. AUD with comorbid MD is characterized by greater malignancy and worse antidepressant effect during psychopharmacotherapy. In the treatment of AUD, both alone and with a comorbidity, clinicians pay insufficient attention to anti-craving pharmacotherapy with antidepressants.

REFERENCES

1. Roshchina O.V., Rozin A.I., Schastnyy E.D., Bokhan N.A. Clinical value of comorbidity of affective disorders and alcohol dependence. *Bulletin of Siberian Medicine*. 2019; 18 (4): 110–118 (in Russ.). DOI: 10.20538/1682-0363-2019-4-110–118.
2. Vasilieva S.N., Simutkin G.G., Schastnyy E.D., Bokhan N.A. Clinical-dynamic features of affective disorders comorbid with alcohol dependence. *International Journal of Mental Health and Addiction*. 2020. DOI: 10.1007/s11469-020-00234-y.
3. Shytov E.A., Kiselev D.N., Shustov D.I. Comorbidity of mental disorders with alcohol and drug abuse (revue of the problem dual diagnose). *I.P. Pavlov Russian Medical Biological Herald*. 2009; 17 (2): 100–105 (in Russ.).
4. Marusin A.V., Kornetov A.N., Swarovskaya M.G., Vagaitseva K.V. Pavlenyuk E.S., Stepanov V.A. Genetic predisposition to alcoholism, schizophrenia and Alzheimer's disease with psychodiagnostic characteristics in Russian population. *Bulletin of Siberian Medicine*. 2016; 15 (5): 83–96 (in Russ.).
5. Voevodin I.V., Mandel A.I., Abolonin A.F., Belokrylov I.I. Psychological risk factors and variants of addictive, anxiety, and depressive disorders formation among the students. *Siberian Herald of Psychiatry and Addiction Psychiatry*. 2018; 4 (101): 21–27 (in Russ.). DOI: 10.26617/1810-3111-2018-4 (101)-21-27.
6. Andryuschenko A.V., Shulyak Yu.A. Alcohol addiction and depression: approaches to diagnostics and therapy. *Medical Council*. 2016; 19: 28–36 (in Russ.). DOI: 10.21518/2079-701X-2016-19-28-36.
7. Moggi F. Epidemiology, etiology and treatment of patients with psychosis and co-morbid substance use disorder. *Therapeutische Umschau*. 2018; 75 (1): 37–43. DOI: 10.1024/0040-5930/a000964.
8. Grazioli V.S., Bagge C.L., Studer J., Bertholet N., Rougemont-Bücking A., Mohler-Kuo M., Daeppen J.B., Gmel G. Depressive symptoms, alcohol use and coping drinking mo-

tives: Examining various pathways to suicide attempts among young men. *Journal of Affective Disorders*. 2018; 232: 243–251. DOI: 10.1016/j.jad.2018.02.028.
9. Fein G. Psychiatric comorbidity in alcohol dependence. *Neuropsychology Review*. 2015; 25 (4): 456–475. DOI: 10.1007/s11065-015-9304-y.
10. Turner S., Mota N., Bolton J., Sareen J. Self-medication with alcohol or drugs for mood and anxiety disorders: a narrative review of the epidemiological literature. *Depression and Anxiety*. 2018; 35 (9): 851–860. DOI: 10.1002/da.22771.
11. Lebedeva E.V., Simutkin G.G., Schastnyy E.D., Repin A.N., Sergienko T.N. Particularly disturbing, depressive disorders and suicidal behavior in patients of elderly and senile age with chronic CAD. *Suicidology*. 2014; 5 (5): 69–76 (in Russ.).
12. Vasilieva S.N., Simutkin G.G., Schastnyy E.D., Ivanova S.A., Bokhan N.A. Suicidal behavior of patients suffering from bipolar affective disorder and comorbid mental diseases. *Suicidology*. 2018; 9 (3): 86–92 (in Russ.). DOI: 10.32878/suiciderus.18-09-03(32)-86-92.
13. Lebedeva E.V., Schastnyy E.D., Simutkin G.G., Repin A.N., Nonka T.G. Clinical description of affective disorders and efficiency of antidepressant therapy. *Bulletin of Siberian Medicine*. 2018; 17 (4): 85–93 (in Russ.). DOI: 10.20538/1682-0363-2018-4-85–93.
14. Buzik O.J., Agibalova T.V. Comorbid diseases in alcohol-dependent patients. *I.P. Pavlov Russian Medical Biological Herald*. 2008; 3: 79–86 (in Russ.).
15. Samushia M.A. Monograph review by Bokhan N.A., Korobitsina T.V. "Associated forms of alcoholism in general hospital network". *Mental Disorders in General Medicine*. 2008; 02: 45–47 (in Russ.).
16. Salmina-Khvostova O.I., Zyablitsev D.V. The significance of motivation, stages of readiness to behavior changes, factors of compliance to treatment of alcohol addiction. *Russian Medical Journal*. 2013; 4: 22–25 (in Russ.).
17. Agibalova T.V., Altshuler V.B., Vinnikova M.A., Dudko T.N., Kozyreva A.V., Kravchenko S.L., Nenastieva A.Yu., Utkin S.I. Federal clinical guidelines for the diagnosis and treatment of addiction syndrome. *RSP*. 2014; 57 (in Russ.).
18. Sivolap Yu.P. Alcohol abuse and related disorders treatment of alcohol dependence. *Neurology, Neuropsychiatry, Psychosomatics*. 2014; 3: 7–9 (in Russ.). DOI: 10.14412/2074-2711-2014-3-4-9.
19. Artemyeva M.S., Maydan A.V. Comparative analysis of modern alcoholism treatment concepts. *Health and Education Millennium*. 2017; 19 (9): 19–21 (in Russ.).
20. Krupitsky E.M. Short-term intensive psychotherapeutic narcologic influence as viewed from positions of evidence-based medicine. *Neurological Bulletin*. 2010; 3: 25–27 (in Russ.).
21. Nenastieva A.Yu. Psychometric scales used in modern clinical addiction medicine. *Journal of Addiction Problems*. 2018; 7 (16): 46–71 (in Russ.).
22. Carton L., Pignon B., Baguet A., Benradia I., Roelandt J.L., Vaiva G., Thomas P., Amad Ali., De Philippe T., Naassila M., Geoffroy P.A., Rolland B. Influence of comorbid alcohol use disorders on the clinical patterns of major depressive disorder: a general population-based study. *Drug and Alcohol Dependence*. 2018; 187: 40–47. DOI: 10.1016/j.drugalcdep.2018.02.009.
23. Claycomb E.M., Charak R., Durham T.A., Armour C., Ly C., Southwick S.M., Elhai J.D., Pietrzakde R.H. The 7-factor hybrid model of DSM-5 PTSD symptoms and alcohol consumption and consequences in a national sample of trauma-exposed veterans. *Journal of Anxiety Disorders*. 2017; 51: 14–21. DOI: 10.1016/j.janxdis.2017.08.001.
24. Destoop M., Morrens M., Coppens V., Dom G. Addiction, Anhedonia, and Comorbid Mood Disorder. A Narrative Review. *Frontiers in Psychiatry*. 2019; 10: 311. DOI: 10.3389/fpsyt.2019.00311.
25. Elsayed N.M., Kim M.J., Fields K.M., Olvera R.L., Hariri A.R., Williamson D.E. Trajectories of alcohol initiation and use during adolescence: the role of stress and amygdala reactivity. *Journal of the American Academy of Child and Adolescent Psychiatry*. 2018; 57 (8): 550–560. DOI: 10.1016/j.jaac.2018.05.011.
26. Blum K., Gondre-Lewis M.C., Baron D., Thanos P.K., Braverman E.R., Neary J., Elman I., Badgaiyan R.D. Introducing precision addiction management of reward deficiency syndrome, the construct that underpins all addictive behaviors. *Frontiers in Psychiatry*. 2018; 9: 548. DOI: 10.3389/fpsyt.2018.00548.
27. Wiener C.D., Moreira F.P., Zago A., Souza L.M., Branco J.C., Oliveira J.F., Silva R.A.D., Portela L.V., Lara D.R., Jansen K., Oses J.P. Mood disorder, anxiety, and suicide risk among subjects with alcohol abuse and/or dependence: a population-based study. *Brazilian Journal of Psychiatry*. 2018; 40 (1): 1–5. DOI: 10.1590/1516-4446-2016-2170.
28. Gofman A.G., Oyfe I.A. Alcoholism and endogenous depression; ed. Smulevich A.B. In: Depression and comorbid disorders. Moscow, 1997: 124–136 (in Russ.).
29. Ponizovsky P.A., Gofman A.G. Depression in patients with alcohol dependence. *Korsakov Journal of Neurology and Psychiatry*. 2015; 115 (7): 146–150 (in Russ.).
30. Rozin A.I., Roshchina O.V., Peshkovskaya A.G., Belokrylov I.I. Comorbid combinations of alcohol dependence and depressive disorders. *Siberian Herald of Psychiatry and Addiction Psychiatry*. 2018; 4 (101): 40–45 (in Russ.). DOI: 10.26617/1810-3111-2018-4(101)-40-45.
31. Cooper S., Robison A.J., Mazei-Robison M.S. Reward circuitry in addiction. *Neurotherapeutics*. 2017; 14 (3): 687–697. DOI: 10.1007/s13311-017-0525-z.
32. Mendelevich V.D. Addiction syndrome: evidence-based therapy. *Narcology*. 2014; 8: 94–103 (in Russ.).

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Roshchina O.V., Rozin A.I. – carrying out of the research, statistical analysis and interpretation of data. Schastnyy E.D. – critical revision for important intellectual content. Bokhan N.A. – final approval of the manuscript for publication.

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The mRNA expression levels of calpains and their activity in malignant and dysplastic epithelium of the upper respiratory tract

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ABSTRACT

Background. The calpain proteolytic system plays an important role in the development of cancer. Detection of early cancer in the upper respiratory tract is often challenging, as symptoms are largely non-specific, and most cases are diagnosed at an advanced stage.

Aim. To identify candidate markers of transition from premalignant lesions to invasive carcinoma, we studied mRNA expression levels of CAPN1 and CAPN2 and the total activity of calpains in the tumor tissues of patients with head and neck squamous cell carcinoma (HNSCC) and in the epithelial dysplasia-affected tissues of patients with chronic diseases of the upper respiratory tract.

Materials and methods. The study included 32 patients with HNSCC (T1-3N0-1M0) and 12 patients with chronic diseases of the upper respiratory system associated with epithelial dysplasia. The expression levels of CAPN1 and CAPN2 were assessed using real-time polymerase chain reaction (PCR). The calpain activity was determined by hydrolysis of the fluorogenic Suc-LLVY-AMC oligopeptide.

Results. The mRNA expression levels of CAPN1 and CAPN2 were, respectively, 3 and 4 times higher in the tumor tissue of patients with HNSCC than in the tissue of patients with endothelial dysplasia in the upper respiratory tract. The level of calpain activity was 4.4 times higher in patients with HNSCC than in patients with epithelial dysplasia of different severity.

Conclusion. The elevated mRNA expression levels of CAPN1 and CAPN2 and their activity in the tumor tissues of patients with HNSCC compared to patients with chronic respiratory diseases associated with epithelial dysplasia are likely to characterize a high potential for transition from precancerous lesion to cancer. To clarify the role of calpains in the carcinogenesis of HNSCC, further studies of intact tissues using animal models are required.

Key words: head and neck squamous cell carcinoma, epithelial dysplasia, calpain activity, mRNA expression of CAPN1 and CAPN2.

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 Biomedical Ethics Committee at Cancer Research Institute, Tomsk NRMC.

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Экспрессия мРНК кальпаинов и их активность в злокачественном и диспластически измененном эпителии верхних дыхательных путей

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РЕЗЮМЕ

Актуальность. Кальпаиновая протеолитическая система играет важную роль в развитии злокачественных опухолей. В настоящее время диагностика рака верхних дыхательных путей на ранних стадиях проводится редко, заболевание часто характеризуется запущенностью опухолевого процесса.

С **целью** поиска кандидатных маркеров перехода предопухолевых заболеваний в злокачественные изучали уровни экспрессии мРНК *CAPN1*, *CAPN2* и общей активности кальпаинов в опухолевой ткани пациентов с плоскоклеточным раком головы и шеи (ПРГШ) и в диспластически измененном эпителии верхних дыхательных путей.

Материалы и методы. В исследование были включены 32 пациента с ПРГШ ($T_{1-3}N_{0-1}M_0$), группу больных с предопухолевой патологией составили 12 пациентов с хроническими заболеваниями верхних отделов дыхательной системы, ассоциированными с диспластическими изменениями эпителия различной степени. Уровень экспрессии мРНК *CAPN1* и *CAPN2* оценивался с помощью полимеразной цепной реакции в режиме реального времени. Активность кальпаинов определяли по гидролизу флуорогенного олигопептида Suc-LLVY-AMC.

Полученные **результаты** показали увеличение уровня экспрессии мРНК *CAPN1* и *CAPN2* (в 3 и 4 раза соответственно) в опухолевой ткани у пациентов с ПРГШ в сравнении с диспластически измененным эпителием верхних дыхательных путей. Также отмечен высокий уровень активности кальпаинов у больных ПРГШ, который в 4,4 раза превышал показатели, полученные для пациентов с диспластическими изменениями эпителия различной степени.

Заключение. Вероятно, увеличение уровня мРНК *CAPN1* и *CAPN2* и общей активности кальпаинов в опухолевых тканях пациентов с ПРГШ в сравнении с пациентами с хроническими заболеваниями, ассоциированными с диспластическими изменениями различной степени, может играть важную роль в процессе перехода предрака в рак. Для полного установления роли кальпаинов в канцерогенезе ПРГШ необходимо дальнейшее проведение подобных исследований в интактной ткани, что возможно только на экспериментальных моделях.

Ключевые слова: плоскоклеточный рак головы и шеи, дисплазия эпителия, активность кальпаинов, экспрессия мРНК *CAPN1* и *CAPN2.*

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

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INTRODUCTION

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common malignancy worldwide, with more than 500,000 new cases diagnosed each year [1, 2]. Most head and neck squamous cell carcinomas arise from the mucosal surfaces of the larynx and hypopharynx [3]. Despite the availability of visual and instrumental studies, diagnosis of the upper respiratory tract cancer at early stages remains challenging, as symptoms are largely non-specific, and most cases are diagnosed at an advanced stage [4]. The mechanism of epithelial dysplasia progression to squamous cell carcinoma is not well understood. This mechanism is thought to represent a stepwise process in which genetic damage is followed by morphological changes in squamous epithelium [5]. The presence of dysplasia in the mucous membrane of the larynx, laryngopharynx, and oropharynx indicates the increased risk of developing cancer [6].

Proteolytic systems that can regulate many molecular and cellular processes have a great influence on tumor transformation. The calpain system, which is involved in cancer development and progression, is an important system of specific intracellular proteolysis [7]. Calpains are cytoplasmic cysteine proteases exhibiting Ca^{2+}-dependent proteolytic activity. Proteolysis implemented by calpains is partial; it does not degrade protein but only changes its structure. Therefore, they are called "modulating proteases" [8].

In the calpain family, there are ubiquitously expressed isoforms, such as μ-calpain (calpain 1) and m-calpain (calpain 2), and tissue-specific isoforms, such as calpain 9, which is found in the digestive tract [9]. Although many of the functions of calpains and mechanisms controlling proteolytic activity remain to be analyzed, experimental studies demonstrated the apparent role of calpains in a number of important cellular processes, including proliferation, differentiation, DNA repair, and apoptosis [10, 11]. Moreover, calpains play an essential role in cancer progression [12–14].

Despite the active study of the calpain system, there are currently not enough data showing the changes in the expression level and activity of calpains in patients with HNSCC and dysplasia-associated chronic diseases. Therefore, the aim of the study was to assess mRNA expression levels of CAPN1 and CAPN2 and their activity in the tumor tissues of patients with HNSCC ($T_{1-3}N_{0-1}M_0$) and in the epithelial dysplasia-affected tissues of patients with chronic diseases of the upper respiratory tract.

MATERIALS AND METHODS

The study included 32 patients with HNSCC ($T_{1-3}N_{0-1}M_0$) and 12 patients with chronic respiratory disease associated with histologically verified epithelial dysplasia (DI-II), who were treated at the Department of Head and Neck Cancer of the Cancer Research Institute (TNRMC RAS, Tomsk, Russia). HNSCC was histologically verified in all patients, who had not previously received any special treatment. The average age of the patients was (56.3 ± 7.2) years.

The study was carried out in compliance with the principles of voluntariness and confidentiality in accordance with the "Fundamentals of the legislation of the Russian Federation on the protection of citizens' health" (Decree of the President of the Russian Federation No. 2288 of 24.12.1993). The permission of the Biomedical Ethics Committee of the Institute was obtained.

Biopsy samples of both cancerous and healthy tissues obtained during videolaryngoscopy served as a study material. The expression levels of calpains (CAPN1, CAPN2) were analyzed using the real-time PCR (RT-PCR) with the intercalating dye SYBR Green I (BioMaster HS-qPCR SYBR Blue (2×); Biolabmix, Novosibirsk). The total RNA pool was isolated from the tissue samples using the LIRA reagent (Biolabmix, Novosibirsk). The concentration and quality of the isolated RNA were evaluated using a NanoDrop 2000C spectrophotometer (ThermoScientific, USA). To obtain cDNA from mRNA, a reverse transcription reaction was performed using the OT M-MuLV-RH reaction mix (Biolabmix, Novosibirsk). Primers for RT-PCR were selected using specialized programs Vector NTI Advance 11.5 and the NCBI database (Table 1).

The expression levels of the target genes were calculated using the $2\Delta\Delta Ct$ equation [15] and expressed in arbitrary units. The housekeeping gene of the GAPDH enzyme was used as the reference gene, and the expression level of each target gene was normalized with respect to the expression of GAPDH.

Table 1

The sequence of the studied gene primers

Gene	Primers
CAPN1 NM_001198868.2	F 5′- AGAGCCTGGGTTACAAG -3′ R 5′- TGTCGTTGAGAGTGAGG -3′
CAPN2 NM_001146068.1	F 5′- ATGCTAGATTCGGACGGGAG-3′ R 5′- TGGAGTTGACAGGGCATCTT-3′
GAPDH NM_001256799.3	F 5′- GGAAGTCAGGTGGAGCGA-3′ R 5′-GCAACAATATCCACTTTACCAGA-3′

Note. NM – RNA sequence number in the National Center for Biotechnology Information (NCBI); F – forward primer; R – reverse primer.

The calpain activity was determined in clarified tissue homogenates by hydrolysis of the fluorogenic Suc-LLVY-AMC oligopeptide (Sigma, USA). The reaction mix containing 3mM Suc-LLVY-AMC and 5 µl supernatant was incubated at 25 °C for 30 min in the presence or absence of 10 mM $CaCl_2$ and 5 mM N-acetyl-Leu-Leu-norleucinal inhibitor (Sigma, USA). The resulting product was recorded with the Hitachi-850 fluorimeter (Japan) at an excitation wavelength of 380 nm and emission of 440 nm. The calpain activity was determined in samples with 10 mM $CaCl_2$ and with an inhibitor. The unit of activity was the amount of the enzyme at which 1 nmol of Suc-LLVY-AMC is hydrolyzed for 1 min. The specific activity was expressed in units of activity per 1 mg of protein. The protein content was determined by the Lowry method.

For statistical analysis, the Statistica 10.0 software package was used. The results shown in the table are presented as the median with the interquartile range *Me* (Q_1–Q_3). Using the Kruskal — Wallis test, statistically significant differences were found between the groups under investigation. For further pairwise comparison, the nonparametric Mann – Whitney test for multiple comparisons (with Bonferroni correction) was applied. The differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

A significant difference in the mRNA expression levels of CAPN1 and CAPN2 between patients with epithelial dysplasia (DI-II) and patients with HNSCC ($T_{1-3}N_{0-1}M_0$) was found (Table 2). CAPN1 and CAPN2 expression levels were, respectively, 3 and 4 times higher in the tumor tissue of patients with HNSCC than in the dysplastic epithelium of the upper respiratory tract. In the tumor tissues of patients with stages $T_2N_{0-1}M_0$ and $T_3N_{0-1}M_0$, the CAPN1 expression level was, respectively, 2 and 4 times higher than that observed in patients with epithelial dysplasia (DI-II). The CAPN2 expression level was 4 times higher in patients with stages $T_1N_{0-1}M_0$ and $T_3N_{0-1}M_0$ than in patients with epithelial dysplasia of varying degree. The highest expression levels for both CAPN1 and CAPN2 were observed in patients with stage $T_3N_{0-1}M_0$. The CAPN1 level increased along with the tumor size. It should be noted that in the malignant epithelium of the upper respiratory tract, the expression level of CAPN2 was higher than that of CAPN1.

The findings of our study are consistent with other studies that indicate that the components of the calpain system are involved in the pathogenesis of head and neck tumors [10, 16, 17]. Calpains are implicated in processes crucial for cancer development, such as impaired intercellular adhesion, actin cytoskeletal rearrangement, morphological transformation, and cell migration, since calpains degrade proteins involved in these processes [8, 10].

Table 2

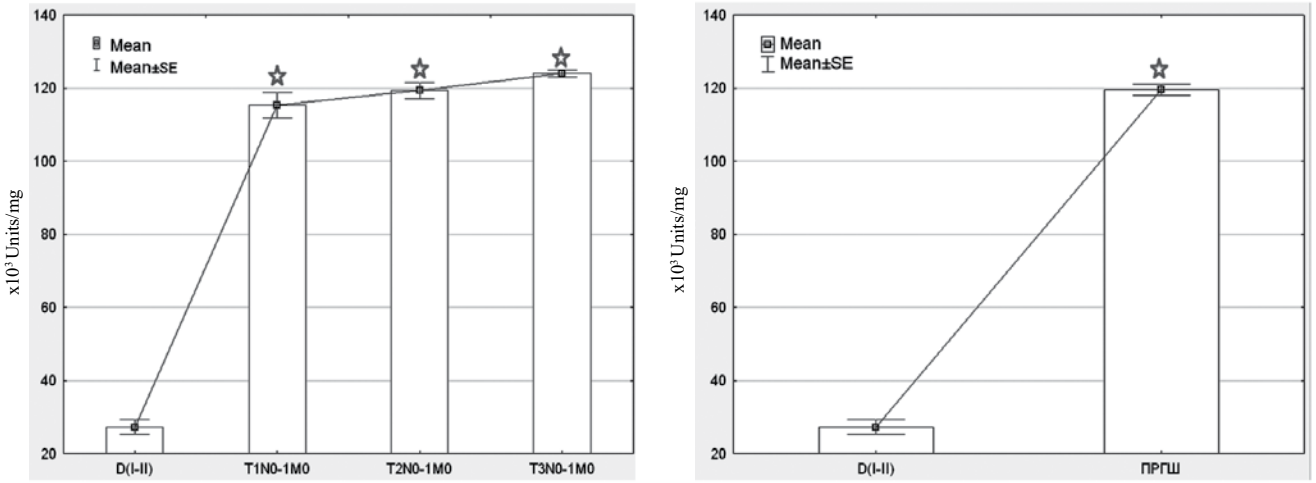
mRNA expression levels of *CAPN1* and *CAPN2* in the tissues of malignant and dysplastic epithelium of the upper respiratory tract

Group	Expression level, conventional units	
	CAPN1	CAPN2
Epithelial dysplasia (DI-II), $n = 12$	0.5 (0.08–0.86)	0.5 (0.06–0.95)
HNSCC ($T_{1-3}N_{0-1}M_0$), $n = 32$	1.58 (0.25–10.98) $p = 0.021$	1.94 (0.25–4.12) $p = 0.045$
$T_1N_{0-1}M_0$, $n = 10$	0.59 (0.25–5.0)	2.09 (0.51–5.68) $p = 0.043$
$T_2N_{0-1}M_0$, $n = 11$	1.0 (0.25–11.88) $p = 0.041$	0.52 (0.19–2.27)
$T_3N_{0-1}M_0$, $n = 11$	1.92 (0.53–10.98) $p = 0.021$	2.14 *(0.28–5.13)* $p = 0.046$

Note. Significance level of differences in the parameters compared to the group "Epithelial dysplasia (DI-II)" – *p*.

Analysis of the total activity of calpains in the biopsy samples showed a significant difference between HNSCC and dysplasia (DI-II) groups (Figure). Diagram A showed changes in the activity of calpains depending on the tumor spread in comparison

with dysplastic changes in the epithelium of the upper respiratory tract. Patients with stage $T_3N_{0-1}M_0$ had the highest rate of calpain activity. Additionally, this diagram showed a tendency towards a rise in the activity of calpains along with the increasing size of the tumor. Diagram B demonstrated that the total activity of calpains in patients with HNSCC was 4.4 times higher (125.7×10^3 units / mg of protein) than that observed in patients with dysplastic epithelial changes.



a *b*

Figure. Calpain activity in the biopsy samples of patients with epithelial dysplasia of the upper respiratory tract (DI-II) and HNSCC (T1-3N0-1M0): significance of differences compared to the group "Epithelial dysplasia (DI-II)", $p < 0.05$

Our data were consistent with the recent studies conducted by V.D.Koval et al., who revealed that the activity of calpains was nearly 12 times higher in patients with endometrial hyperplasia than in patients with endometrial cancer [18]. Compared to the healthy tissue, the increased activity of the calpain system components was reported in many cancers, such as meningioma, renal cell carcinoma, colorectal adenocarcinoma, endometrial cancer, gastric cancer, and breast cancer [10, 12, 13, 19]. Involvement of calpains in development of malignant tumors is determined by their essential role in many physiological cellular processes. Calpains potentially recognize more than 200 substrates, as confirmed by *in vitro* studies [11].

Among the proteins identified as calpain substrates, there are transcription factors, transmembrane receptors, signaling pathway components, and cytoskeletal proteins. The calpain proteases and proteasomes function in a coordinated manner. In this case a complex develops in the malignant tissue which is called the cancer degradome and represented by enzymes of various types of catalysis [7]. The components of the cancer degradome provide effective proteolysis during tumor progression, including invasion and metastasis.

CONCLUSION

Our results show significant differences in the expression levels and activity of the calpain system components between malignant and dysplastic epithelium of the upper respiratory tract. The elevated mRNA expression levels of CAPN1 and CAPN2 and overall calpain activity in the tumor tissues of HNSCC patients (compared to patients with chronic respiratory diseases associated with epithelial dysplasia) are likely to characterize a high potential for transition from a precancerous lesion to cancer. However, to fully establish the role of calpains in HNSCC carcinogenesis, it is necessary to conduct similar studies in the intact tissues using animal models. The data obtained indicate that the calpain system is directly involved in the development of head and neck cancer. Further development of criteria for potentially premalignant respiratory lesions posing a high risk of

malignant transformation is promising for successful prevention of HNSCC.

REFERENCES

1. Leemans C.R., Braakhuis B.J.M., Brakenhoff R.H. The molecular biology of head and neck cancer. *Nature Reviews Cancer.* 2011; 11 (1): 9–22. DOI: 10.1038/nrc2982.
2. Alsahafi E., Begg K., Amelio I., Raulf N., Lucarelli P., Sauter T., Tavassoli M. Clinical update on head and neck cancer: molecular biology and ongoing challenges. *Cell Death Dis.* 2019; 10 (8): 540. DOI: 10.1038/s41419-019-1769-9.
3. Kaprin A.D., Starinskiy V.V., Petrova G.V. Malignant neoplasms in Russia in 2018 (morbidity and mortality). Moscow: P. Hertsen Moscow Research Oncology Institute – branch of FSBI "NMRRC" of the Ministry of Health of the Russian Federation; 2019: 250 (in Russ.).
4. Frolova I.G., Choinzonov E.L., Goldberg V.E., Chizhevskaya S.Yu., Chernov V.I., Goldberg A.V., Belevich Yu.V. Imaging techniques for the detection of lymph node metastasis in patients with laryngeal and hypopharyngeal cancer. *Siberian Journal of Oncology.* 2018; 17 (3):101–108 (in Russ.). DOI: 10.21294/1814-4861-2018-17-3-101-108.
5. Trivedi S., Rosen C.A., Ferris R.L. Current understanding of the tumor microenvironment of laryngeal dysplasia and progression to invasive cancer. *Curr. Opin. Otolaryngol. Head Neck Surg.* 2016; 24 (2): 121–127. DOI: 10.1097/MOO.0000000000000245.
6. Cheremisina O.V., Choinzonov E.L., Pankova O.V., Menshikov K.Yu. Chronic hypeplastic laryngitis as a criterion for defining groups at high risk of laryngeal cancer. *Russian Otorhinolaryngology.* 2013; 63 (2): 84–89 (in Russ.).
7. Kakurina G.V., Kondakova I.V., Choinzonov E.L. Degradome components in progression of squamous cell carcinoma of the head and neck. *Annals of the Russian Academy of Medical Sciences.* 2015; 70 (6):684–693 (in Russ.). DOI: 10.15690/vramn563.
8. Sorimachi H., Hata S., Ono Y. Calpain chronicle – an enzyme family under multidisciplinary characterization. *Proc. Jpn. Acad. Ser. B Phys. Biol. Sci.* 2011; 87 (6): 287– 327. DOI: 10.2183/pjab.87.287.
9. Macqueen D.J., Wilcox A.H. Characterization of the definitive classical calpain family of vertebrates using phylogenetic, evolutionary and expression analyses. *Open Biol.* 2014; 4 (4): 130219. DOI: 10.1098/rsob.130219.
10. Moretti D., Del Bello B., Allavena G., Maellaro E. Calpains and cancer: Friends or enemies? *Archives of Biochemistry and Biophysics.* 2014; 564: 26–36. DOI: 10.1016/j.abb.2014.09.018.
11. Suzuki K., Hata S., Kawabata Y., Sorimachi H. Structure, Activation, and Biology of Calpain. *Diabetes.* 2004; 53 (suppl 1): S12-S18. DOI: 10.2337/diabetes.53.2007.S12.
12. Ivanova E.V., Kondakova I.V., Spirina L.V., Afanas'ev S.G., Avgustinovich A.V., Cheremisina O.V. Chymotrypsin-Like Activity of Proteasomes and Total Calpain Activity in Gastric and Colorectal Cancer. *Bull. Exp. Biol. Med.* 2014; 157 (6): 781–784. DOI: 10.1007/s10517-014-2666-y.
13. Shashova E.E., Kolegova E.S., Zav'yalov A.A., Slonimskaya E.M., Kondakova I.V. Changes in the Activity of Proteasomes and Calpains in Metastases of Human Lung Cancer and Breast Cancer. *Bull. Exp. Biol. Med.* 2017; 163 (4): 486–489. DOI: 10.1007/s10517-017-3834-7.
14. Zhang S., Deen S., Storr S.J., Chondrou P.S., Nicholls H., Yao A., Rungsakaolert P., Martin S.G. Calpain system protein expression and activity in ovarian cancer. *J. Cancer Res. Clin. Oncol.* 2019; 145 (2): 345–361. DOI: 10.1007/s00432-018-2794-2.
15. Livak K.J., Schmittgen T.D. Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the $2^{-\Delta\Delta CT}$ Method. *Methods.* 2001; 25 (4): 402–408. DOI: 10.1006/meth.2001.1262.
16. Ma D., Fang J., Liu Y., Song J-J., Wang Y-Q., Xia J., Cheng B., Wang Z. High level of calpain1 promotes cancer cell invasion and migration in oral squamous cell carcinoma. *Oncology Letters.* 2017; 13 (6): 4017–4026. DOI: 10.3892/ol.2017.5970.
17. Sletov A.A., Mozheiko R.A. Features of differential diagnostics of cancer of the mucous membrane of the oral cavity with the use of specific markers of tumor progression. *Science Almanac.* 2017; 28 (2–3):389–397 (in Russ.). DOI: 10.17117/na.2017.02.03.389.
18. Koval' V.D., Spirina L.V., Kondakova I.V., Kolomiets L.A., Shpileva O.V. Proteasome and calpain activities in endometrial hyperplasia and endometrial cancer. *Molecular Medicine.* 2012; (4):45–48 (in Russ.).
19. Shashova E.E., Doroshenko A.V., Bondar L.N., Slonimskaya E.M., Kondakova I.V. Proteasomal and calpain proteolysis systems in different molecular subtypes of breast cancer. *Siberian Journal of Oncology.* 2017; 16 (3): 33–39 (in Russ.). DOI: 10.21294/1814-4861-2017-16-3-33-39.

Authors contribution

Sidenko E.A. – carrying out of laboratory research, determination of the calpain gene expression, drafting of the article. G.V. Kakurina – determination of the calpain gene expression, analysis and interpretation of data. Cheremisina O.V. – collection of biopsy material from patients with laryngeal and laryngopharyngeal cancer and precancerous diseases of the upper respiratory tract. Spirina L.V. – carrying out of laboratory research, determination of the calpain activity. Shashova E.E. – statistical processing of data. Korshunov D.A. – preparation

of the illustrative material, drafting of the article. Kondakova I.V. – conception and design, substantiation of the manuscript, critical revision for important intellectual content, final approval of the manuscript for publication.

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Nonclinical study of the new immunotropic drug effectiveness in salmonella infection treatment

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ABSTRACT

The aim of the study was to evaluate the immunoregulatory activity of the experimental drug based on ultra-high dilutions of antibodies to MHC I and MHC II molecules against *Salmonella enteritidis rif92*.

Materials and methods. The drug tested: a sample of ultra-high water-alcohol dilutions of antibodies to MHC I and MHC II molecules applied to lactose powder (the theoretical level of the initial antibody concentration reduction is at least 10^{24} times). A model of non-lethal salmonella infection in chickens was induced by administering a virulent strain of *Salmonella enteritidis rif92* with a concentration of 2.5×10^9 CFU / g in the volume of 0.5 ml / bird. The following groups were formed (n = 15 in each group): 1 – drug; 2 – drug + antibiotic at the median effective dose (ED 50); 3 – placebo; 4 – placebo + antibiotic at ED50; 5 – intact control. The duration of the experiment was 12 days. The studied parameters included the survival rate during the observation period; daily body weight; feed consumption for the entire period; pathogen concentration in the litter on day 3, 6, and 9; the presence and concentration of the pathogen in the liver and cecum on day 12; and the index of antimicrobial activity on day 12.

Results. In the groups receiving the experimental drug, the infectious process proceeded in a milder form and the bacterial load in chickens was lower. The bacterial count in the litter was reduced by two orders compared to the respective control when the drug was added both alone and in combination with the antibiotic. A protective effect of the experimental drug on the liver of the infected chickens was detected.

Conclusion. A pronounced immunoregulatory activity of the studied drug against *Salmonella enteritidis rif92* in chickens was demonstrated for the first time. The results obtained allow to consider the drug as a promising agent for the treatment of salmonella infection.

Key words: salmonellosis, ultra-high dilutions of antibodies, MHC class I and II molecules, antibiotics, chickens.

Conflict of interests. N.V. Petrova, E.A. Karelina, K.K. Ganina, and S.A. Tarasov are employees of MATERIA MEDICA HOLDING LLC. O.I. Epstein is the founder and the President of MATERIA MEDICA HOLDING LLC. The decision to publish the results of the study belongs to MATERIA MEDICA HOLDING LLC. Patent applications for the substances and the drug have been submitted by MATERIA MEDICA HOLDING LLC and O.I. Epstein.

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Доклиническое изучение эффективности нового иммунотропного препарата при лечении сальмонеллезной инфекции

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РЕЗЮМЕ

Цель исследования – оценить иммунорегуляторную активность экспериментального препарата на основе сверхвысоких разведений антител к молекулам МНС I и II в отношении *Salmonella enteritidis rif92*.

Материалы и методы. Изучаемый препарат: образец сверхвысоких водно-спиртовых разведений антител к молекулам МНС I и II, нанесенных на порошок лактозы (теоретический уровень снижения концентрации исходных антител как минимум в 10^{24} раз). Модель – нелетальная сальмонеллезная инфекция у цыплят. Заражение проводили вирулентным штаммом *S. enteritidis rif92* концентрацией 2,5 × 10^9 КОЕ/г в объеме 0,5 мл/голову. Группы (n = 15 в каждой): 1 – препарат; 2 − препарат + антибиотик в дозировке 50%-й эффективной дозы (ЭД50); 3 − плацебо; 4 − плацебо + антибиотик в дозировке ЭД50; 5 − интактный контроль. Продолжительность эксперимента 12 сут. Изучаемые показатели: выживаемость в течение периода наблюдения, масса тела ежедневно, затраты корма за весь период, концентрация возбудителя в помете на 3, 6, 9-е сут, наличие и концентрация возбудителя в печени и слепых отростках тонкого кишечника, а также индекс антимикробной активности на 12-е сут.

Результаты. В группах с введением экспериментального препарата инфекционный процесс проходил в более легкой форме, бактериальная нагрузка у цыплят была ниже. Обсемененность помета снижалась на два порядка по сравнению с соответствующим контролем при добавлении препарата как в виде монотерапии, так и в сочетании с антибиотиком. Выявлено протективное действие препарата на печень зараженных цыплят. Заключение**.** Впервые продемонстрирована выраженная иммунорегуляторная активность изучаемого препарата в отношении *Salmonella enteritidis rif92* у цыплят. Полученные результаты позволяют рассматривать данный препарат в качестве перспективного агента для терапии сальмонеллезной инфекции.

Ключевые слова: сальмонеллез, сверхвысокие разведения антител, молекулы МНС I и II классов, антибиотики, цыплята.

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INTRODUCTION

Salmonella infection tends to result from consumption of low-quality food, primarily eggs and poultry meat [1, 2–5]. The infectious process usually proceeds without complications, but a severe form can be observed in patients with impaired immune status, as well as in children and elderly people [1, 6–7]. If the disease has a mild or moderate course, antimicrobial therapy is not recommended in healthy people [8], since most antimicrobial drugs are active against salmonella only during the incubation period and at the beginning of the disease [9]. In addition, excessive use of antimicrobials contributes to the development of pathogen resistance to drugs through multiple molecular and genetic mechanisms [1, 8, 10–12]. Thus, the use of drugs that affect the targets expressed by immune cells can be considered as a relevant and promising direction in treatment of salmonellosis [13–17].

We conducted an *in vivo* study using a model of non-lethal salmonella infection in chickens, which was aimed at assessing the activity of a new drug based on ultra-high dilutions of antibodies to MHC class I and II molecules. The drug was developed by MATERIA MEDICA HOLDING LLC and has a modulatory effect aimed at the targets of immune cells.

MATERIALS AND METHODS

The experimental drug was a lactose powder saturated with ultra-high dilutions of antibodies to MHC class I and II molecules and obtained using the following technology: affinity-purified rabbit polyclonal antibodies to MHC I and MHC II were used as the initial substance and subsequently utilized for the preparation of ultra-high dilutions. To obtain a 100-fold dilution, the substances were diluted in an aqueous-alcohol solution in the ratio of 1 : 100 with vigorous stirring. The final dilutions of antibodies to MHC I and MHC II contained a mixture of the 12^{th}, 30^{th} and 50^{th} centesimal dilutions.

Thus, if the identified special physical and chemical features typical of highly diluted substances [18–20] are not taken into account, the theoretical level of reduction in the concentration of the initial antibodies can be 10^{24} times. Lactose monohydrate was saturated with the resulting dilutions using the fluidized bed unit. Lactose powder with an aqueous alcohol solution applied on it, which was obtained with a similar technology of ultra-high dilutions in purified water, was used as a placebo. The samples of the drug and placebo were supplied and tested blinded. Unblinding was carried out after the end of the experiment and statistical analysis of the data obtained.

For the study, 2.5% aqueous solutions of the drug and placebo were prepared and administered orally to chickens once a day at a dose of 0.2 ml per bird. Ciprofloxacin hydrochloride was used as an antibacterial drug. In preliminary studies, its efficacy against the pathogen was confirmed and a median effective dose (ED50) was calculated. Inoculation was carried out using a virulent *Salmonella enteritidis rif92* strain obtained from the State Collection of Pathogenic Microorganisms and Cell Cultures (SCPM-Obolensk) with a non-lethal concentration of 2.5×10^9 CFU / g in the volume of 0.5 ml / bird.

Five groups of 15-day-old cross Cobb broiler chickens obtained from Novo-Petrovskaya Poultry Farm LLC were used in the study. For *in vivo* models of salmonellosis, rodents are usually proposed [21], but the used strain of *Salmonella enteritidis* is associated with poultry and poultry products, which are a source of human infection [4], and it is one of the main pathogens of food toxicoinfection in humans [22]. Infections in mice develop in the absence of pronounced symptoms of diarrhea, so the rodent model may not be sufficiently informative [23].

We employed methods that allowed to assess the bacterial load of internal organs in order to quantify virulence: the gastrointestinal tract of chickens is an optimal system for studying intestinal zooanthroponotic infections [24]. Taking into account the intended treatment, the following groups of chickens were formed in the experiment: 1 – drug; 2 – drug + antibiotic; 3 – placebo; 4 – placebo + antibiotic; 5 – intact control. All groups were under the same housing conditions. The duration of the study was 12 days (from day 1 to day 12 of the chickens' life).

The chickens were quarantined for the first two days. On day 3, they were randomized into groups and infected with *Salmonella enteritidis rif92* (except for the intact group). On days 4–9 of life, the studied drug or placebo was administered to chickens of groups 1–4. In addition, on days 5–9 of life, the chickens of the 2^{nd} and 4^{th} groups received ciprofloxacin hydrochloride orally at ED50 (0.5 mg / kg body weight) in the volume of 0.2 ml. The experimental drug or placebo, respectively, and the antibiotic were administered at one-hour intervals. The following parameters were assessed: the survival rate during the experiment; daily body weight; feed consumption per 1 kg of weight gain over the entire observation peri-

od; the concentration of *Salmonella enteritidis rif92* in the litter on days 3, 6, 9 of the experiment; the presence and concentration of the pathogen in the liver and cecum on day 12, and the index of antimicrobial activity (IAA).

Colonization of the intestine by *Salmonella enteritidis rif92* was monitored by bacteriological analysis of the feces of the infected chickens. On days 3, 6, and 9, the pool of feces of the entire group was studied; on day 12, it was studied individually after euthanasia. The persistence level of *Salmonella enteritidis rif92* was estimated according to the number of bacteria in one gram of the litter. The presence and concentration of *Salmonella enteritidis rif92* in the intestine were determined according to ISO 6887-1983 *General Guidance for the Preparation of Dilutions for Microbiological Examination*.

The colonies grown on the nutrient media were counted and identified using salmonella diagnostic sera during mass spectrometry analysis.

IAA was calculated as a ratio of the microbial cells contained in the organ homogenate in the control group to those in the experimental group at the end of the observation period [25].

Statistical analysis was performed using the Microsoft R Open 3.4.4 platform. Based on the primary survival rate data, a statistical model was developed for comparing the groups using the log-rank test. The Holm – Bonferroni method was used for multiplicity adjustment. According to the body mass index, the arithmetic mean and the standard error of the mean were calculated for each group. The groups were compared using two-factor linear models and post-hoc Tukey's honestly significant difference (HSD) test. To analyze the concentration of the pathogen in the liver and intestinal contents of the infected chickens, the arithmetic mean and standard error of the mean were calculated. The groups were compared using the Kruskal – Wallis test and the Wilcoxon signed-rank test. The differences between the groups were considered statistically significant at $p < 0.05$.

RESULTS

The survival rate of chickens in all the groups was 100%. The average values of the chickens' body weight from day 1 to day 9 of the study were comparable in all the groups. Starting from day 10, the body weight in the drug group was higher than in the other groups. At the same time, statistically significant differences were observed: on day 10, the drug group as opposed to the placebo group (260.0 ± 72 g vs. 228.8 ± 8.7 g); on day 11 – as opposed to the intact group (297.0 ± 8.4 g vs. 263.0 ± 15.8 g); on day 12 – as opposed to the placebo group (325.8 ± 9.6 g vs. 291.9 ± 10.2 g) and the intact control (325.8 ± 9.6 g vs. 287.1 ± 16.2 g). The remaining groups were comparable in terms of body weight.

The best values for feed consumption per 1 kg of body weight gain were obtained in the drug group (1.28 kg), in the drug + antibiotic group (1.29 kg), and in the placebo + antibiotic group (1.27 kg), while this value was 1.39 kg in the placebo group, and 1.50 kg in the intact control group.

The dynamics of *Salmonella enteritidis rif 9* concentration in chickens' litter is given in Table 1.

Table 1

***Salmonella enteritidis* concentration per gram of fecal pool post infection (CFU / g)**

Groups	Day 3	Day 6	Day 9
Drug, $n = 15$	5×10^6	2×10^2	74
Drug + antibiotic, $n = 15$	5×10^6	1×10^2	32
Placebo, $n = 15$	7×10^6	6×10^4	4×10^2
Placebo + antibiotic, $n = 15$	5×10^6	5×10^4	39
Intact control, $n = 15$	-	-	-

On day 6 post infection, in the groups where the experimental drug was administered both alone and in combination with the antibiotic, the salmonella concentration was reduced by 4 orders of magnitude, while in the placebo + antibiotic group – only by 2 orders of magnitude. On day 9, the presence of *Salmonella enteritidis* in the chickens' litter remained only in the placebo group. In the other groups, the concentration of the pathogen was represented by single colonies.

The data on the number of infected chickens, the bacterial count of *Salmonella enteritidis* in the liver, and the presence of the pathogen in the intestinal contents for all the groups at the end of the experiment (on day 12 post infection) are given in Table 2.

On day 12 post infection, the pathogen in the intestine was observed in almost half of the chickens in the placebo group. The addition of the antibiotic reduced the percentage of infected chickens, but the best results were obtained in the drug and drug + antibiotic groups, and in the latter, the concentration of the pathogen was minimal. As for the pathogen detected in the liver, low percentage of invasion was observed in all the groups, but the best results were obtained in the drug + antibiotic group.

Table 2

The presence of *Salmonella enteritidis* in the infected chickens after euthanasia and the index of antimicrobial activity

Groups	Presence of *S. enteritidis* in the liver		Index of antimicrobial activity	Presence of *S. enteritidis* in the intestinal contents		Index of antimicrobial activity
	n (%)	Concentration, CFU/g ($M \pm SE$)		*n* (%)	Concentration, CFU/g ($M \pm SE$)	
Drug	2 (13.3)	18.5 ± 14.5	10.6	2 (13.3)	$(5.5 \pm 2.5) \times 10^3$	0.4
Drug + antibiotic	1 (6.7)	56.0 ± 0	3.6	4 (26.7)	$(5.0 \pm 1.2) \times 10^2$	4.6
Placebo	3 (20.0)	195.7 ± 52.3	-	7 (46.7)	$(2.3 \pm 6.3) \times 10^2$	-
Placebo + antibiotic	1 (6.7)	200.0 ± 0	0.9	5 (33.3)	$(3.8 \pm 1.7) \times 10^3$	0.6
Intact control	–	–	–	–	–	–

The antimicrobial activity of the antibiotic administered at ED50 was low (the placebo + antibiotic group). However, the addition of the drug increased IAA by 4.0 and 7.7 times in the liver and intestine, respectively. We also observed high IAA of the experimental drug during liver examination, which indicates its protective effect in case of salmonella invasion.

DISCUSSION

The targets of the experimental drug examined in this study are MHC class I and class II molecules. Based on the previously shown properties of this drug class [26, 27], the experimental drug obviously influences its targets by activating the processing and presentation of the antigen and forming an adequate immune response during the infectious process. MHC class I molecules present peptide antigenic determinants to naive CD8+ killer T cells, while MHC class II molecules – to naive CD4+ T helper cells and regulatory T cells [28]. The MHC system molecules are now considered as some of the most promising markers of specific adaptive immune responses, including antigen processing and presentation. The mechanisms of their functioning are investigated in the studies on infectious diseases [29–32].

In this study, experimental infection of chickens with a reduced dose of *Salmonella enteritidis rif92* resulted in the development of a non-lethal infection, which is manifested by prolonged (in some chickens, until the last day of the study) presence of the pathogen in the gastrointestinal tract. The difference between the groups in terms of body weight is worth noting as an indicator of the overall condition. In the chickens treated with the experimental drug, it was significantly higher than in the placebo and intact control groups as well as in the antibiotic groups. This indirectly confirms the development of milder infectious process in the group that received the experimental drug.

The best results in terms of feed consumption per 1 kg of weight gain were observed in the groups where chickens were treated with the experimental drug or the antibiotic, as well as a combination of them. The pathogenesis of this model is associated with a violation of the morphological and functional characteristics of the gastrointestinal tract, so a decrease in feed consumption indicates a higher ability of the intestine to digest and absorb nutrients and, consequently, a lower negative impact of infection. The effectiveness of treating chickens with the experimental drug in terms of nutrient absorption was comparable to that of antibiotic therapy or a combination of them.

The therapy with the experimental drug reduced the time of chickens' recovery. Thus, day 6 post infection was characterized by a biologically significant decrease in the concentration of the pathogen in the litter by two orders of magnitude in the groups receiving the experimental drug both alone and in combination with a reduced dose of the antibiotic compared to the groups receiving placebo.

Adding the experimental drug to the antibiotic increased the level of antimicrobial activity of the latter by 4 and 7.7 times in the liver and intestine, respectively. At the same time, the intrinsic antimicrobial activity of the experimental drug in the liver was high. The decrease in salmonella invasion in the liver may be associated with the protective effect of the drug on the walls of the gastrointestinal tract, which prevented the penetration of the pathogen into the bloodstream and internal organs. The results of studies by other authors [13–15, 33] also confirm the assumption about immune mechanisms in the defense against salmonella.

CONCLUSION

Pronounced immunoregulatory activity of the studied drug against *Salmonella enteritidis rif92* was shown for the first time. The results obtained allow to

consider this drug as a promising agent for treatment of salmonellosis both alone and in combination with an antibiotic.

REFERENCES

1. URL: https://www.who.int/ru/news-room/fact-sheets/detail/salmonella-(non-typhoidal) (access date: 29.01.2020).
2. Pimenov N.V., Laishevtcev A.I., Pimenova V.V. the role of farming poultry's salmonella pathogens in infection and pathology of human disease. *Russian Journal of Agricultural and Socio-Economic Sciences*. 2017; 2 (62): 282–289 (in Russ.). DOI: 10.18551/rjoas.2017-02.33.
3. Fisinin V.I. Providing biocontainment in poultry production. *Ptitceprom. Special Issue*. 2017; S1: 58–60 (in Russ.).
4. Antunes P., Mourão J., Campos J., Peixe L. Salmonellosis: the role of poultry meat. *Clinical Microbiology and Infection.* 2016; 22 (2): 110–121. DOI:10.1016/j.cmi.2015.12.004.
5. EFSA and ECDC (European Food Safety Authority and European Centre for Disease Prevention and Control), 2018. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2017. *EFSA Journal*. 2018; 16 (12): 262. DOI: 10.2903/j.efsa.2018.5500.
6. Prokkoeva J.A. Features of bacteriological studies on the background of the probiotic biocomplex usage. *BIO*. 2018; 11: 9–11(in Russ.).
7. Chen Y., Glass K., Liu B., Hope K., Kirk M. Salmonella Infection in Middle-Aged and Older Adults: Incidence and Risk Factors from the 45 and Up Study. *Foodborne Pathogens and Disease.* 2016; 13 (12): 689–694. DOI: 10.1089/fpd.2016.2170.
8. Acheson D., Hohmann E.L. Nontyphoidal Salmonellosis. *Clinical Infectious Diseases.* 2001; 32 (2): 263–269. DOI: 10.1086/318457.
9. Polomoshnov N.A., Malysheva L.A. Effectiveness of probiotics for the prevention of salmonellosis. *Veterinarnaya patologiya*. 2012; 1 (39): 52–56 (in Russ.).
10. Kaftyreva L.A., Egorova S.A., Makarova M.A., Zabrovskaya A.V., Matveeva Z.N., Suzhaeva L.V., Voitenkova E.V. diversity of antimicrobial resistance mechanisms in salmonella.. *Russian Journal of Infection and Immunity*. 2011; 1 (4): 303–310 (in Russ.). DOI: 10.15789/2220-7619-2011-4-303-310.
11. Liljebjelke K.A., Hofacre C.L., White D.G., Ayers S., Lee M.D., Maurer J.J. Diversity of antimicrobial resistance phenotypes in salmonella isolated from commercial poultry farms. *Frontiers in Veterinary Science.* 2017; 4: 96. DOI: 10.3389/fvets.2017.00096.
12. Wei Z., Xu X., Yan M., Chang H., Li Y., Kan B., Zeng M. *Salmonella typhimurium* and *Salmonella enteritidis* infections in sporadic diarrhea in children: source tracing and resistance to third-generation cephalosporins and ciprofloxacin. *Foodborne Pathogens and Disease*. 2019; 16 (4): 244–255. DOI: 10.1089/fpd.2018.2557.
13. McSorley S.J., Ehst B.D., Yu Y., Gewirtz A.T. Bacterial flagellin is an effective adjuvant for CD4+ T Cells *in vivo. The Journal of Immunology.* 2002; 169 (7): 3914–3919. DOI: 10.4049/jimmunol.169.7.3914.
14. Pham O.H., McSorley, S.J. Protective host immune responses to Salmonella infection. *Future Microbiology.* 2015; 10 (1): 101–110. DOI: 10.2217/fmb.14.98.
15. Salazar-Gonzalez R.-M., Srinivasan A., Griffin A., Muralimohan G., Ertel, J.M., Ravindran R., Vella A.T., McSorley, S.J. Salmonella flagellin induces bystander activation of splenic dendritic cells and hinders bacterial replication *in vivo. The Journal of Immunology.* 2007; 179 (9): 6169–6175. DOI: 10.4049/jimmunol.179.9.6169.
16. Novokshonov A.A., Sokolova N.V., Galeeva E.V., Kurbanova G.M., Portnyh O.Yu., Uchaikin V.F. Immunotherapy in acute intestinal infections in children. The experience of new "Gepon" immunomodulatory drug usage. *Children infections*. 2003; 1: 32–36 (in Russ.).
17. Pavelkina V.F., Erovichenkov A.A., Pak S.G. The development of pathogenic therapy in the diseases of bacterial etiology. *Journal Infectology*. 2012; 4 (3): 67–75 (in Russ.).
18. Bunkin N.F., Shkirin A.V., Penkov N.V., Chirikov S.N., Ignatiev P.S., Kozlov V.A. The physical nature of mesoscopic inhomogeneities in highly diluted aqueous suspensions of protein particles. *Physics of Wave Phenomena*. 2019; 27 (2): 102–112. DOI: 10.3103/S1541308X19020043.
19. Chikramane P.S., Kalita D., Suresh A.K., Kane S.G., Bellare J.R. Why extreme dilutions reach non-zero asymptotes: a nanoparticulate hypothesis based on froth flotation. *Langmuir*. 2012; 28 (45): 15864-15875. DOI: 10.1021/la303477s.
20. Ryzhkina I.S., Murtazina L.I., Kiseleva U.V., Konovalov A.I. Self-organization and physical and chemical properties of water-antibody dilutions to interferon gamma in ultra high dilution. *Doklady akademii nauk.* 2015; 462 (2): 185–189 (in Russ.). DOI: 10.7868/S0869565215140170.
21. Higginson E.E., Simon R., Tennant S.M. Animal models for salmonellosis: applications in vaccine research. *Clinical and Vaccine Immunology*. 2016; 23 (9): 746–756. DOI: 10.1128/cvi.00258-16.
22. Guard-Petter J. The chicken, the egg and Salmonella enteritidis. *Environmental Microbiology*. 2001; 3 (7): 421–430. DOI: 10.1046/j.1462-2920.2001.00213.x.
23. Wallis T.S., Galyov E.E. Molecular basis of Salmonella-induced enteritis. *Molecular Microbiology.* 2000; 36 (5): 997–1005. DOI: 10.1046/j.1365-2958.2000.01892.x.
24. Card R.M., Cawthraw S.A., Nunez-Garcia J., Ellis, R. J., Kay, G., Pallen, M. J., Woodward M.J., Anjum, M. F. An *in vitro* chicken gut model demonstrates transfer of a multidrug resistance plasmid from salmonella to commensal *Escherichia coli. mBio*. 2017: 8 (4): e00777–007817. DOI: 10.1128/mbio.00777-17.
25. Okhapkina V.Yu., Pyatkova N.V., Fedotov A.K. Method of Accelerated Evaluation of Antimicrobial Preparation Efficacy against Experimental. *Problems of Particularly Dangerous Infections*. 2016; 2: 79–82 (in Russ.). DOI: 10.21055/0370-1069-2016-2-79-82.
26. Zilov V.G., Sudakov K.V., Epstein O.I. Elements of informational biology and medicine. M.: MGUL, 2001: 248 (in Russ.).
27. Epstein O.I. Release-activity fenomenon and the hypothesis of special homeostasis. *Uspehi fiziologicheskih nauk.* 2013; 44 (3): 54–76 (in Russ.).
28. Garib F.Yu., Rizopulu A.P. Interactions of pathogenic bacteria with innate immune reactions of host. *Infection and Immunity*. 2012; 2 (3): 581–596 (in Russ.). DOI: 10.15789/2220-7619-2012-3-581-596.

29. Kostyuchenko M.V., Ponomarenko D.G., Rakitina E.L., Logvinenko O.V., Sannikova I.V., Dejneka D.A., Golub O.G. Perspective of in vitro lymphocytes antigenicity evaluation for the diagnostics of acute brucellosis. *Infection and Immunity*. 2017; 7 (1): 91–96 (in Russ.). DOI: 10.15789/2220-7619-2017-1-91-96.
30. Blander J.M. The comings and goings of MHC class I molecules herald a new dawn in cross-presentation. *Immunological Reviews.* 2016; 272 (1): 65–79. DOI: 10.1111/imr.12428.
31. Faivre V., Lukaszewicz A.-C., Payen D. Downregula31. tion of blood monocyte HLA-DR in ICU patients is also present in bone marrow cells. *PLoS One.* 2016; 11 (11): 15. DOI: 10.1371/journal.pone.0164489.
32. Zhu M., Dai J., Wang C., Wang Y., Qin N., Ma H., Song C., Zhai X., Yang Y., Liu J., Liu L., Li S., Liu J., Yang H., Zhu F., Shi Y., Shen H., Jin G., Zhou W., Hu Z. Fine mapping the MHC region identified four independent variants modifying susceptibility to chronic hepatitis B in Han Chinese. *Human Molecular Genetics.* 2016; 25 (6): 1225–1232. DOI: 10.1093/hmg/ddw003.
33. Fisinin V.I., Surai P. Gut immunity in birds: facts and reflections (review). *Agricultural Biology*. 2013; 48 (4): 3–25 (in Russ.).

Authors contribution

Teimurazov M.G. – planning and carrying out of the laboratory research, analysis and interpretation of the data. Petrova N.V. – planning of the research design, analysis of the results. Karelina E.A.– planning of the research design, analysis of the results, drafting of the article. Ganina K.K. – statistical processing of the results. Tarasov S.A. – management of the project. Epstein O.I. – development of the drug concept, comments on the manuscript.

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Predictors of an adverse course of heart failure with preserved left ventricular ejection fraction in patients with obstructive sleep apnea syndrome

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ABSTRACT

Aim. To study the relationship of obstructive respiratory disorders during sleep with subclinical development of right ventricular dysfunction and pulmonary hypertension, as well as with the risk of an adverse course of chronic heart failure (CHF) with preserved left ventricular ejection fraction (LVEF).

Materials and methods. The study included 86 men with moderate and severe forms of obstructive sleep apnea syndrome (OSAS) (with an apnea / hypopnea index (AHI) > 15 per hour). All patients had abdominal obesity and hypertension. Upon inclusion in the study, all patients underwent polysomnography and echocardiography according to the standard protocol with an additional assessment of the fractional area change in the right ventricular myocardium (ΔSRV) and the right ventricular stroke work index (RVSWI). Also, the content of the N-terminal brain natriuretic peptide precursor (NT-proBNP) in the blood serum was determined by enzyme immunoassay analysis. A six-minute walk test (6MWT) was performed after inclusion in the study and after 12 months of follow-up. Depending on the course of CHF during the follow-up, retrospectively, the patients were divided into 2 groups: with an unfavorable (n = 33) and favorable (n = 53) prognosis.

Results. A significant relationship between AHI and ΔSRV, RVSWI, NT-proBNP, and 6MWT was revealed. Based on the results of one-way correlation analysis, it was found that ΔSRV (odds ratio (OR) 2.51; 95% confidence interval (CI) 2.42–3.24; p = 0.0009), NT-proBNP (OR 1.92; 95% CI 1.32–2.78; p = 0.003), and AHI (OR 3.93; 95% CI 2.87–4.11; p = 0.018) were predictors of an adverse course of CHF. In a multivariate analysis, it was found that AHI was an independent predictor of an adverse course of CHF (OR 3.49; 95% CI 2.17–11.73; p = 0.0008), while the addition of NT-proBNP improved risk stratification of an adverse course of CHF (OR 4.66; 95% CI 3.87–13.11; $p < 0.0001$).

Conclusion. The fractional area change in the right ventricular myocardium (ΔSRV) can be considered as a non-invasive marker for determining the emerging right ventricular dysfunction and predicting adverse cardiovascular events in patients with preserved LVEF and OSAS. Moreover, the combined use of echocardiographic (ΔSRV) and laboratory (NT-proBNP) markers can improve risk stratification of CHF progression.

Key words: obstructive sleep apnea syndrome, chronic heart failure with preserved ejection fraction, right ventricular dysfunction, pulmonary 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 patients signed an informed consent to participate in the study. The study was approved by the local Ethics Committee at the Clinical Hospital "Russian Railways – Medicine", Novosibirsk (Protocol No. 27 of 16.04.2018).

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Предикторы неблагоприятного течения сердечной недостаточности с сохраненной фракцией выброса левого желудочка у больных с синдромом обструктивного апноэ во сне

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РЕЗЮМЕ

Цель. Изучить взаимосвязь обструктивных нарушений дыхания во сне с развитием дисфункции правого желудочка (ПЖ) и легочной гипертензии, а также с риском неблагоприятного течения хронической сердечной недостаточности (ХСН) с сохраненной фракцией выброса левого желудочка (ФВ ЛЖ).

Материалы и методы. В исследование включены 86 мужчин со среднетяжелой и тяжелой формами синдрома обструктивного апноэ во сне (СОАС) (с индексом апноэ/гипопноэ (ИАГ) более 15 в час). Все пациенты имели абдоминальное ожирение и артериальную гипертензию. При включении в исследование всем больным выполнено полисомнографическое исследование, а также эхокардиография по стандартному протоколу с дополнительной оценкой фракционного изменения площади и индекса работы миокарда правого желудочка (ΔSПЖ и ИРМПЖ), определено содержание предшественника мозгового натрийуретического пептида (NT-proBNP) в сыворотке крови методом иммуноферментного анализа. Тест 6-минутной ходьбы (ТШХ) выполняли после включения в исследование и через 12 мес наблюдения. В зависимости от характера течения ХСН за период наблюдения, ретроспективно, пациенты были разделены на две группы: с неблагоприятным (n = 33) и благоприятным (n = 53) прогнозом.

Результаты. Выявлена значимая взаимосвязь между ИАГ и ΔSПЖ, ИРМПЖ, ТШХ, уровнем NT-proBNP. На основании результатов однофакторного корреляционного анализа установлено, что ΔSПЖ (отношение шансов (ОШ) 2,51; 95%-й доверительный интервал (ДИ) 2,42–3,24; p = 0,0009), NT-proBNP 1,92; 95%-й ДИ 1,32–2,78; p = 0,003), ИАГ (ОШ 3,93; 95%-й ДИ 2,87–4,11; p = 0,018) были предикторами неблагоприятного течения ХСН. При проведении многофакторного анализа установлено, что независимым предиктором неблагоприятного течения ХСН являлся ИАГ (ОШ 3,49; 95%-й ДИ 2,17–11,73; p = 0,0008), при этом добавление NT-proBNP улучшало стратификацию риска неблагоприятного течения ХСН (ОШ 4,66; 95%-й ДИ 3,87–13,11; p < 0,0001).

Заключение. Фракционное изменение площади ПЖ ΔSПЖ можно рассматривать в качестве неинвазивного маркера для определения формирующейся правожелудочковой дисфункции и прогнозирования неблагоприятных сердечно-сосудистых событий у больных с сохраненной ФВ ЛЖ и СОАС. При этом комбинированное использование эхокардиографического (ΔSПЖ) и лабораторного (NT-proBNP) маркеров позволяет улучшить стратификацию риска прогрессирования ХСН.

Ключевые слова: синдром обструктивного апноэ во сне, хроническая сердечная недостаточность с сохраненной фракцией выброса, правожелудочковая дисфункция, легочная гипертензия.

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

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

Соответствие принципам этики. Все пациенты подписали информированное согласие на участие в исследовании. Исследование одобрено этическим комитетом ЧУЗ «Клиническая больница ‘‘РЖД-Медицина” г. Новосибирск» (протокол № 27 от 16.04.2018).

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INTRODUCTION

Obstructive sleep apnea syndrome (OSAS) is a metabolic disorder associated with abdominal obesity and an increased risk of cardiovascular complications. A number of large, clinically controlled, prospective studies have demonstrated the relationship between obstructive sleep apnea and arterial hypertension, heart rhythm and conduction disturbances, as well as an increased risk of sudden cardiac death at night [1–5].

The significance of OSAS is determined by its wide prevalence, high frequency of severe metabolic and cardiopulmonary complications, and mortality [6, 7]. Thus, in the United States, more than 40 million patients with sleep disorders are observed, of which about 10 million people suffer from OSAS [8]. In Russia, there are no accurate data on the epidemiology of OSAS, but given high prevalence of the main risk factors for this syndrome, such as obesity, smoking, and thyroid diseases, it can be assumed that this syndrome is characterized by quite high prevalence among the Russian population.

One of the important links in the pathogenesis of sleep apnea syndrome is overload of the right ventricle due to a periodic increase in intra-abdominal pressure during apnea. Another important pathogenetic mechanism is development of pulmonary hypertension against the background of nocturnal intermittent hypoxemia, that leads to hyperactivation of the sympathetic nervous system, activation of the renin-angiotensin-aldosterone system (RAAS), development of the endothelial dysfunction with increased expression of endothelin-1, and activation of hypoxic pulmonary vasoconstriction [9, 10].

In addition, endothelial dysfunction caused by acidosis of the vascular wall is accompanied by overexpression of vasoconstrictors (thromboxane A2, endothelin-1) and inhibition of the production of vasodilators (nitric oxide and prostacyclin) [11]. It was shown that in OSAS, an episode of apnea is followed by a period of hyperventilation with a characteristic rise in the negative pressure in the chest and an increase in the right ventricular inflow, which subsequently leads to dilatation of the inferior vena cava and the right atrium, right ventricular hypertrophy, and activation of NT-proBNP [12, 13]. It is possible that these factors initiate the emergence and progression of global right ventricular dysfunction and pulmonary hypertension.

However, the complexity of interpreting right ventricular dysfunction and the mechanisms of pulmonary hypertension progression as prognostic factors of OSAS is determined by the fact that this syndrome is often accompanied by other risk factors for cardiovascular pathology, in particular, with obesity, insulin resistance, arterial hypertension, chronic obstructive pulmonary disease, and portal hypertension [14, 15]. Consequently, in cardiology practice, the use of modern approaches to multifactorial risk stratification of cardiovascular complications for selecting the optimal pathogen-specific therapy has significant advantages over assessing individual parameters, such as the functional class of pulmonary arterial hypertension (PAH) (according to the World Health Organization (WHO), or 6MWT). The effect of OSAS on the cardiovascular system is mediated by a number of factors. Therefore, well-designed clinical trials are necessary to assess this effect in detail and develop specific approaches to prevention and treatment of cardiovascular complications of OSAS.

It is assumed that an increase in the activity of the RAAS is associated with an increased risk of adverse cardiovascular events, as well as with a decrease in renal sensitivity to atrial and cerebral natriuretic peptides [3]. All these neurohumoral disorders lead to pathological remodeling of the pulmonary microvasculature, contributing to the progression of right ven-

tricular dysfunction and pulmonary hypertension. The presence of the listed pathogenetic factors contributes to the formation of chronic cor pulmonale.

Until now, it is not completely clear what the main reason for the initiation and progression of pulmonary hypertension in OSAS is: the actual periodic episodes of respiratory arrest during sleep or the severity of chronic arterial hypoxemia associated with them. At the same time, the assessment of subclinical right ventricular dysfunction and pulmonary hypertension seems to be an important innovative strategy for early personalized diagnosis, prevention, and treatment of various cardiovascular pathologies, as well as a useful tool for assessing the effectiveness of the pathogen-specific therapy used [16].

The aim of the study was to assess the relationship of obstructive sleep breathing disorders with subclinical development of right ventricular dysfunction and pulmonary hypertension, as well as with the risk of an adverse course of CHF with preserved LVEF.

MATERIALS AND METHODS

The study protocol was approved by the local Ethics Committee at the Clinical Hospital "Russian Railways – Medicine" of Novosibirsk (Protocol No. 27 of 16.04.2018). All patients signed an informed consent to participate in the study. The study included males who met the inclusion / exclusion criteria below.

Inclusion criteria: 1) moderate and severe OSAS (with AHI > 15 per hour); 2) arterial hypertension (including patients with stabilization of blood pressure (BP) against the background of antihypertensive therapy) 3) abdominal obesity, waist circumference (WC) ≥ 92 cm, body mass index (BMI) ≥ 30 kg / m^2.

Exclusion criteria: 1) primary pulmonary hypertension; 2) history of pulmonary embolism with pulmonary hypertension (systolic pressure in the right ventricle ≥ 45 mm Hg); 3) severe bronchial asthma, chronic obstructive pulmonary disease (COPD); 4) lesions of the cardiac valvular apparatus (insufficiency of the mitral, tricuspid or aortic valves ≥ II degree); 5) hypertrophic and dilated cardiomyopathy; 6) coronary artery disease (CAD); 7) chronic atrial fibrillation; 8) decompensated CHF with reduced LVEF; 9) pathology of the thyroid gland, severe renal (Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation < 30 ml / min / m^2) and liver failure; 8) refusal to participate in the research.

In order to diagnose OSAS, polysomnography of nocturnal sleep was performed in all patients using the Somnolab2PSG diagnostic system (Weinemann, Germany). The severity of OSAS was assessed by AHI; the study included patients with moderate (14 < AHI < 30 per hour) and severe (AHI ≥ 30 per hour) OSAS. Also, according to the sleep study results, the level of average night saturation (SPO_2av.), desaturation index, and the presence of cardiac arrhythmias at night were assessed. All patients included in the study underwent 6MWT. Determination of the NT-proBNP content in the serum *in vitro* was performed by enzyme-linked immunosorbent assay (ELISA) using NTproBNP-ELISA-BEST reagents (VEKTOR-BEST, Russia) on the Multiskan FC microplate photometer (China).

The study included 86 males with moderate and severe OSAS (with AHI > 15 per hour) with the average age of 52 [31.0; 78.0] years. All patients included in the study were diagnosed with abdominal obesity (WC > 92 cm), BMI exceeded 30 kg / m^2. In all cases, arterial hypertension was identified, but against the background of optimally selected antihypertensive therapy at the time of inclusion in the study, the patients achieved target BP levels. In 33.7% of the patients (n = 29), functional class (FC) I CHF (according to New York Heart Association (NYHA)) was diagnosed, in 39.5% (n = 34) – FC II CHF (according to NYHA), in the rest of the cases (n = 23), the 6MWT distance was more than 550 meters. At the same time, the NT-proBNP levels in all cases exceeded the reference values > 125 pg / ml.

After 12 months of prospective follow-up, retrospectively, depending on the course of CHF, the patients were divided into 2 groups. Group 1 (n = 33) included patients with an unfavorable course of CHF, group 2 (n = 53) – patients with a favorable course of the disease. The criteria for an unfavorable course of CHF were hospitalization for decompensated CHF or progression of the pathology according to the 6MWT data (deterioration of the NYHA functional class).

The clinical and demographic characteristics of the examined patients with OSAS at the time of inclusion in the study are presented in Table 1. The groups were comparable in terms of the main characteristics, however, higher AHI (p = 0.0001) and NT-proBNP (p = 0.024) levels were associated with an unfavorable course of CHF.

Echocardiography (EchoCG) was performed in all patients according to the standard protocol on the EPIQ device (Philips Ultrasound, Inc., USA). The following parameters were assessed: the sizes of the left and right heart chambers, LVEF (according to Simpson method), left ventricular myocardial mass index,

interventricular septal thickness, wall thickness of the left and right ventricles, and systolic pressure in the pulmonary artery (determined according to the degree of tricuspid regurgitation using the continuous wave method). Evaluation of global systolic dysfunction of the right ventricle (RV) was also carried out by analyzing fractional changes in the RV area and the right ventricular myocardial work index. The fractional change in the RV area was calculated using the formula ΔSRV = 100 × (EDA – ESA / EDA), where EDA is the end-diastolic area of the RV and ESA is the end-systolic area of the RV. The right ventricular stroke work index (RVSWI) (right ventricular myocardial performance index) was calculated as the ratio of the sum of isovolumic relaxation (IVR) time and isovolumic contraction time (IVCT) to the ejection time (ET): RVSWI = (IVR + IVCT) \ ET (normal 0.28 ± 0.04).

Statistical processing of the study results was carried out using the STATISTICA 10.0 and Medcale 11.5.0.0 software. To check the statistical hypotheses when comparing 2 independent quantitative variables, the Mann – Whitney test was used, to compare 2 dependent variables, the Wilcoxon test was applied. Quantitative data were presented as the median and the interquartile range *Me* [Q_{25}; Q_{75}]. Qualitative data were presented as percentages and absolute values. For analysis of qualitative features, contingency tables were used with calculation of Pearson's χ^2 criterion. If there were cells with an expected frequency of less than 5, the two-sided Fisher's exact test was applied.

Table 1

Clinical and demographic characteristics of the examined patients

Parameter	Group 1, *n* = 33	Group 2, *n* = 53	*p*
Age, years, *Me* [Q_{25}; Q_{75}]	52 [33; 71]	50 [31; 78]	0.717
Body mass index, kg / m^2, *Me* [Q_{25}; Q_{75}]	36.1 [30.1; 74.8]	36.8 [30.06; 77.2]	0.268
AHI, per hour, *Me* [Q_{25}; Q_{75}]	46.0 [20.6; 85]	27.0 [14.0; 98.0]	**0.0001**
SPO_2av., %, *Me* [Q_{25}; Q_{75}]	92 [83; 95.5]	93 [76; 96]	0.148
Desaturation index, *Me* [Q_{25}; Q_{75}]	44.8 [13.0; 85]	27.5 [4; 78]	0.0005
FC I CHF (NYHA), *n* (%) FC II CHF (NYHA), *n* (%)	8 (24.2) 15 (45.5)	21 (39.6) 19 (35.8)	0.142 0.069
NT-proBNP, pg/ml	338 [168; 678]	278 [177; 815]	**0.024**
According to WHO: FC PAH of the 1st degree, *n* (%) FC PAH of the 2nd degree, *n* (%) FC PAH of the 3rd degree, *n* (%)	 12 (36.4) 11 (33.3) 1 (3.0)	 29 (54.7) 18 (33.9) 4 (7.5)	 0.097 0.058 0.105
6-minute walk test, m, *Me* [Q_{25}; Q_{75}]	416 [318; 634]	527 [318; 640]	0.014
SBPav., mm Hg, *Me* [Q_{25}; Q_{75}] DBPav., mm Hg, *Me* [Q_{25}; Q_{75}]	132 [128; 138] 88 [75; 94]	134 [128; 136] 88 [78; 95]	0.376 0.431
COPD, *n* (%)	9 (27.3)	13 (24.5)	0.345
Smoking, *n* (%)	12 (36.4)	15 (28.3)	0.877
Dyslipidemia, *n* (%)	17 (51.5)	23 (43.4)	0.453
Diabetes mellitus, *n* (%)	6 (18.2)	9 (17.0)	0.120
VPB (Lown's grade II–III), *n* (%)	8 (24.2)	13 (24.5)	0.245
Atrial fibrillation, *n* (%)	7 (21.2)	9 (17.0)	0.654

Note. AHI – apnea / hypopnea index (according to polysomnography data), SPO_2av. – average night saturation (according to polysomnography data), FC – functional class, CHF – chronic heart failure, NT-proBNP – N-terminal brain natriuretic peptide precursor, PAH – pulmonary arterial hypertension, SBPav. – average daily systolic blood pressure, DBPav. – average daily diastolic blood pressure, VPB – ventricular premature beats, COPD – chronic obstructive pulmonary disease.

Comparison of the frequencies of adverse events in the groups was carried out using the Kaplan – Meier curves; the log-rank test was used to compare the two curves. To identify predictors of the development of unfavorable endpoints, the univariate analysis was used. To identify independent predictors, the method of multivariate analysis with calculating the odds ratio (OR) was applied. To determine the cutoff predictors of adverse cardiovascular events, the ROC analysis was used with the calculation of the area under the curve (AUC). The critical *p*-value significance level for all analyses was taken equal to 0.05.

RESULTS

Correlation analysis at the stage of inclusion in the study revealed the relationship between AHI and BMI ($r = 0.362$; $p = 0.0006$), left atrial volume ($r = 0.570$; $p < 0.00001$), ΔSRV ($r = –0.527$; $p < 0.00001$), RVSWI ($r = –0.377$; $p = 0.0003$), NT-proBNP ($r = 0.611$; $p < 0.00001$), and 6MWT ($r = –0.511$; $p < 0.00001$).

The main structural and functional EchoCG parameters of the left ventricle (LV) between the groups were comparable. Significant associations of RV performance indicators – ΔSRV ($p = 0.031$) and RVSWI ($p = 0.022$) – with an unfavorable clinical course of CHF were revealed (Table 2).

The therapy received by patients at the time of inclusion in the study was optimal and in line with current recommendations [17]. The groups were comparable in terms of the main groups of drugs used for treatment of hypertension and CHF (Table 3).

Table 2

Echocardiographic characteristics of the patients at the time of inclusion in the study

Parameter	Group 1, $n = 33$	Group 2, $n = 53$	p
Left atrium, mm, *Me* [Q_{25}; Q_{75}]	58 [55; 66]	5.5 [5.3; 6.0]	0.051
LVEF, %, *Me* [Q_{25}; Q_{75}]	58 [51; 66]	58 [52; 62]	0.902
LV EDD, mm, *Me* [Q_{25}; Q_{75}]	62 [56; 69]	58 [55; 63]	0.051
Interventricular septum, mm, *Me* [Q_{25}; Q_{75}]	13 [12; 14]	12 [11; 14]	0.195
Posterior wall of LV, mm, *Me* [Q_{25}; Q_{75}]	11 [10; 12]	11 [10; 12]	0.330
LV myocardial mass index, g / m^2, *Me* [Q_{25}; Q_{75}]	114 [91.5; 134.5]	113 [98; 139]	0.811
Diastolic dysfunction, *n* (%)	17 (51.5)	18 (34.0)	0.107
Left atrial volume, cm^2, *Me* [Q_{25}; Q_{75}]	20.8 [18.8; 22.8]	18.4 [16.2; 22.6]	0.057
ΔSRV, %, *Me* [Q_{25}; Q_{75}]	40 [35; 47]	44 [40; 47]	0.031
RVSWI, *Me* [Q_{25}; Q_{75}]	0.25 [0.22; 0.25]	0.25 [0.24; 0.26]	0.022
RVSP, mm Hg, *Me* [Q_{25}; Q_{75}]	30 [24; 41]	28 [21; 37]	0.321
RV anterior wall thickness, mm, *Me* [Q_{25}; Q_{75}]	5.0 [4.0; 6.0]	4.0 [4.0; 5.0]	0.186
RV EDD, mm, *Me* [Q_{25}; Q_{75}]	26 [21; 36]	29 [20; 33]	0.608

Note. LV – left ventricle, RV – right ventricle, LVEF – left ventricular ejection fraction, EDD – end-diastolic dimension, ΔSRV – fractional area change of the right ventricle, RVSWI – right ventricular myocardial stroke work index, RVSP – systolic pressure in the right ventricle.

Table 3

Therapy received by patients at the time of inclusion in the study, *n* (%)

Group of drugs	Group 1, $n = 33$	Group 2, $n = 53$	p
ACE inhibitors	20 (60.6%)	33 (62.3%)	0.581
Beta-blockers	22 (66.6%)	32 (60.4%)	0.472
Diuretics	16 (48.5%)	26 (49%)	0.748
Calcium antagonists	14 (42.4%)	20 (37.7%)	0.665
AT^2-receptor antagonists	17 (51.5%)	25 (47.2%)	0.678

Note. ACE – angiotensin-converting enzyme.

According to the univariate analysis, ΔSRV (OR 2.51; 95% CI 2.42–3.24; $p = 0.0009$), NT-proBNP (OR 1.92; 95% CI 1.32–2, 78; $p = 0.003$), and AHI (OR 3.93; 95% CI 2.87–4.11; $p = 0.018$) were predictors of an unfavorable course of CHF, while RVSWI (OR 2.53; 95% CI 1.98–4.08; $p = 0.0009$) turned out to be an insignificant predictor (OR 1.08; 95% CI 0.98–1.17; $p = 0.082$).

According to the ROC analysis, the cutoff point characterizing the unfavorable course of CHF, was the AHI value ≥ 33.5 episodes per hour (sensitivity 75.8%, specificity 67.9%, AUC – 0.732; $p < 0.0001$), ΔSRV ≥ 18.6% (sensitivity 75.8%, specificity 54.7%, AUC – 0.62; $p = 0.047$), and NT-proBNP ≥ 311 pg / ml (sensitivity 63.6%, specificity 73.6%, AUC – 0.645; $p < 0.0001$). When comparing the ROC curves, AHI remained the most significant predictor of CHF progression ($p = 0.007$), as opposed to NT-proBNP and ΔSRV (Fig. 1).

To identify the association of a higher AHI with an unfavorable course of CHF, the Kaplan – Meier analysis was carried out. The patients were divided according to the cutoff level: group A ($n = 38$) – less than 33.5, group B ($n = 48$) – more than 33.5. According to the results of the Kaplan – Meier analysis (Fig. 2), it was found that the frequency of the unfavorable course of CHF in the groups was statistically significantly different ($p = 0.014$). It was shown that AHI was associated with higher frequency of CHF progression during 12 months of follow-up.

According to the multivariate analysis with the inclusion of risk factors for CHF progression (BMI, weight, LVEF, carbohydrate metabolism disorders,

NT-proBNP levels, etc.), the AHI remained an independent predictor of an unfavorable course of CHF (OR 3.49; 95% CI 2.17–11.73; $p = 0.0008$), while the addition of NT-proBNP significantly improved risk stratification for an unfavorable course of CHF (OR 4.66; 95% CI 3.87–13.11; $p < 0.0001$).

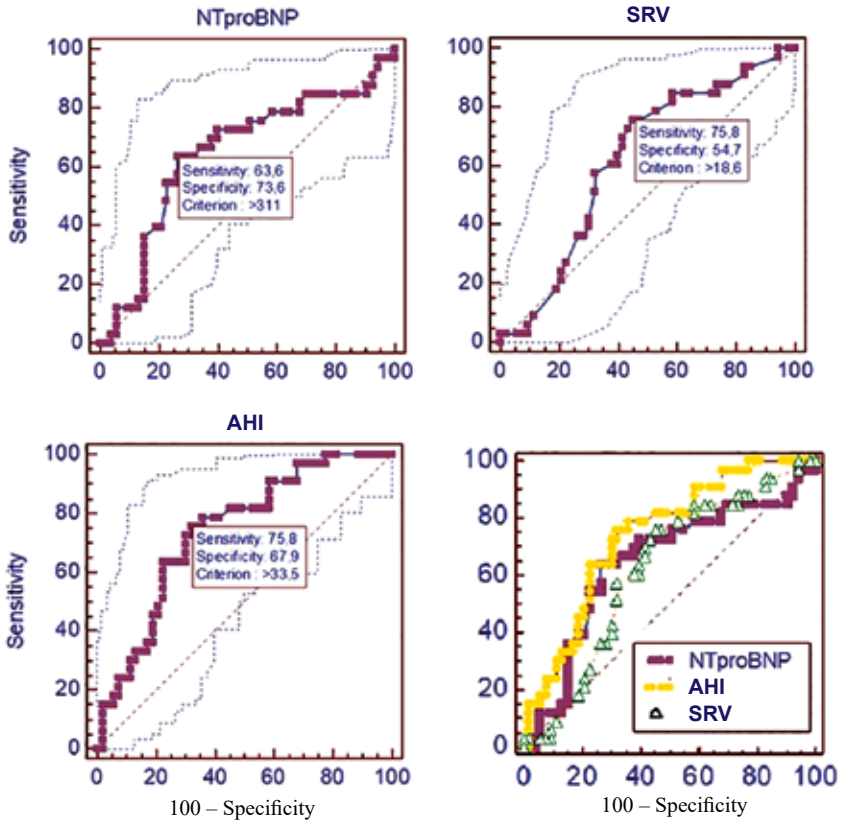

Fig. 1. Predictors of an adverse course of chronic heart failure (ROC-analysis): AHI – the apnea / hypopnea index (according to polysomnography), NT-proBNP – the N-terminal brain natriuretic peptide precursor, ΔSRV – fractional area change in the right ventricle (here and in Fig. 2)

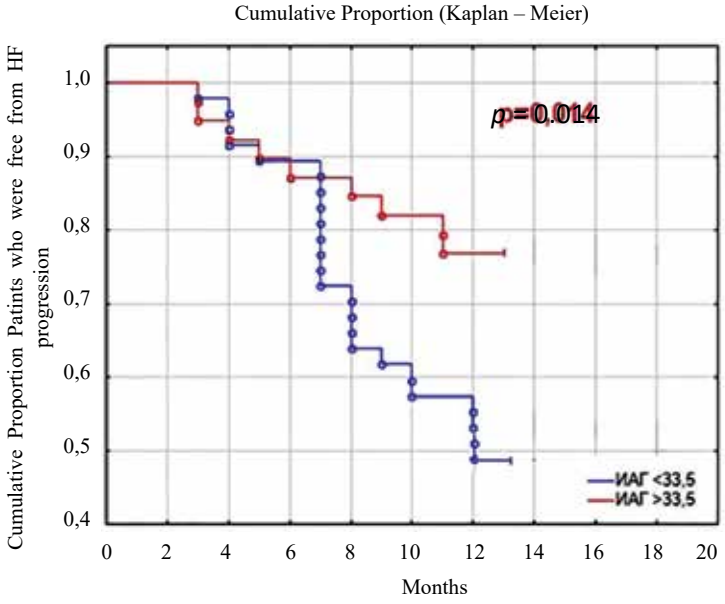

Fig. 2. Analysis of the frequency of an adverse course of CHF depending on the apnea / hypopnea index (Kaplan – Meier)

DISCUSSION

The study revealed the presence of a relationship between the severity of obstructive breathing disorders during sleep, assessed by AHI and echocardiographic (ΔSRV, RVSWI) parameters of right ventricular dysfunction, and the level of NT-proBNP. On the contrary, no significant correlations of the above-mentioned indicators with the severity of nocturnal hypoxemia (SPO_2av.) were found. This may indicate a key pathogenetic role of a prolonged excessive increase in intra-abdominal and intra-thoracic pressure during repeated apnea episodes in the development of right ventricular dysfunction in OSAS.

From this point of view, it is the frequency of apnea episodes, and not blood oxygenation, that seems to be the main factor determining right ventricular dysfunction and the clinical course of the disease in general. The importance of chronic nocturnal hypoxemia as a factor forming the mechanism of persistent pulmonary hypertension fades into the background. This is consistent with the data of a number of already published studies [18, 19].

As the analysis of the obtained data showed, the severity of obstructive breathing disorders during sleep, the main parameter of which is AHI, significantly correlates with the unfavorable clinical course of CHF with preserved ejection fraction. The most unfavorable course was observed in patients with severe OSAS (AHI > 33.5 per hour according to the Kaplan – Meier analysis). This association is probably determined by the commonality of a number of pathogenetic links in OSAS and the pathogenesis of the classic cardiovascular continuum: hyperactivation of the sympathetic nervous system, oxidative stress, and triggering of systemic inflammatory responses. Along with this, statistical analysis did not reveal significant correlations between the severity of nocturnal hypoxemia (SPO_2av.) and the course of CHF in the studied patients, which again may indicate a secondary role of nocturnal hypoxemia caused by obstructive apnea in the formation of right ventricular dysfunction in these patients.

The absence of reliable associations between the severity of pulmonary hypertension and the clinical course of the disease is worth noting. This is possibly determined by the method for assessing the degree of pulmonary hypertension chosen for this study. The assessment was carried out by calculation according to the degree of tricuspid regurgitation. At the same time, the degree of tricuspid regurgitation could be mediated to a certain extent with right ventricular dysfunction itself, and to clarify the value of pulmonary hypertension as a marker of the clinical course of OSAS, it is necessary to consider, apparently, another, more accurate variant of assessing this parameter.

At the same time, the results of the study demonstrated that the ΔSRV echocardiographic parameter of right ventricular dysfunction and the NT-proBNP laboratory marker are independent predictors of an unfavorable clinical course of CHF with preserved EF and OSAS. Considering the pathogenetic concept of the initial lesion of the right heart [20], which is currently available in the works of most researchers, the facts obtained in this study seem quite logical. The appearance of structural and functional disorders in the work of the right heart may indicate a previous pathogenetic effect of OSAS, significant in time and strength, and be accompanied by appropriate clinical presentation, or indicate its imminent emergence. In this case, the markers of right ventricular dysfunction can appear much earlier than the corresponding disorders in the left ventricle and serve as a criterion for the severity of the syndrome and an important prognostic sign.

A number of studies showed the prognostic significance of various forms of pulmonary hypertension as a marker of an unfavorable prognosis of cardiovascular mortality in patients with cardiovascular diseases, such as acquired heart defects, coronary artery disease, and CHF [20, 21]. However, at this point in time, the formation of chronic pulmonary hypertension and right ventricular dysfunction against the background of OSAS, as well as the prognostic role of these disorders are poorly understood. Data on the epidemiology and prevalence of pulmonary hypertension in OSAS both in Russia and in economically developed countries are very contradictory [18, 19, 22, 23].

A number of researchers consider an increase in the pulmonary artery pressure in the development of OSAS during the rapid eye movement (REM) sleep phase, regardless of the degree of arterial hypoxemia, a proven fact [24]. In addition, some researchers believe that OSAS induces the development of pulmonary hypertension mainly or exclusively in patients with COPD or in OSAS associated with primary pulmonary hypertension [25–27].

In practical terms, the emergence of the markers of right ventricular dysfunction can be useful in choosing a treatment strategy for these patients, as a signal for more aggressive treatment tactics with an earlier

decision on starting continuous positive airway pressure (CPAP) therapy. At the same time, the severity of nocturnal hypoxemia did not show such significant associations either with the markers of right ventricular dysfunction or with the clinical course of the disease. It is obvious that hypoxemia was mediated by OSAS and was secondary in nature.

From this point of view, when determining the treatment strategy, the preferred choice is CPAP therapy rather than prolonged oxygen inhalation, which is consistent with existing clinical guidelines and consensus documents [28, 29]. Undoubtedly, for clarifying the prognostic role of individual parameters of right ventricular dysfunction as well as for a more detailed study of the pathogenesis of cardiovascular complications in OSAS, it seems promising to evaluate these echocardiographic parameters at runtime with a longer follow-up period for this category of patients.

CONCLUSION

In the study, significant correlations between the severity of obstructive breathing disorders during sleep and echocardiographic and laboratory markers of developing right ventricular dysfunction in patients with OSAS were found, which may indicate an important pathogenetic role of these disorders in development of cardiovascular complications in the studied pathology. Evaluation of the clinical course of the disease revealed the relationship between the echocardiographic (ΔSRV) parameters of right ventricular dysfunction and the laboratory (NT-proBNP) markers with an adverse clinical course in this category of patients.

The data obtained make it possible to evaluate these markers as independent predictors of an adverse clinical course of the disease. In the future, they can be used to stratify the clinical risk of heart failure with preserved left ventricular ejection fraction and determine the treatment strategy in patients with OSAS.

REFERENCES

1. Butt M., Dwivedi G., Khair O., Lip G.Y.H. Obstructive sleep apnea and cardiovascular disease. *Int. J. Cardiol.* 2010; 139: 7–16. DOI: 10.1016/j.ijcard.2009.05.021.
2. Chazova I.E., Litvin A.Yu. Obstructive sleep apnea syndrome and related cardiovascular complications. *Cardiology*. 2002; 11: 86–92 (in Russ.).
3. Marin J.M., Agusti A., Villar I., Forner M., Nieto D., Carrizo S.J., Barbé F., Vicente E., Wei Y., Nieto F.J., Jelic S. Association between treated and untreated obstructive sleep apnea and risk of hypertension. *JAMA*. 2012; 307 (20): 2169–2176. DOI: 10.1097/sa.0b013e31827f34f1.
4. Mentz R.J., Fiuzat M. Sleep-disordered breathing in patients with heart failure. *Heart Fail. Clin.* 2014; 10 (2): 243–250. DOI: 10.1016/j.hfc.2013.10.001.
5. Bitter T., Westerheide N., Prinz C., Hossain M.S., Vogt .J, Langer C., Horstkotte D., Oldenburg O. Cheyne-stokes respiration and obstructive sleep apnoea are independent risk factors for malignant ventricular arrhythmias requiring appropriate cardioverter–defibrillator therapies in patients with congestive heart failure. *Eur. Heart J.* 2011; 32: 61–74. DOI: 10.1093/eurheartj/ehq327.
6. Lindberg. E., Gislason T. Epidemiology of sleep-related obstructive breathing. *Sleep Med. Rev.* 2000; 4: 321–327. DOI: 10.1053/smrv.2000.0118.
7. Lindberg E. Epidemiology of OSA. *Eur. Respir. Mon.* 2010; 50: 51–68. DOI: 10.1183/1025448x.00025909.
8. Peppard P.E., Young T., Barnet J.H., Palta M., Hagen E.W., Hla K.M. Increased prevalence of sleep-disordered breathing in adults. *Am. J. Epidemiol*. 2013; 177 (9): 1006–1014. DOI: 10.1093/aje/kws342.
9. Fletcher E.C. Effect of episodic hypoxia on sympathetic activity and blood pressure. *Respir. Physiol.* 2000; 119 (2–3): 189–197. DOI: 10.1016/s0034-5687(99)00114-0.
10. Ziegler M.G., Mills P.J., Loredo J.S. Ancoli-Israel S., Dimsdale J.E. Effect of continuous positive airway pressure on sympathetic nervous activity in patients with obstructive sleep apnea. *Chest.* 2001; 120 (3): 887–893. DOI: 10.1378/chest.120.3.887.
11. Kasai T., Bradley T.D. Obstructive sleep apnea and heart failure: pathophysiologic and therapeutic implications. *J. Am. Coll. Cardiol*. 2011; 57 (2): 119–127. DOI: 10.1016/j.jacc.2010.08.627.
12. Frantz R.P., Farber H.W., Badesch D.B., Elliott C.G., Frost A.E., McGoon M.D., Zhao C., Mink D.R., Selej M., Benza R.L. Baseline and serial brain natriuretic peptide level predicts 5-year overall survival in patients with pulmonary arterial hypertension. *Chest.* 2018; 154 (1): 126–135. DOI: 10.1016/j.chest.2018.01.009.
13. Tugcu A., Yildirimturk O., Tayyareci Y. Demiroglu C., Aytekin S. Evaluation of subclinical right ventricular dysfunction in obstructive sleep apnea patients using velocity vector imaging. *Circ. J*. 2010; 74 (2): 312–319. DOI: 10.1253/circj.cj-09-0562.
14. Van Deursen V.M., Urso R., Laroche C., Damman K., Dahlström U., Tavazzi L.,. Maggioni A.P., Voors A.A. Comorbidities in patients with heart failure: an analysis of the European Heart Failure Pilot Survey. *Eur. J. Heart Fail.* 2014; 16 (1): 103–111. DOI: 10.1016/s0735-1097(13)60738-x.
15. Schulz R., Grebe M., Eisele H.J., Mayer K., Weissmen N. Obstructive sleep apnea-related cardiovascular disease. *Med. Klin. (Munich)*. 2006; 101 (4): 321–327. DOI: 10.1159/000325110.
16. Williams B., Mancia G., Spiering W., Agabiti Rosei E., Azizi M., Burnier M., Clement D.L., Coca A., de Simone G., Dominiczak A., Kahan T., Mahfoud F., Redon J., Ruilope L., Zanchetti A., Kerins M., Kjeldsen S.E., Kreutz R., Laurent S., Lip G.Y.H., McManus R., Narkiewicz K., Ruschitzka .F, Schmieder R.E., Shlyakhto E., Tsioufis C., Aboyans V.,

Desormais I. ESC scientific document group. 2018 ESC/ESH guidelines for the management of arterial hypertension. *Eur. Heart J.* 2018; 39 (33): 3021–3104. DOI: 10.1093/eurheartj/ehy339.
17. Lim H.E., Kim Y.H., Kim S.H., Kim E.J., Pak H.N., Kim Y.H., Baik I., Shin C. Impact of obstructive sleep apnea on the atrial electromechanical activation time. *Circ. J.* 2009; 73 (2): 249–255. DOI: 10.1253/circj.cj-08-0813.
18. Sanner B.M., Konermann M., Sturm A., Muller H.J., Zidek W. Right ventricular dysfunction in patients with obstructive sleep apnea syndrome. *Eur. Respir. J.* 1997; 10 (9): 945–951. DOI: 10.1183/09031936.97.10092079.
19. Romero-Corral A., Somers V.K., Pellikka P.A., Olson E.J., Bailey K.R., Korinek J., Orban M., Sierra-Johnson J., Kato M., Amin R.S., Lopez-Jimenez F. Decreased right and left ventricular myocardial performance in obstructive sleep apnea *Chest.* 2007; 132 (6): 1863–1870. DOI: 10.1378/chest.07-0966.
20. Awdish R., Cajigas H., Definition, epidemiology and registries of pulmonary hypertension. *Heart Fail. Rev*. 2016; 21 (3): 223–228. DOI: 10.1007/s10741-015-9510-y.
21. Benza R.L., Miller D.P., Gomberg-MaitlandM., Frantz R.P., Foreman A.J., Coffey C.S., Frost A., Barst R.J., Badesch D.B., Elliott C.G., Liou T.G., McGoon M.D. Predicting survival in pulmonary arterial hypertension: insights from the Registry to Evaluate Early and Long-Term Pulmonary Arterial Hypertension Disease Management (REVEAL). *Circulation.* 2010; 122 (2); 164–172. DOI: 10.1161/circulationaha.109.898122.
22. Tugcu A., Guzel D., Yildirimturk O., Aytekin S. Evaluation of right ventricular systolic and diastolic function in patients with newly diagnosed obstructive sleep apnea syndrome without hypertension. *Cardiology.* 2009; 113 (3): 184–192. DOI: 10.1159/000193146.
23. Weitzenblum E., Krieger J., Apprill M., Vallée E., Ehrhart M., Ratomaharo J., Oswald M., Kurtz D. Daytime pulmonary hypertension in patients with obstructive sleep apnea syndrome. *Am. Rev. Respir. Dis.* 1988; 138 (2): 345–349. DOI: 10.1164/ajrccm/138.2.345.
24. Chaouat A., Weitzenblum E., Krieger J., Oswald M., Kessler R. Pulmonary hemodynamics in the obstructive sleep apnea syndrome: Results in 220 consecutive patients. *Chest.* 1996; 109 (2): 380–386. DOI: 10.1378/chest.109.2.380.
25. Bradley T.D., Rutherford R., Grossman R.F., Lue F., Zamel N., Moldofsky H., Zamel N., Phillipson E.A. Physiological determinants of nocturnal arterial oxygenation in patients with obstructive sleep apnea. *J. Appl. Physiol*. 1985; 59 (5): 1364–1368. DOI: 10.1152/jappl.1985.59.5.1364.
26. Fletcher E.C., Schaaf J.W., Miller J., Fletcher J.C. Long-term cardiopulmonary sequelae in patients with sleep apnea and chronic lung disease. *Am. Rev. Respir. Dis.* 1987; 135 (3): 525–533. DOI: 10.1378/chest.92.4.604.
27. Arias M.A., García-Río F., Alonso-Fernández A., Martínez I., Villamor J. Pulmonary hypertension in obstructive sleep apnoea: Effects of continuous positive airway pressure: a randomized, controlled cross-over study. *Eur. Heart J.* 2006; 27 (9): 1106–1113. DOI: 10.1093/eurheartj/ehi807.
28. Epstein L.J., Kristo D., Strollo P.J. Jr, Friedman N., Malhotra A., Patil S.P., Ramar K., Rogers R., Schwab R.J., Weaver E.M., Weinstein M.D., Adult Obstructive Sleep Apnea Task Force of the American Academy of Sleep Medicine. Clinical guideline for the evaluation, management and long-term care of obstructive sleep apnea in adults. *J. Clin. Sleep Med.* 2009; 5 (3): 263–276. DOI: 10.5664/jcsm.27497.
29. Nakamura S., Asai K., Kubota Y., Murai K., Takano H., Tsukada Y.T., Shimizuet W. Impact of sleep-disordered breathing and efficacy of positive airway pressure on mortality in patients with chronic heart failure and sleepdisordered breathing: a meta-analysis. *Clin. Res. Cardiol.* 2015; 104 (3): 208–216. DOI: 10.1007/s00392-014-0774-3.

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High residual platelet aggregation in patients with coronary artery disease: a new methodological approach to detection

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ABSTRACT

Aim. To develop a new methodological approach to assessment of collagen-induced platelet aggregation in patients with coronary artery disease (CAD) and to determine the quality of various methods for detecting high residual platelet reactivity (HRPR) to predict the risk of myocardial perfusion disturbance.

Materials and methods. 36 patients (10 men and 26 women) aged 41–83 years and having stable CAD were examined. All patients had been undergoing continuous antiaggregation therapy for 6 months. We evaluated platelet aggregation using a laser analyzer with collagen as an aggregation inducer by the standard method 1 and our own patented method 2. The degree of platelet aggregation (%) and the size of aggregates in relative units (r.u.) in platelet-rich plasma were estimated. Myocardial perfusion scintigraphy with 99mTc-methoxy-isobutylisonitrile was performed according to a two-day stress-rest protocol. The summed stress score (SSS) values were used for analysis. SSS < 4 was regarded as normal myocardial perfusion.

Results. The degree of platelet aggregation according to method 1 was 12 (5; 64)%, the aggregate size was 3 (2; 7) r.u. The degree of platelet aggregation according to method 2 was 44 (13; 78)%, and the aggregate size was 5 (4; 8) r.u. Method 2 allowed to diagnose the presence of myocardial ischemia with an aggregation degree ≥ 44.9% with sensitivity of 84% and specificity of 92% (area under the curve (AUC) = 0.89; $p < 0.0001$; odds ratio (OR) 2.18; 95% confidence interval (CI) 0.57–0.98) and an increase in aggregate size ≥ 4.80 r.u. with sensitivity of 84% and specificity of 84% (AUC = 0.95; $p < 0.00001$; OR 5.83; 95% CI 0.72–0.99).

Conclusion. In patients with CAD, the detection of high rates of collagen-induced platelet aggregation using the patented technique is associated with the risk of impaired myocardial perfusion. The developed new methodological approach to detection of HRPR allowed to determine high risk of atherothrombotic complications in additional 22% of the examined patients.

Key words: aggregation, platelet, collagen, coronary artery disease, residual reactivity.

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. Each patient signed an informed consent to participate in the study. The study was approved by the Ethics Committee at Cardiology Research Institute, Tomsk NRMC (Protocol No. 139 of 18.11.2015).

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Высокая остаточная агрегационная активность тромбоцитов у пациентов с ишемической болезнью сердца: новый методический подход к выявлению

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РЕЗЮМЕ

Цель – разработать новый методический подход к оценке коллаген-индуцированной агрегации тромбоцитов у пациентов с ишемической болезнью сердца (ИБС) и определить информативность методик выявления высокой остаточной реактивности тромбоцитов для прогнозирования риска нарушения миокардиальной перфузии.

Материалы и методы. Обследованы 36 пациентов (10 мужчин и 26 женщин) в возрасте 41–83 лет со стабильной формой ИБС, находящихся на непрерывной антиагрегационной терапии в течение 6 мес. Оценку агрегации тромбоцитов проводили на лазерном анализаторе с индуктором агрегации коллагеном по стандартной методике 1 и по собственной запатентованной методике – методике 2. Оценивали степень агрегации тромбоцитов (%) и размер агрегатов (отн. ед.) в суспензии тромбоцитов. Перфузионную сцинтиграфию миокарда с 99mTc-метокси-изобутилизонитрилом выполняли по двухдневному протоколу «нагрузка – покой». Для анализа использовали значения SSS, при SSS < 4 делали вывод о нормальной миокардиальной перфузии.

Результаты. Степень агрегации тромбоцитов по методике 1 составила 12 (5; 64)%, размер агрегата – 3 (2; 7) отн. ед. Степень агрегации тромбоцитов по методике 2 составила 44 (13; 78)%, а размер агрегата – 5 (4; 8) отн. ед. Методика 2 позволила диагностировать наличие нарушений миокардиальной перфузии при степени агрегации ≥44,9% с чувствительностью 84% и специфичностью 92% (AUC = 0,89; $p < 0,0001$; отношение шансов (ОШ) 2,18; 95%-й доверительный интервал (ДИ) 0,57–0,98) и увеличение размеров агрегатов ≥4,80 отн. ед. с чувствительностью 84% и специфичностью 84% (AUC = 0,95; $p < 0,00001$; ОШ 5,83; 95%-й ДИ 0,72–0,99).

Заключение. У пациентов с ишемической болезнью сердца выявление высоких показателей коллаген-индуцированной агрегации тромбоцитов с помощью запатентованной методики ассоциируется с риском нарушения миокардиальной перфузии. Разработанный новый методический подход по выявлению высокой остаточной реактивности тромбоцитов позволил определить наличие высокого риска развития атеротромботических осложнений дополнительно у 22% обследованных пациентов.

Ключевые слова: агрегация, тромбоцит, коллаген, ишемическая болезнь сердца, остаточная реактивность.

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

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Соответствие принципам этики. Информированное согласие получено от каждого пациента. Исследование одобрено этическим комитетом НИИ кардиологии Томского НИМЦ (протокол № 139 от 18.11.2015).

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INTRODUCTION

Coronary artery disease (CAD) remains the most common disease of the cardiovascular system, with a high risk of vascular events and death. High residual platelet reactivity (HRPR) in CAD patients is associated with development of ischemic complications, which was proven by numerous studies and meta-analysis data [1, 2]. However, in the daily practice of a cardiologist, platelet function is not evaluated due to a weak evidence base. Monitoring of platelet aggregation is advisable only in certain clinical situations (class IIb recommendations) [1, 2]. Nevertheless, despite the recommendations, the discussion about the routine use of platelet function testing continues. It is known that the gold standard for assessing platelet aggregation is light transmission aggregometry [1]. However, the sensitivity of the currently used methods for assessing residual platelet aggregation is often insufficient.

In this work, along with the standard examination of patients with CAD, the assessment of HRPR values by light transmission aggregometry using two methodological approaches was performed. The threshold values of platelet aggregation parameters were determined. Their relationship with myocardial perfusion disorders was evaluated according to myocardial perfusion scintigraphy. Knowledge in this research area is relevant both for clinical and fundamental medicine as well as for development of new diagnostic methods.

The aim of the study was to develop a new methodological approach to assessment of collagen-induced platelet aggregation in CAD patients and to determine the quality of various methods for HRPR detection to predict the risk of myocardial perfusion disturbance.

MATERIALS AND METHODS

A cross-sectional (single-stage) study was performed. The patients were recruited on the premises of Cardiology Research Institute in accordance with the principles of the Declaration of Helsinki. 36 patients with stable CAD who had been receiving continuous antiaggregation therapy for 6 months were examined. The study included patients aged 41–83 years (10 men and 26 women). All the examined patients received regular combination therapy in accordance with current guidelines for CAD treatment. Laboratory and instrumental methods of investigation, including platelet aggregation and ECG-synchronized myocardial perfusion scintigraphy, were used in all the patients in accordance with the recommendations for CAD diagnosis and treatment. The criteria for inclusion in the study were stable CAD and continuous antiaggregation therapy for 6 months (cardiomagnil, 75mg). The criteria for exclusion from the study encompassed non-adherence to the therapy; acute vascular complications less than 6 months ago; severe comorbidities; clinical and laboratory signs of acute inflammation; serum creatinine levels higher than 120 μmol / l; atrial fibrillation; ventricular arrhythmia of high grades in the Lown grading system, and refusal to participate in the study.

A special study to assess platelet aggregation was performed using the Born method, modified by Z.A. Gabbasov on a two-channel laser analyzer 220 LA (BIOLA SCIENTIFIC, Russia) using two methods. Method 1 (standard approach): collagen was used as an aggregation inducer; it was introduced once at a final concentration of 2 μmol / l for 10 seconds. Method 2: five-fold introduction of collagen at 2 μmol / l for 10 seconds with platelet aggregation being measured at 1st, 2nd, 3rd, and 4th minutes of the research. The new methodological approach is described in detail in the patent for the invention RUS 2686700 of 01.08.2018 [4].

Peripheral venous blood with 3.8% sodium citrate as an anticoagulant was used to isolate the platelet suspension. Experimental values of light transmission were determined for each patient's blood sample, where platelet-poor plasma was taken as 100% and platelet-rich plasma was taken as 0% of aggregation in this patient. The maximum value of light transmission was used to determine the degree of platelet aggregation (%). The average aggregate size (r.u.) was used to determine the size of an aggregate. Aggregation values determined by the light transmission curve in the range of 45–100% indicated HRRP in the patients.

Myocardial perfusion scintigraphy (MPS) with 99mTc-methoxy-isobutylisonitrile (99mTc-MIBI) was performed according to the two-day stress-rest protocol. The research was carried out on a hybrid 64-slice Discovery NM/CT 570c scanner (GE Healthcare, USA), equipped with a gamma camera with cadmium zinc telluride (CZT) detectors. An intravenous infusion of adenosine at a dose of 140 mg / kg /min for 4 minutes was used as a stress test. Myocardial perfusion was assessed using specialized software Corridor 4DM SPECT (INVIA, Ann Arbor, MI, USA). According to the generally

accepted approach, myocardial perfusion disorders were determined by the summed stress score (SSS), summed rest score (SRS), and summed difference score (SDS) for the entire left ventricular myocardium [5]. SSS values < 4 were regarded as normal myocardial perfusion.

Statistical data processing was performed using SPSS statistical packages (version 19) and Statistica 10.0. The Shapiro – Wilk test was used to evaluate the distribution of quantitative features. The distribution of quantitative aggregation indicators did not follow the normal distribution law; the aggregation data was represented as the median and the interquartile range *Me* (Q_1; Q_3). MPS data were presented as an absolute value and a relative value (*n*, %). The significance of differences for paired or dependent samples was evaluated using the Wilcoxon T-test. The nonparametric Spearman test was used to evaluate the correlation between variables. ROC analysis was used to determine the sensitivity and specificity of aggregation levels in risk stratification of adverse cardiovascular events. The AUC value > 0.70 was considered significant. To identify factors that have a significant impact on the course of the disease, the odds ratio (OR) was calculated with a 95% confidence interval (CI). The differences between the samples were considered statistically significant at $p < 0.05$.

RESULTS

In the group of the examined patients, the following cardiovascular risk factors were widely distributed: smoking – 27 (75 %) patients, overweight and obesity – 14 (39 %) patients, hypertension – 31 (86 %) patients, dyslipidemia – 33 (92 %) patients, type 2 diabetes – 12 (33 %) patients. Patients with FC III and II angina pectoris (15 (42%) and 11 (30%) patients, respectively) predominated. In the anamnesis, 8 (22%) patients had a Q-myocardial infarction (MI) that occurred 6 months or more before the study. In the majority of cases, the included patients were diagnosed with a multivascular lesion of the coronary arteries (30 (83 %) patients).

The study of platelet aggregation in CAD patients revealed significant differences between the parameters of platelet aggregation and the size of aggregates obtained during the implementation of the standard method, as opposed to the patented method.

Using the new methodological approach developed by us (method 2), it was found that CAD patients showed a significant increase in the size of aggregates and a rise in the degree of platelet aggregation in comparison with the corresponding values obtained during method 1 implementation (Table). Method 1 helped to identify HRPR in 9 (25%) patients. When using method 2, HRPR was detected in additional 8 (22%) patients, which amounted to 47% of all patients.

The indications for MPS in 16 (39%) patients were the diagnosis of CAD with pretest probability of 16–85%, in 12 (33%) patients – assessment of myocardial perfusion and the state of coronary stents, in 8 patients (22%) – assessment of coronary artery bypass grafts. According to MPS data, in 7 (19%) patients, myocardial perfusion at stress was within the normal values (SSS < 4). Minimal myocardial perfusion disturbance (SSS 4–8) was observed in 14 (39%) patients, moderate (SSS 9–13) – in 9 (25%) patients, and severe (SSS > 13) – in 6 (22%) patients.

Table

Parameters of platelet aggregation and logistic regression of patients with CAD according to two methods

Parameter	Method 1			Method 2		
	Aggregation values *Me* (Q_1; Q_3)	OR	95% CI	Aggregation values *Me* (Q_1; Q_3)	OR	95% CI
Degree of aggregation, %	12 (5; 64)	1.46	0.58–0.93	44 (13; 78)*	2.18	0.57–0.98
Size of aggregate, r.u.	3 (2; 7)	1.79	0.61–0.95	5 (4; 8)*	5.83	0.72–0.99

* difference between the methods with the level of statistical significance $p < 0.05$.

A correlation analysis showed the presence of associations between the aggregation degree and the size of the aggregate according to method 1 and the value of SSS of an average-strength relationship ($r = 0.54$ and $r = 0.61$, respectively; $p < 0.002$). Method 2 revealed a high-strength relationship ($r = 0.78$ and $r = 0.61$, respectively; $p < 0.002$).

The results of a logistic regression analysis showed that platelet aggregation parameters obtained in method 2 were associated with an increased risk of myocardial ischemia (Table).

To study and compare the diagnostic and prognostic characteristics (sensitivity and specificity) of various methods for evaluating aggregation activity,

a ROC analysis was performed. The indicator of the presence (absence) of myocardial ischemia was used as a predictor. According to the results of the ROC analysis, the study of platelet aggregation in CAD patients using method 1 revealed the presence of myocardial perfusion disturbances with an increase in the degree of aggregation ≥ 16.6% ($p < 0.0001$) and the size of the aggregates ≥ 2.97 r.u. ($p < 0.0004$). Method 2 was characterized by greater specificity. Thus, it was shown that method 2 allowed for diagnosis of myocardial perfusion disturbances at the degree of aggregation ≥ 44.9% ($p < 0.0001$) and an increase in the size of the aggregates ≥ 4.80 r.u. ($p < 0.0001$) (Figure).

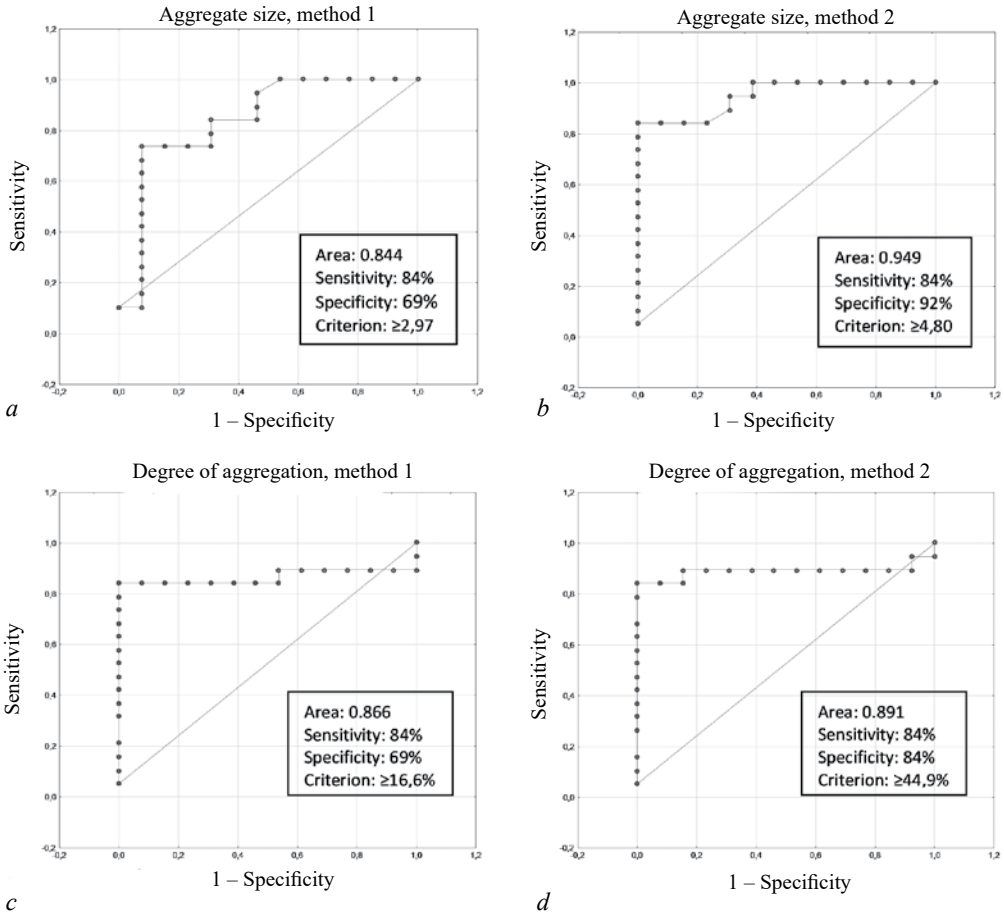

Figure. ROC analysis of platelet aggregation in patients with CAD using two methods as a predictor of myocardial ischemia: *a* – aggregate size (method 1), *b* – aggregate size (method 2), *c* – degree of aggregation (method 1), *d* – degree of aggregation (method 2)

DISCUSSION

Modern therapy in a hospital setting is very expensive, so the search for simple and inexpensive diagnostic tests is becoming more and more relevant. The discussion about the feasibility of studying platelet aggregation in CAD patients is still ongoing, which determines the need for research in this area.

The present study is an open, single-center, and cross-sectional observation. In the conducted study, light transmission aggregometry determined the threshold values of aggregation parameters for this subpopulation of CAD patients. Reaching these parameters meant that patients had HRPR. According to the standard method 1, the conclusion about the presence of myocardial ischemia can be made at the degree of aggregation ≥ 16.6% and the average size of aggregates ≥ 2.97 r.u. In the study of aggregation by method 2, the threshold values were 44.9 % for the degree of aggregation and 4.80 r.u. for the size of the aggregates. In addition, it was shown that standard aggregation research methods are not always sufficient to detect HRPR. The use of increased concentrations of a collagen inducer, added five times during the platelet aggregation study, increases the accuracy of evaluating collagen-induced aggregation in patients with CAD.

Data on the relationship between the risk of developing cardiovascular complications and insufficient suppression of platelet activity in CAD patients remain contradictory. The results of several independent meta-analyses involving more than 10,000 patients showed that HRPR was associated with a significant increase in the incidence of MI, stent thrombosis, and death from cardiovascular causes [2, 5, 6]. At the same time, there is evidence that there is no relationship between cardiovascular risk and HRPR in patients.

Therefore, the VerifyNow French Registry (VERIFRENCHY) was published, where the prognostic value of assessing the platelet function was studied. The results of a one-year follow-up did not reveal significant differences in the frequency of certain (probable) stent thrombosis, cardiovascular death, or MI (1,001 patients, VerifyNow device, Instrumentation Laboratory, USA) [1]. From our point of view, the negative results obtained in these studies may be associated with the fact that all currently used methods of aggregometry have limited sensitivity, specificity, usability, and predictive value.

In accordance with the data obtained to date, the process of formation of platelet aggregates proceeds in the following way. Damage to a vessel exposes collagen on its wall, which is both a substrate and a strong activator of platelet aggregation. Platelets adhere to the damaged endothelium of the vascular wall with the help of specific collagen receptors, which is one of the triggers in the development of a parietal thrombus of the coronary arteries. Subsequently, the platelets activate one other, forming a platelet thrombus [7, 8]. Therefore, we believe that only repeated addition of the collagen inducer to platelet-rich plasma in the study of platelet aggregation activity can provide objective information about the presence of HRPR in patients. The use of a new methodological approach developed by us with additional introduction of a collagen aggregation inducer in assessment of collagen-induced platelet aggregation allows to obtain additional information about the risk of myocardial ischemia, which determines the novelty of our study.

The ROC analysis results showed that our own patented method (method 2) is more specific for stratifying the risk of developing myocardial ischemia in patients with CAD. The data obtained are consistent with the results of studies by various authors that confirmed the association of HRPR with the development of adverse cardiac complications [1, 2, 5].

Comparison of methods demonstrated that repeated addition of a collagen inducer can detect platelets with high residual activity and a tendency to form large aggregates. The lack of response to a single addition of collagen may be due to partial activation of platelets with a tendency to subsequent disaggregation. Performing an aggregation study using adenosine diphosphate (ADP) or arachidonic acid as inducers may lead to a false conclusion about the effectiveness of antiaggregation therapy, while in reality the propensity of platelets to activate in response to interaction with the damaged endothelium remains elevated. Currently, clinical data indicate that neither acetylsalicylic acid nor clopidogrel in standard doses in the absence of platelet activity control can fully guarantee the effectiveness of antiaggregation therapy aimed at reducing the risk of recurrent acute vascular events [5, 7]. Medications that effectively act on collagen receptors are not currently patented and are not used.

From our viewpoint, the detection of HRPR using a new methodological approach will not only determine an increased cardiovascular risk in patients, but also suggest possible reasons for the ineffectiveness of antiaggregation therapy.

Limitations of the study include its cross-sectional design and a relatively small number of patients examined. However, the results emphasize the need for further research to study the clinical consequences of HRPR in patients with CAD and to improve methods for primary and secondary prevention of cardiovascular events.

CONCLUSION

This paper analyzes the clinical and prognostic significance of HRPR in patients with CAD. We showed that the detection of high rates of collagen-induced platelet aggregation in the patients using our own patented method is associated with the risk of impaired perfusion according to myocardial perfusion scintigraphy. The study of platelet aggregation showed that the newly developed methodological approach to detection of HRPR allowed to determine a high risk of atherothrombotic complications in additional 22% of patients, compared to the standard method.

REFERENCES

1. Mirzaev K.B., Andreev D.A., Sichev D.A. The evaluation of platelet aggregation in clinical practice. *Rational Pharmacotherapy in Cardiology*. 2015; 11(1): 85–91 (in Russ.). DOI: 10.20996/1819-6446-2015-11-1-85-91.
2. Stone G.W., Witzenbichler B., Weisz G., Rinaldi M.J., Neumann F.-J., Metzger D.C. et al. Platelet reactivity and clinical outcomes after coronary artery implantation of drug-eluting stents (ADAPT-DES): a prospective multicentre registry study. *Lancet*. 2013; 382 (9892): 614–623. DOI: 10.1016/S0140-6736(13)61170-8.
3. Trubacheva O.A., Suslova T.E., Kologrivova I.V., Gusakova A.M., Pavlyukova E.N., Schneider O. L., Petrova I.V. Method for determining thrombocyte resistance to antiaggregant preparations in patients with ischemic heart disease. Russian patent RU 2 686 700 C1 (in Russ.). Publ. 30.04.2019.
4. Zavadovsky K.V., Gulya M.O., Saushkin V.V., Saushkina Yu.V., Lishmanov Yu.B. Superimposed single-photon emission computed tomography and X-ray computed tomography of the heart: Methodical aspects. *Journal of Radiology and Nuclear Medicine.* 2016; 97 (4): 235–242 (in Russ.). DOI: 10.20862/0042-4676-2016-97-4-8-15.
5. Udell J.A., Bonaca M.P., Collet J.-P., Lincoff A.M., Kereiakes D.J., Costa F. et al. Long-term dual antiplatelet therapy for secondary prevention of cardiovascular events in the subgroup of patients with previous myocardial infarction: a collaborative meta-analysis of randomized trials. *European Heart Journal*. 2016; 37 (4): 390–399. DOI: 10.1093/eurheartj/ehv443.
6. Aradi D., Storey R.F., Komocsi A., Trenk D., Gulba D., Kiss R.G. et al. Expert position paper on the role of platelet function testing in patients undergoing percutaneous coronary intervention. *European Heart Journal*. 2014; 35 (4): 209–215. DOI: 10.1093/eurheartj/eht375.
7. Jastrzebska M., Marcinowska Z., Oledzki S. et al. Variable gender-dependent platelet responses to combined antiplatelet therapy in patients with stable coronary-artery disease. *Journal of Physiology and Pharmacology*. 2018; 69 (4): 595–605. DOI: 10.26402/jpp.2018.4.10.
8. Bakunovich A.V., Bulanova K.Ya., Lobanok L.M. Molecular mechanisms of platelet aggregation. *Journal of the Belarusian State University. Ecology*. 2017; 4: 40–51 (in Russ.).

Authors contribution

Trubacheva O.A., Gusakova A.M., Kologrivova I.V. – conception and design, carrying out of the experimental part of the study, analysis and interpretation of data, drafting of the manuscript. Suslova T.E., Zavadovsky K.V., Petrova I.V. – critical revision of the manuscript for important intellectual content and final approval of the manuscript for publication. Schneider O.L. – selection of patients, interviewing of patients, carrying out of the required list of examinations.

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The role of ^{18}F-FDG PET / CT in evaluation of therapy effectiveness and prognosis of lymphomas

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ABSTRACT

Aim. To determine the diagnostic value of positron emission tomography (PET) / computed tomography (CT) with F-18 fluorodeoxyglucose (^{18}F-FDG) for monitoring the effectiveness and prognosis of lymphoma therapy.

Materials and methods. Retrospective data of ^{18}F-FDG PET/CT (before treatment (PET1), after two cycles (PET2), and after completion of chemotherapy (PET3)) in 30 people with lymphomas were analyzed.

Results and discussion. A complete metabolic response in PET2 (PET2–) was observed in 21 patients (70%). In 9 patients in PET2–, a partial metabolic response (6 people), lack of metabolic response (2 people), or metabolic progression (1 person) were detected. These patients comprised the PET2+ group.

After chemotherapy, a complete metabolic response (PET3–) was diagnosed in 26 patients (87%). This effect was achieved in 21 patients (100%) with PET2– and in 5 patients (66%) with PET2+. Of the 9 patients in the PET2+ group, in 4 (44%) patients, a partial metabolic response or no metabolic response was diagnosed. Further monitoring of these patients showed that progression was detected in 2 cases, and in 2 patients, further treatment resulted in complete remission.

A two-year follow-up of patients revealed that remission was observed in 20 (67%) patients. The analysis of the results of PET2 showed that a relapse of the disease was observed in 6 (67%) PET2+ patients and remission was noted in 3 (33%) patients. In PET2– patients, a relapse was diagnosed in 4 (19%) persons, and remission was established in 17 (81%) patients.

Conclusion. Early PET / CT with ^{18}F-FDG allows to predict the effect of lymphoma treatment. The method can be recommended for monitoring lymphoma therapy.

Key words: Hodgkin's lymphoma, non-Hodgkin's lymphoma, computed tomography, positron emission tomography, monitoring of lymphoma therapy, prognosis of lymphoma therapy.

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

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Роль позитронной эмиссионной и компьютерной томографии с ^{18}F-флуоро-2-дезокси-D-глюкозой в оценке эффективности терапии и прогнозе лимфом

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РЕЗЮМЕ

Цель. Определение диагностической значимости позитронной эмиссионной и компьютерной томографии (ПЭТ/КТ) с меченной ^{18}F-флуоро-2-дезокси-D-глюкозой (^{18}F-ФДГ) в оценке эффективности и прогнозе лечения лимфом.

Материалы и методы. Проанализированы ретроспективные данные ПЭТ/КТ с ^{18}F-ФДГ 30 человек со злокачественными лимфомами: до лечения (ПЭТ1), через два курса (ПЭТ2) и после завершения полихимиотерапии (ПЭТ3).

Результаты и обсуждение. При анализе результатов ПЭТ2 полный метаболический ответ на два курса химиотерапии (ПЭТ2–) наблюдался у 21 (70%) пациента. У 9 пациентов через два цикла химиотерапии были установлены: частичный метаболический ответ (6 человек), отсутствие метаболического ответа (2 человека) или метаболическое прогрессировании (1 человек). Эти больные составили группу ПЭТ2+.

После окончания химиотерапии полный метаболический ответ (ПЭТ3–) был диагностирован у 26 (87%) пациентов. Такой эффект был достигнут у 21 (100%) больного с ПЭТ2– и 5 (66%) человек с ПЭТ2+. Из 9 пациентов группы ПЭТ2+ у 4 (44%) пациентов после завершения химиотерапии был диагностирован частичный метаболический ответ или его отсутствие. Дальнейшее наблюдение за этими пациентами показало, что в двух случаях было диагностировано прогрессирование, а у 2 больных последующее лечение привело к полной ремиссии.

При двухлетнем наблюдении за пациентами обнаружено, что ремиссия наблюдалась у 20 (67%) пациентов. Анализ результатов ПЭТ2 показал, что при ПЭТ2+ рецидив заболевания наблюдался в 6 (67%) случаях, ремиссия – в 3 (33%). В то время как при ПЭТ2– рецидив диагностирован у 4 (19%) человек, ремиссия установлена у 17 (81%).

Заключение. ПЭТ/КТ с ^{18}F-ФДГ, выполненная на ранних этапах химиотерапии, позволяет предсказать эффект лечения у пациентов со злокачественными лимфомами. Метод показан к широкому использованию в клинической практике на этапах терапии этой патологии.

Ключевые слова: лимфома Ходжкина, Неходжкинские лимфомы, компьютерная томография, позитронная эмиссионная томография, мониторинг терапии лимфом, прогноз терапии лимфом.

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

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INTRODUCTION

Today, in Russia, the percentage of malignant neoplasms of the lymphatic and hematopoietic tissue accounts for 5% and 4.6% of tumors detected annually in men and women, respectively. In 2016, the incidence of this pathology in the Russian Federation was 19.58 per 100 thousand population, while the average annual growth rate was 1.78% [1]. Every year lymphomas become the cause of death in 5% of all patients with tumor diseases. Hodgkin's lymphoma (HL) is the most common lymphoproliferative disease (30%). Of non-Hodgkin's lymphomas (NHL), diffuse large B-cell lymphoma and follicular lymphoma are most commonly diagnosed (33% and 22%, respectively). The incidence of other types of lymphomas is less than 10% [2].

Usually the effect of lymphoma treatment is monitored by dynamic assessment of the tumor size using anatomical imaging techniques, most commonly computed tomography (CT). At the same time, CT is not optimal for this purpose. Thus, after completion of therapy, in more than 60% of patients with HL and 40% of patients with aggressive NHL, according to CT data, a residual tumor mass is visualized, which may contain areas of fibrosis and necrosis, as well as tumor cells [3]. At the same time, CT cannot differentiate a viable tumor mass from the residual scar tissue. In addition, anatomical imaging techniques usually do not allow to determine a tumor response at early stages of treatment, since reduction of the tumor volume takes time.

Therefore, there is a growing interest in new methods for diagnosing lymphoproliferative diseases. This fully applies to positron emission tomography (PET) with ^{18}F-fluoro-2-deoxy-d-glucose (^{18}F-FDG) [4, 5] and single-photon emission computed tomography (SPECT) with ^{99m}Tc-1-thio-d-glucose [6–8]. The data of modern studies show high efficiency and confirm the prognostic value of these methods, which makes it possible to determine the prevalence of lymphomas, assess the effectiveness of the therapy, and determine the presence or absence of indications for radiation therapy. Thus, according to the literature, PET with ^{18}F-FDG allows to visualize increased metabolic activity in 30–64% of patients with residual tumor masses after completion of the therapy [4]. The presence of such hypermetabolic formations in 62–100% of cases is accompanied by a relapse after the first-line chemotherapy [9].

The widespread use of PET in the Russian Federation is currently limited due to the high cost of the procedure and the insufficient number of PET centers, which are located mainly in the European part of the country. Considering high efficiency and demand for this method in recent years, modern PET centers have been built in the Eastern part of Russia, including the Nuclear Medicine Center of the Federal Siberian Research Clinical Center under FMBA of Russia (FSRCC FMBA of Russia) in Krasnoyarsk. The opening of this center made PET available to residents of the Siberian Federal District.

The aim of this study was to determine the diagnostic value of ^{18}F-FDG PET / CT in assessing the effectiveness and prognosis of lymphoma treatment.

MATERIALS AND METHODS

Retrospective data of ^{18}F-FDG PET / CT of 30 people with malignant lymphomas were analyzed: before treatment (PET1), after 2 cycles of polychemotherapy (PET2), and after completion (from 6 to 8 cycles) of polychemotherapy (PET3). PET2 was performed before the introduction of the third cycle of chemotherapy, and PET3 was performed 2 weeks after the last dose. In 12 cases, additional ^{18}F-FDG PET / CT (PET4) was performed at the stages of dynamic observation of patients (6–12 months after treatment completion). The examination was carried out at the Nuclear Medicine Center of the FSRCC FMBA of Russia (Krasnoyarsk) from 2015 to 2017. The study involved 13 men and 17 women aged 19–74 years (average age was 42 years). All patients underwent an immunohistochemical examination, according to which 12 patients had HL, and the remaining 18 had aggressive NHL: diffuse large B-cell lymphoma (11 cases) and follicular lymphoma (7 cases). In accordance with the data of the initial clinical and instrumental studies (clinical examination, CT, magnetic resonance imaging (MRI), ultrasound, bone marrow biopsy), stage I of the disease was established in 3 patients (10%), stage II – in 8 (27%) patients, stage III – in 8 (27%) patients, and stage IV – in 11 (36%) patients.

Reference methods for result verification were histological examination or, if it was impossible to perform it, long-term (at least a year) clinical monitoring of the patient with a series of control instrumental examinations (CT, MRI, ultrasound, ^{18}F-FDG PET / CT).

Most patients received R-CHOP treatment (14 patients) (rituximab, cyclophosphamide, doxorubicin, vincristine, prednisolone). 9 patients had the BEACOPP regimen (bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, prednisolone), 4 patients underwent RB (rituximab, bendamustine) treatment, and 3 patients received ABVD (adriamycin, bleomycin, vinblastine, and dacarbazine) therapy.

Routine methods for assessing the effectiveness of treatment included a clinical examination of the patient, laboratory tests, and CT of the chest wall and abdominal cavity. This assessment was carried out after 2–3 cycles of chemotherapy and after completion of the entire treatment program. In accordance with the "Russian clinical guidelines for the diagnosis and treatment of lymphoproliferative diseases" [10], the patient's condition was assessed as complete remission (CR), uncertain complete remission (uCR), partial remission (PR), stabilization (St), relapse (after CR or uCR), or progression (after PR or St).

^{18}F-FDG PET / CT was performed in the whole body mode (from the level of the eye sockets to the middle third of the thigh) with simultaneous low-dose CT to correct the attenuation. The study was carried out on a PET / CT scanner Discovery PET / CT 600. Eating was allowed no later than 6 hours before the study. The radiopharmaceutical ^{18}F-FDG was administered intravenously at a dose of 300–550 MBq, and after 60–90 minutes, scanning was performed. The obtained images were reconstructed using standard software. The results of all studies were interpreted and analyzed by specialists in nuclear medicine and radiology.

The results of PET2 and PET3 were assessed as follows: a complete metabolic response (1, 2, and 3 points on a five-point Deauville scale in lymph nodes or extranodal sites with or without residual tumor mass); a partial metabolic response (4 or 5 points on the Deauville scale with visually reduced uptake of the radiopharmaceutical compared to baseline and residual tumor mass of any size); lack of a metabolic response (4 or 5 points on the Deauville scale without a significant change in ^{18}F-FDG uptake compared to the baseline value), and metabolic progression (4 or 5 points on the Deauville scale with an increase in ^{18}F-FDG uptake compared to the baseline value and / or the emergence of new metabolically active foci associated with lymphoma) [11].

To study the prognostic value of the results of patients' examination at the stages of treatment, progression-free survival (the time from the diagnosis to the first signs of progression or a relapse or to disease-related death) (PFS) was chosen as an endpoint. The survival curves were constructed using the Kaplan – Meier method. The differences between the groups were analyzed using a log rank test.

RESULTS

According to the reference verification methods, 191 lymph nodes involved in the pathogenetic process were found in 30 patients with lymphoma. Most often, there was a lesion in the cervical (21%), supra- and subclavian (20%), mediastinal (13%), axillary (10%), and bronchopulmonary (10%) lymph nodes. Lesions in the mesenteric (6%), inguinal (6%), and paraaortic (5%) nodes were less commonly diagnosed.

According to PET / CT, pathological accumulation of ^{18}F-FDG in the lymph nodes was observed in all patients included in the study. 169 (88%) of 191 affected nodes were hypermetabolic. ^{18}F-FDG PET /CT was most effective in diagnosing the state of the iliac, mediastinal, mesenteric, and inguinal lymph nodes, when its sensitivity exceeded 90%.

In 16 patients, 33 extranodal lesions were diagnosed using reference verification methods. Most often, there was dissemination of malignant lymphomas in the lungs (10 patients), spleen (11 patients), and red bone marrow (7 patients); in individual cases, it was observed in the liver and soft tissues.

According to PET / CT, pathological ^{18}F-FDG uptake was observed in 30 (91%) of 33 extranodal foci. The PET / CT method was most effective in diagnosing the state of the red bone marrow, soft tissues, lungs, and spleen, when its sensitivity was 90% or more. When assessing the state of the liver, it was possible to visualize 2 pathological foci out of 3.

When analyzing the PET2 results, a complete metabolic response after two cycles of chemotherapy (PET2–) was observed in 21 patients (70%): in 9 patients (75%) with HL and 12 patients (67%) with NHL (Fig. 1). In this group of patients, patients with early stages of lymphoma (stages I-II) were more common, (9 (82%) out of 11), as well as persons without signs of extranodal lesions (11 (79%) out of 14). In stage III-IV lymphoma and in the presence of extranodal lesions, PET2– occurred in 12 (63%) and 10 (62%) patients, respectively (Table 1).

Table 1

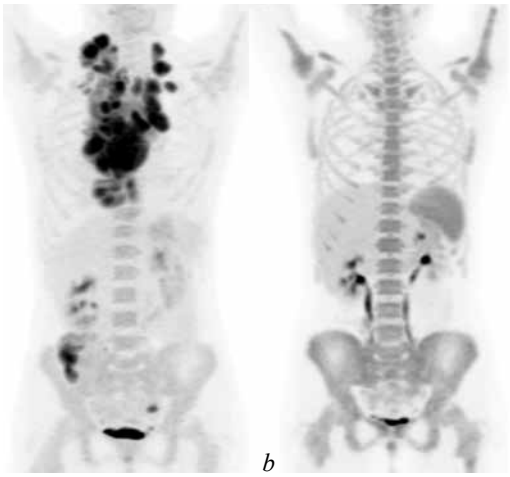
Interim ^{18}F-FDG PET/CT and ^{18}F-FDG PET/CT results after chemotherapy in lymphoma patients

Patients	LH		NLH		LH + NLH		Total
	PET2–	PET2+	PET2–	PET2+	PET2–	PET2+	
Total in the group	9	3	12	6	21	9	30
PET3–	9	2	12	3	21	5	26
PET3+	0	1	0	3	0	4	4
Early stages (I-II)	3	1	6	1	9	2	11
PET3–	3	1	6	0	9	1	10
PET3+	0	0	0	1	0	1	1
Advanced stages (III-IV)	6	2	6	5	12	7	19
PET3–	6	1	6	3	12	4	16
PET3+	0	1	0	2	0	3	3
Extranodal lesions –	5	1	6	2	11	3	14
PET3–	5	1	6	2	11	3	14
PET3+	0	0	0	0	0	0	0
Extranodal lesions +	4	2	6	4	10	6	16
PET3–	4	1	6	1	10	2	12
PET3+	0	1	0	3	0	4	4

In 9 patients, after 2 cycles of chemotherapy, a partial metabolic response (6 people), no metabolic response (2 people), or metabolic progression (1 person) were established (Fig. 2). These patients made up the PET2+ group. This group included 3 patients (25%) with HL and 6 patients (33%) with NHL. Signs of tumor metabolic activity on interim PET / CT scans were observed in 2 (18%) cases with early stages of lymphoma and in 7 (37%) patients with advanced stages of the disease. In addition, positive interim PET was more common in the presence of extranodal lesions (6 (38%) patients) than in their absence (3 (21%) patients) (Table 1). After the end of chemotherapy, a complete metabolic response (PET3–) was diagnosed in 26 patients (87%). This effect was achieved in 21 patients (100%) with PET2– and in 5 people (66%) with PET2+ (Fig. 3, Table 1).

In the PET2+ group after completion of chemotherapy, 4 (44%) patients had a partial metabolic response (3 people) or no metabolic response (1 person) (Fig. 1, Table 1). Follow-up of these patients showed that in 2 cases, progression was diagnosed, and in 2 patients, further treatment led to complete remission.

During a two-year follow-up of patients, it was found that remission was observed in 20 (67%) patients (complete remission in 15 people, uncertain complete remission in 5 patients). The "remission" group included 10 (83%) patients with HL and 10 (56%) individuals with NHL. The "relapse" group consisted of 10 (40%) people: 6 patients had a relapse of the disease, and 4 people had progression (Fig. 4, 5, Table 2). More often, a relapse or progression was observed in NHL – 8 (56%) cases, less often, in HL – in 2 (17%) patients (Fig. 6, Table 2). Patients with early stages of lymphoma more often were in the "remission" group than patients with advanced stages of the disease, 72% and 63%, respectively. It should also be noted that remission was more often observed in patients with no extranodal lesions (76%) than in those with extranodal lesions (56%) (Table 2).



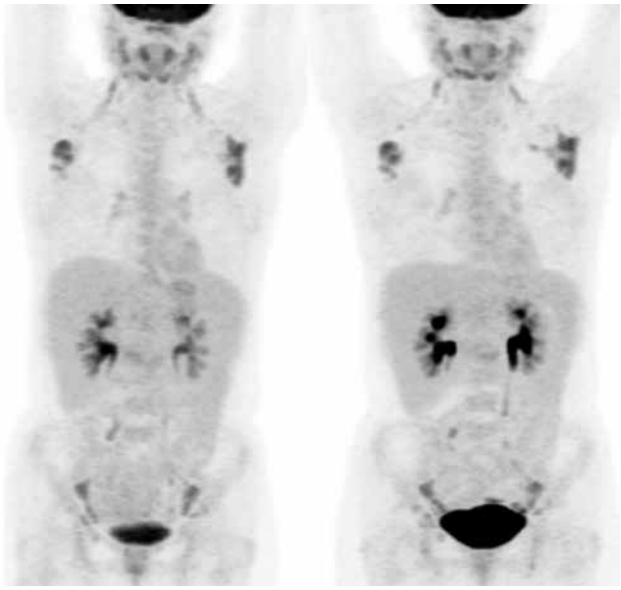
a *b*

Fig. 1. Patient T., 25 years old. Diagnosis: Hodgkin's lymphoma: *a* – PET1: signs of lymphoproliferative disease with multiple metabolically active lesions of the anterior and posterior mediastinum, cervical, supraclavicular and subclavian, bronchopulmonary, and intraperitoneal lymph nodes; *b* – PET2; complete metabolic regression

Table 2

Interim ^{18}F-FDG PET/CT and follow-up prognosis in lymphoma patients

Patients	LH		NLH		LH + NLH		Total
	PET2–	PET2+	PET2–	PET2+	PET2–	PET2+	
Total in the group	9	3	12	6	21	9	30
Remission	9	1	8	2	17	3	20
Relapse	0	2	4	4	4	6	10
Early stages (I-II)	3	1	6	1	9	2	11
Remission	3	0	4	1	7	1	8
Relapse	0	1	2	0	2	1	3
Advanced stages (III-IV)	6	2	6	5	12	7	19
Remission	6	1	4	1	10	2	12
Relapse	0	1	2	4	2	5	7
Extranodal lesions –	5	1	6	2	11	3	14
Remission	4	1	5	1	9	2	11
Relapse	1	0	1	1	2	1	3
Extranodal lesions +	4	2	6	4	10	6	16
Remission	3	0	4	2	7	2	9
Relapse	1	2	2	2	3	4	7



a *b*

Fig. 2. Patient C., 38 years old. Diagnosis: Hodgkin's lymphoma, nodular sclerosis: *a* – PET1: signs of lymphoproliferative disease with a metabolically active lesion of the cervical, axillary, iliac, and inguinal lymph nodes; *b* – PET2: compared to PET1– no changes

Analysis of the PET2 results showed that in patients with PET2+, a relapse was observed in 6 (67%) cases, and remission – in 3 (33%) cases. At the same time, in patients with PET2–, the opposite pattern took place: 4 (19%) people were diagnosed with a relapse and 17 (81%) had remission (Fig. 4, 5).

DISCUSSION

The results of our study confirm the global experience of using ^{18}F-FDG PET / CT. In lymphomas, the metabolic activity of the tumor changes quite rapidly after the start of treatment, even before a change in tumor size is detected [12]. Many studies have shown that ^{18}F-FDG PET / CT performed at early stages of chemotherapy (interim ^{18}F-FDG PET / CT) predicts the effectiveness of treatment. Such stratification of patients makes it possible to personalize the treatment strategy and has a positive effect on the outcome of the disease [13]. In patients with lymphoma, an interim ^{18}F-FDG PET / CT scan is usually performed after one to four (as a rule two) chemotherapy cycles out of six to eight planned ones.

Interim ^{18}F-FDG PET / CT helps to differentiate between patients with favorable lymphoma who need standard therapy and high-risk patients who require more intensive treatment with high-dose chemotherapy regimens. The method has proven itself well in determining the sensitivity of tumor tissue to chemotherapy, especially in patients with an advanced stage of the disease and an unfavorable course of the lymphoproliferative process, who may need additional radiation therapy [14]. In addition, in low-risk patients, interim ^{18}F-FDG PET / CT can reduce side effects and unnecessary toxicity associated with treatment, making it possible to choose the gentlest protocols and reduce the number of cycles. According to the ESMO guidelines, in patients with HL [14], interim ^{18}F-FDG PET / CT after 1–2 cycles of chemotherapy can identify a group with a high probability of achieving a complete metabolic response after completion of treatment and without indications for consolidation radiotherapy [13]. At advanced stages of the disease, this method is used to identify patients who need to change the chemotherapy regimen.

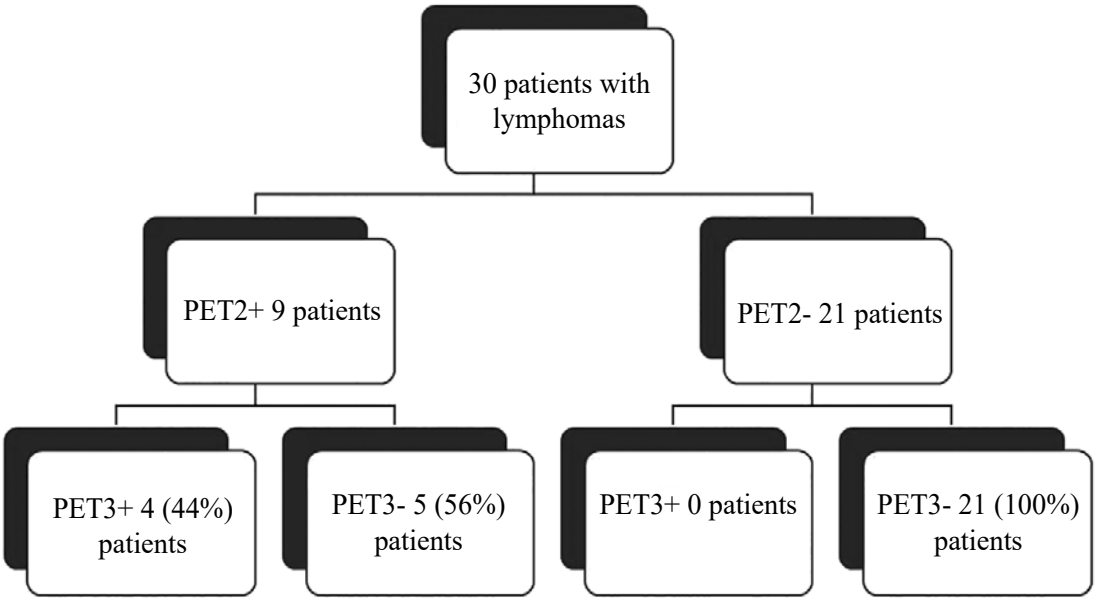


Fig. 3. Results of ^{18}F-FDG PET/CT after lymphoma chemotherapy in PET2+ and PET2– groups

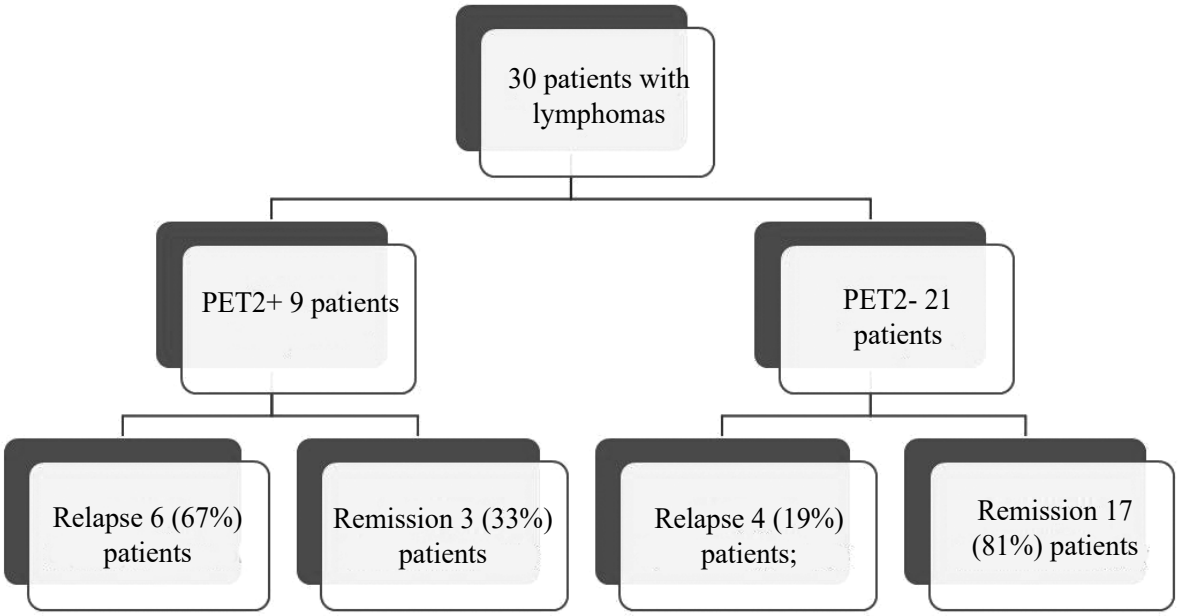


Fig. 4. Results of long-term follow-up of PET2+ and PET2– groups of lymphoma patients

According to a study by J. Radford et al. [13], performed on the basis of the analysis of the ^{18}F-FDG PET / CT findings in 602 patients with early stage HL who received ABVD chemotherapy, it was shown that if after 3 cycles the metabolic activity in the tumor foci was not detected, then further radiation therapy was unnecessary and progression-free survival (PFS) remained unchanged. A retrospective study of 260 HL patients treated with ABVD therapy confirmed the predictive role of interim ^{18}F-FDG PET/CT using Deauville's criteria for predicting a treatment response [16].

In another study, observation of patients with advanced stage HL showed that a complete metabolic tumor response after two cycles of ABVD chemotherapy had high negative predictive value (NPV) (94%) and positive predictive value (PPV) (73%) with predicting 3-year PFS [17]. Similar data were presented in an article by J. Markova et al., in which the analysis of the interim ^{18}F-FDG PET / CT findings in patients

with advanced HL after 4 cycles of BEACOPP chemotherapy demonstrated that a complete metabolic response in predicting 4-year PFS had NPV and PPV levels of 98% and 96%, respectively [18]. In addition to its high predictive value for assessing PFS, interim ^{18}F-FDG PET / CT can be used to determine the indications for consolidation radiotherapy [18].

CONCLUSION

Interim ^{18}F-FDG PET / CT with high accuracy allows to predict the effect of malignant lymphoma treatment. The method is recommended for widespread use in clinical practice for monitoring the stages of therapy for this pathology.

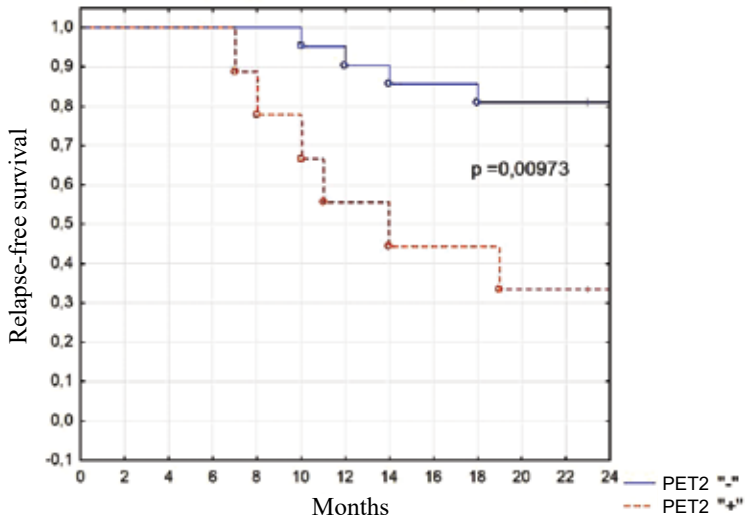
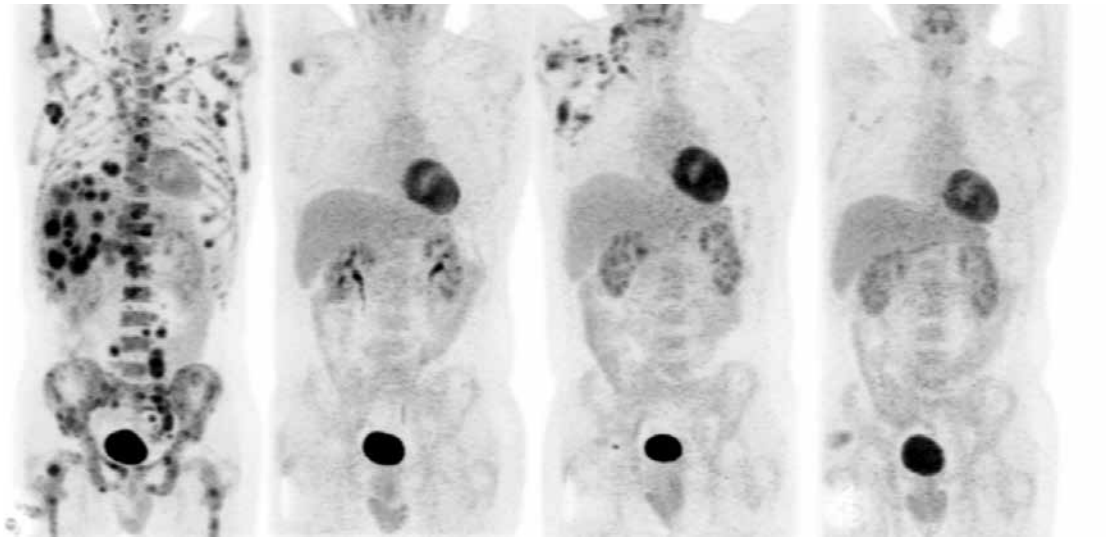


Fig. 5. Two-year relapse-free survival in patients with lymphomas with (PET2–) and without (PET2+) a metabolic response to two cycles of chemotherapy



a *b* *c* *d*

Fig. 6. Patient K., 31 years old. Diagnosis: Hodgkin's lymphoma with damage to peripheral lymph nodes and skin: *a* – PET1: signs of lymphoproliferative disease with multiple metabolically active lesions of the liver, lymph nodes above and below the diaphragm, and bone marrow; *b* – PET2: signs of lymphoproliferative disease with a metabolically active lesion of the acromial process of the right scapula. Compared to PET1 – positive changes; *c* – PET3: metabolic activity in the cervical, supraclavicular, and axillary lymph nodes on the right, a single inguinal lymph node on the right, in the acromial process of the right scapula. Compared to PET2 – negative changes; *d* – PET4: a metabolically active lesion in single right axillary lymph nodes, regarded as a manifestation of Hodgkin's lymphoma. Compared to PET3 – positive changes

REFERENCES

1. Kaprin A.D., Starinskiy V.V., Petrova G.V. Malignant neoplasms in Russia in 2016 (morbidity and mortality). Moscow: P. Hertsen Moscow Oncology Research Institute of the NMRC Radiology of the Ministry of Health of the Russian Federation, 2018: 250 (in Russ.).
2. Rukavicin O.A. Hematology: national guidelines. Moscow: Geotar-Media. 2015: 912 (in Russ.).
3. Zucca E., Copie-Bergman C., Ricardi U., Thieblemont C., Raderer M., Ladetto M. Gastric marginal zone lymphoma of MALT type: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. *Annals of Oncology*. 2013; 24: 144–148. DOI: 10.1093/annonc/mdt343.
4. Chernov V.I., Dudnikova E.A., Gol'dberg V.E., Kravchuk T.L., Danilova A.V., Zel'chan R.V., Medvedeva A.A., Sinilkin I.G., Bragina O.D., Popova N.O., Gol'dberg A.V. Positron emission tomography in the diagnosis and monitoring of lymphoproliferative diseases. *Medical Radiology and Radiation Safety.* 2018; 6 (63): 41–50 (in Russ.). DOI: 10.12737/article_5c0b8d72a8bb98.40545646.
5. Chanchikova N.G., Dudnikova E.A., Karlova E.A., Savel'eva A.S., Silkina O.A., Zel'chan R.V., Sinilkin I.G., Bragina O.D., Medvedeva A.A., Chernov V.I. Possibilities of PET/CT with ^{18}F-FDG in the diagnosis and staging of lymphomas. *Problems in Oncology.* 2019; 65 (1):147–153 (in Russ.).
6. Chernov V.I., Dudnikova E.A., Zelchan R.V., Kravchuk T.L., Danilova A.V., Medvedeva A.A., Sinilkin I.G., Bragina O.D., Goldberg V.E., Goldberg A.V., Frolova I.G. The first experience of using 99mTc-1-thio-d-glucose for single-photon emission computed tomography imaging of lymphomas. *Siberian Journal of Oncology*. 2018; 17 (4): 81–87. DOI: 10.21294/1814-4861-2018-17-4-81-87.
7. Chernov V.I., Dudnikova E.A., Gol'dberg V.E., Kravchuk T.L., Danilova A.V., Zel'chan R.V., Medvedeva A.A., Sinilkin I.G., Bragina O.D., Belevich Ju.V., Koroleva E.S. Single-photon emission computed tomography in the diagnosis and monitoring of lymphoproliferative diseases. *Medical Radiology and Radiation Safety.* 2019; 64(3): 58–63 (in Russ.). DOI: 10.12737/article_5c0b8d72a8bb98.40545646.
8. Zelchan R., Chernov V., Medvedeva A., Sinilkin I., Stasyuk E., Rogov A., Il'ina E., Skuridin V., Bragina O. Study of a glucose derivative labeled with technetium-99m as potential radiopharmaceutical for cancer diagnosis EP634. *Eur. J. Nucl. Med. Mol. Imaging.* 2016; 43 (1): S466.
9. Sasaki M., Kuwabara Y., Koga H., Nakagawa M., Chen T., Kaneko K., Havashi K., Nakamura K., Masuda K. Clinical impact of whole body FDG-PET on the staging and therapeutic decision making for malignant lymphoma. *Annals of Nuclear Medicine.* 2002; 16 (5): 337–345. DOI: 10.1007/BF02988618.
10. Poddubnaya I.V., Savchenko V.G. Russian clinical guidelines for the diagnosis and treatment of lymphoproliferative diseases. Moscow: MediaMedica, 2013:104 (in Russ.).
11. Cheson B.D., Fisher R.I., Barrington S.F., Cavalli F., Schwartz L.H., Zucca E., Lister T.F. Recommendations for initial evaluation, staging, and response assessment of Hodgkin and non-Hodgkin lymphoma: the Lugano classification. *Journal of Clinical Oncology.* 2014; 32 (27): 3059–3068. DOI: 10.1200/JCO.2013.54.8800.
12. Romer W., Hanauske A.R., Ziegler S., Thodtmann R., Weber W., Fuchs C., Enne W., Herz M., Nerl C., Garbrecht M., Schwaiger M. Positron emission tomography in non-Hodgkin's lymphoma: assessment of chemotherapy with fluorodeoxyglucose. *Blood*. 1998; 91 (12): 4464–4471.
13. Radford J., Illidge T., Counsell N., Hancock B., Pettengell R., Johnson P., Wimperis J., Culligan D., Popova B., Smith P., McMillan A., Brownell A., Kruger A., Lister A., Hoskin P., O'Doherty M., Barrington S. Results of a Trial of PET-Directed Therapy for Early-Stage Hodgkin's Lymphoma. *New England Journal of Medicine*. 2015; 372 (17): 1598–1607. DOI: 10.1056/NEJMoa1408648.
14. Kostakoglu L., Cheson B.D. State-of-the-art research on "Lymphomas: Role of molecular imaging for staging, prognostic evaluation, and treatment response". *Front. Oncol.* 2013; 3: 212. DOI: 10.3389/fonc.2013.00212.
15. Dreyling M., Ghielmini M., Marcus R., Salles G., Vitolo U., Ladetto M., ESMO Guidelines Working Group. Newly diagnosed and relapsed follicular lymphoma: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. *Annals of Oncology.* 2014; 25 (3): 76–82. DOI: 10.1093/annonc/mdu200.
16. Gallamini A., Barrington S.F., Biggi A., Chauvie S., Kostakoglu L., Gregianin M., Meignan M., Mikhaeel G.N., Loft A., Zaucha J.M., Seymour J.F., Hofman M.S., Rigacci L., Pulsoni A., Coleman M., Dann E.J., Trentin L., Casasnovas O., Rusconi C., Brice P., Bolis S., Viviani S., Salvi F., Luminari S., Hutchings M. The predictive role of interim positron emission tomography for Hodgkin lymphoma treatment outcome is confirmed using the interpretation criteria of the Deauville five-point scale. *Haematologica.* 2014; 99 (6): 1107–1113. DOI: 10.3324/haematol.2013.103218.
17. Biggi A., Gallamini A., Chauvie S., Hutchings M., Kostakoglu L., Gregianin M., Meignan M., Malkowski B., Hofman M.S., Barrington S.F. International validation study for interim PET in ABVD-treated, advanced-stage hodgkin lymphoma: interpretation criteria and concordance rate among reviewers. *J. Nucl. Med.* 2013; 54 (5): 683–690. DOI: 10.2967/jnumed.112.110890.
18. Markova J., Kahraman D., Kobe C., Skopalova M., Mocikova H., Klaskova K., Dedeckova K., Eich H., Böll B., Dietlein M., Kozak T. Role of [18F]-fluoro-2-deoxy-D-glucose positron emission tomography in early and late therapy assessment of patients with advanced Hodgkin lymphoma treated with bleomycin, etoposide, adriamycin, cyclophosphamide, vincristine, procarbazine and prednisone. *Leuk. Lymphoma*. 2012; 53 (1): 64–70. DOI: 10.3109/10428194.2011.603444.

Authors contribution

Chanchikova N.G. – final approval of the manuscript for publication. Chernov V.I. – conception and design. Dudnikova E.A., Zelchan R.V., Bragina O.D., Medvedeva A.A., Berezneeva E.V. – analysis and interpretation of data. Karlova E.A., Savelyeva A.S., Silkina O.A. – substantiation of the manuscript, critical revision of the manuscript for important intellectual content.

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Pathogenetic factors of ulcerative colitis: mainstream for 2020

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ABSTRACT

The causes of ulcerative colitis are still unknown. Scientists made important advances in understanding the pathogenesis of this disease in the 21st century. Complex involvement of an impaired immune response in relation to antigens of the intestinal microbiota in genetically predisposed individuals under the influence of certain environmental factors was revealed. The factors that disrupt the epithelial barrier and alter the composition of the intestinal microbiota trigger the onset of the disease, thereby stimulating an impaired immune response. Recent studies have discovered completely new hypotheses of its origin and development, gradually interpreting the already known pathogenetic mechanisms of the disease. In this review, we focused on the new concepts in the pathogenesis of ulcerative colitis. We examined genetic, environmental, barrier, and microbial factors. We went into detail on the structure and role of the epithelial barrier and identified specific genes that are involved in the regulation of the intestinal epithelial barrier function in ulcerative colitis. We studied the literature containing information on relevant studies in PubMed and Google Scholar citation systems, using such key words as ulcerative colitis, colon microbiota, barrier function, genetic predisposition, and predisposing factors.

Key words: ulcerative colitis, inflammatory bowel disease, microbiota, pathogenesis, predisposing factors, genetic predisposition.

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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Факторы патогенеза язвенного колита: мейнстрим-2020

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РЕЗЮМЕ

Причины возникновения язвенного колита до сих пор неизвестны. Значительные успехи в понимании патогенеза этого заболевания достигнуты в XXI в. и доказывают комплексное участие нарушенного иммунного ответа по отношению к антигенам собственной кишечной микрофлоры у генетически предрасположенных лиц под воздействием определенных факторов внешней среды. Дебют заболевания провоцируется факто-

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рами, которые нарушают эпителиальный барьер и изменяют состав микробиоты кишечника, тем самым стимулируя аномальный иммунный ответ. Исследования последних лет открывают как абсолютно новые гипотезы его возникновения и развития, так и подробно расшифровывают уже известные механизмы патогенеза болезни. В представленном обзоре мы сосредоточились на новых концепциях патогенеза язвенного колита – генетических, экологических, барьерных и микробиомных факторах. Подробно представили строение и роль эпителиального барьера, обозначили специфические гены, которые участвуют в регуляции барьерной функции эпителия кишечника при язвенном колите. Поиск литературы, содержащей информацию о соответствующих исследованиях, проводился в системах PubMed и Google Scholar по следующим ключевым словам: язвенный колит, микробиота толстой кишки, барьерная функция, генетическая предрасположенность, предрасполагающие факторы.

Ключевые слова: язвенный колит, воспалительные заболевания кишечника, микробиота, патогенез, предрасполагающие факторы, генетическая предрасположенность.

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

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INTRODUCTION

The first description of ulcerative colitis (UC) was presented by Samuel Wilks in 1859 under the title “Morbid appearances in the intestine of Miss Bankes” in the Medical Times and Gazette. In 1875, S. Wilks, together with V. Moxon, described morphological presentation of this disease [1]. Despite a long history of studying UC, the causes of this disorder still remain unknown. The generally accepted modern concept of UC development includes genetic predisposition, epithelial barrier defects, dysregulation of immune responses, intestinal dysbiosis, and environmental factors [2].

The number of patients with inflammatory bowel diseases (IBD) is increasing every year and may reach 30 million people in the world by 2025 [3, 4]. In Russia, the incidence of UC is 2–3 cases per 100 thousand people [5]. High incidence of IBD in economically developed countries is explained by a combination of such factors as improvement of socioeconomic and sanitary conditions in modern society, changes in diet, availability of endoscopic examination, and the level of awareness among both patients and doctors about this medical condition [6]. Epidemiological studies reveal an increase in the incidence of UC in regions where it was at low level before, and where the Western way of life and nutrition is gradually predominating, such as countries of Asia and South America [7].

A tremendous interest of scientists from all over the world in the study of UC has been persistent for many years and requires large investments. To date, progress has been made in understanding this disease, new hypotheses of its emergence have appeared, and the mechanisms of the pathogenesis have been gradually unveiled. For example, it has been proven that the appendectomy at a young age has protective effects against UC development, given the surgery was performed for acute appendicitis [8]. In addition, based on the results of a meta-analysis of four studies, it has been recently discovered that there is an association of IBD with Parkinson’s disease. The studies showed that the risk of developing Parkinson’s disease in IBD patients was significantly higher than in the control group [9]. Moreover, the risk of developing Parkinson’s disease in patients with UC was 30%, and in patients with Crohn’s disease (CD), it was 28%.

Recent studies have been aimed at identifying new targets of etiotropic and pathogen-specific drug therapy to increase the effectiveness of treatment. Emergence of new treatment methods, such as immunosuppressive and biological therapy, resulted in significant pathomorphosis of UC. The disease is aggravating even with appropriate treatment, resulting in development of life-threatening complications, a continuously relapsing course, and universal forms in the majority of patients. The aim of this review was to summarize the data that form the modern understanding of the pathogenetic mechanisms in UC.

GENETIC FACTORS

Genetic studies, including genome-wide association studies (GWAS), whole genome sequencing (WGS), and genetic mapping, have identified 260 susceptibility loci associated with IBD [10–13]. Conventionally, genes that are involved in the development of UC can be divided into the following groups: genes encoding an immune response; genes encoding intestinal barrier function (the so-called barrier genes); genes encoding the quantitative and qualitative composition of the intestinal microbiota. The examination of patients using modern methods of genetic testing allowed scientists to come to the following conclusions.

Firstly, most of the genetic factors were found to be common for UC and CD [14]. Genes encode both innate and adaptive immune pathways, cytokine signaling, and immune response (for example, IL23-R, IL-12, JAK2, CARD9, TNFSF18, and IL-10). In addition, many genes (70%) are associated with other autoimmune diseases, such as ankylosing spondylitis and psoriasis.

Secondly, the strongest genetic signals within UC-specific loci are associated with the human leukocyte antigen (HLA) region on chromosome six. Sixteen HLA allelic associations (mainly class II), specific for UC, were described in genetic mapping [15]. It is known that many UC-specific genes are involved in the regulation of the epithelial barrier function. Studies showed that they are associated with colon involvement in UC and CD [16]. This indicates the key role of aberrant adaptive immune responses and epithelial barrier dysfunction in the pathogenesis of UC.

A group of scientists from Belgium conducted a study in 2017 [17], which analyzed various components of the intestinal epithelial barrier in IBD patients in terms of genetic predisposition. 128 genes associated with epithelial dysfunction were selected, of which 25 were associated with the mucous layer, 34 – with tight junction proteins, 5 – with adherens junctions, 14 – with desmosomes (intercellular junctions), 4 – with hemidesmosomes (half desmosomes), 17 – with cytoskeleton, 9 – with extracellular matrix, and 20 – with regulatory proteins. Analysis of the barrier genes revealed the potential role of *MUC21*, *MUC22, GNA12*, and *HNF4A* genes and loci in the emergence of UC. In the inactive phase of the disease, a persistent change in the expression of *MUC1* and *MUC4* in biopsies of patients with IBD was found, which served as an evidence of their crucial role in the recurrence of IBD (Figure).

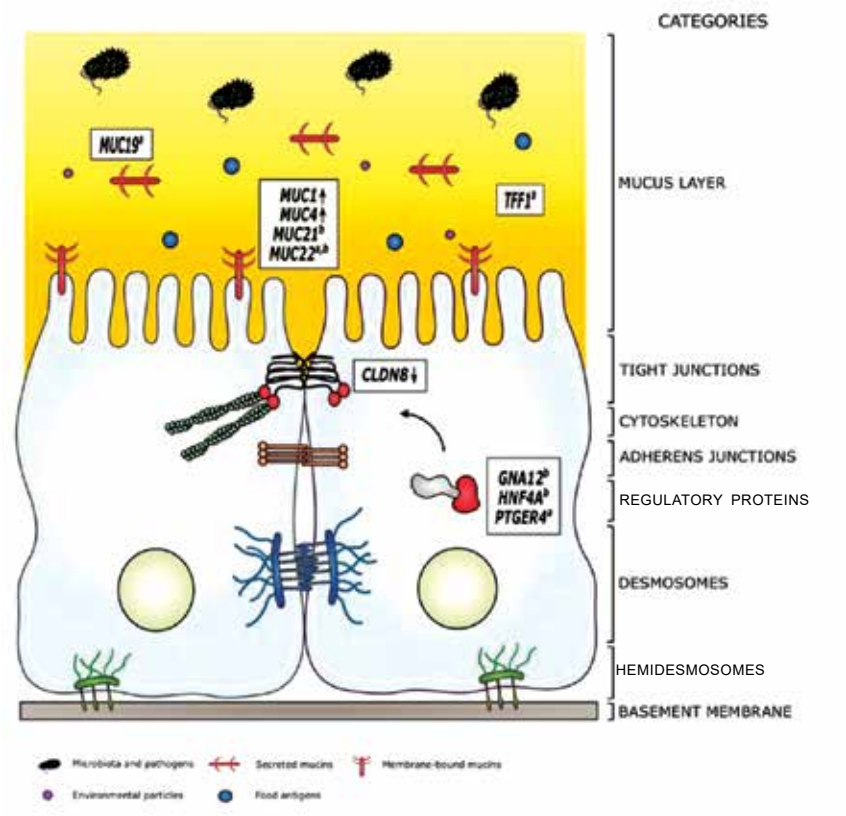

Figure. The role of epithelial barrier genes in the onset of IBD

One of the latest significant discoveries was whole genome sequencing (WGS) performed in almost 2,000 UC patients, which revealed a new and rare variant of a missense mutation (present in 0.6% of cases) in the adenylyl cyclase 7 (*ADCY7*) gene, which doubles the risk of UC development [18].

Thirdly, despite the identification of many susceptibility loci, genetics explains only 8–19% of disease heritability in IBD [19], CD being more common than UC. The concordance rate among monozygotic twins in UC is only 6.3% (compared to almost 60% in CD). Taken together, genetic factors provide a small but definite increase in susceptibility to UC. However, many patients do not have a genetic predisposition, if they are assessed using a polygenic risk score that takes into account all susceptibility loci [20].

Fourthly, non-genetic factors, in particular, epigenetics, which will be discussed below, play an important role in UC emergence [21].

EPIGENETICS

As mentioned above, genetic factors do not explain the occurrence of UC in all patients. In recent years, susceptibility to IBD has been supplemented by new data on epigenetic reprogramming. In response to external stimuli, such as nutrition, psychological stress, and physical activity, this mechanism gives commands to genes to increase or, on the contrary, weaken their activity. Thus, epigenetics studies the changes in gene activity, while the DNA structure remains the same. The main epigenetic mechanisms that control gene expression are DNA methylation, histone modification, and noncoding RNAs [22].

Changes in DNA methylation of the gene promoters are functionally involved in the regulation of gene expression in patients with IBD, mainly with UC. This provided a new look at the pathogenesis of the disease. The first studies concerning epigenetics in IBD were devoted to carcinogenesis and the development of neoplasia in patients [23]. It was proven that a higher level of DNA methylation in *AGTR1*, *WNT2*, and *SLIT2* genes was associated with an increased risk of cancer in patients with UC [24].

A number of studies in this area demonstrate that it is the DNA methylation landscape in genes that determines the severity and nature of IBD [25, 26]. Epigenetic changes are correlated with clinical features and outcomes of IBD, such as the extent of the lesion and the phenotype of the disease. For example, a higher level of *MDR1* methylation was independently associated with universe UC, severe attacks of the disease, and young age of the disease onset [27], while a higher level of *PAR2* methylation in the rectal mucosa was associated with the steroid-dependent or steroid-refractory UC [28].

Nevertheless, there are no convincing and unambiguous data of evidence-based medicine on the influence of epigenetics on the emergence and nature of IBD, since there are many technical difficulties in reproducing the sequences and heterogeneity in the analyzed population (the sampling technique and the studied material differ). For the same reasons, it is not yet possible to conduct meta-analyses on this topic.

INFLUENCE OF ENVIRONMENTAL FACTORS

An increase in the number of UC patients occurs in parallel with changes in lifestyle [29] and basic approaches to nutrition in modern society, namely, widespread use of convenience foods, high-calorie foods, taste modifiers, animal proteins, sugar and refined carbohydrates, artificial sweeteners, a variety of modern cooking and food preservation technologies, emulsifiers, and a lack of dietary fiber in the diet [30]. The general concept of UC association with nutrition is based on data of epidemiological studies and is referred to as westernization of the diet.

Westernization also concerns living conditions and lifestyle in general, for example, the impact of environmental pollution, the availability of antibacterial drugs, and a decrease in physical activity [31]. One of the theories explaining a rise in autoimmune diseases in general and UC, in particular, is the hygiene hypothesis formulated by the English epidemiologist David Strachan [32]. This concept reveals the possibility of an excessive immune response and development of autoimmune diseases following a decrease in antigenic exposure due to improvement of the sanitary conditions in the environment and widespread use of antibacterial drugs and detergents.

It was suggested that stress can initiate or induce a new attack of IBD and is a potential trigger of UC [33]. This association is supported by numerous studies that showed that stressful events in a person's life can trigger a disease [34, 35]. Psychological stressors increase intestinal permeability, weaken tight junctions, and increase bacterial translocation into the intestinal wall.

Finally, scientists have been studying the protective effect of tobacco smoking on the occurrence of UC for a long time. The likelihood of UC occurrence in nonsmokers is higher than in smokers. When giving up smoking, the relative risk of UC development increases by 4.4 times [36].

CHANGE IN THE INTESTINAL MICROBIOTA

A combination of various lifestyle factors in the era of postindustrial society has a significant impact on the microbial composition of the intestine and leads to a change in its diversity in UC. A group of scientists from the United States proved that under the influence of triggers, in particular, emulsifiers, the intestinal mucosal barrier decreases, increasing the number of microbes with proinflammatory and mucolytic activity, resulting in the development of inflammation and emergence of IBD, metabolic syndrome, and, possibly, other chronic inflammatory diseases [37].

A study in 2010 showed significant differences in gut microbiota in children living in rural communities of Burkina Faso compared to children living in Europe. Gut microbiota of African children was rich in *Bacteroides* and poor in *Firmicutes* and *Enterobacteriaceae*, while the results obtained from European children were opposite [38]. With a high probability, it can be assumed that this situation is determined by dietary habits. In Africa, foods with high fiber content prevail, and in Europe, the traditional Western diet prevails.

An important characteristic of gut microbiota of each person is its individual variability, due to genetic predisposition. Based on molecular analysis by 16s RNA sequencing, it was found that gut microbiota is represented by four known types of bacteria: *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, and *Proteobacteria*. In adults, two types of bacteria are predominant: *Bacteroidetes* and *Firmicutes* [39]. Research data were published in 2017 [40], revealing a decrease in the diversity of fungal and bacterial components of microbiota in IBD patients; and in patients with UC, these changes were more pronounced than in patients with CD. Patients with UC exhibit a decrease in the proportion of microorganisms with anti-inflammatory activity that synthesize short-chain fatty acids (SCFAs), such as *Firmicutes*, and an increase in the proportion of pathobionts, which include *Proteobacteria*. Within the phylum *Firmicutes*, the proportion also changes: *Roseburia* and *Faecalibacterium* of the *Ruminococcacceae* and *Lachnospiraceae* families decrease and the content of *Ruminococcus gnavus* increases [41]. An increase in the content of opportunistic bacteria *Enterobacteraceae* and *Esherichia coli* is noted within the phylum *Proteobacteria* [42]. Other studies demonstrate an increase in the content of sulfate-reducing bacteria *Desulfo vibrio* with excessive production of hydrogen sulfide, which negatively affects proliferation, apoptosis, and differentiation of colonic epithelial cells [43].

In addition to bacteria, microbiota of the colon consists of viruses, fungi, and archaea, which are also an essential part of the intestinal microbial composition [44]. Archaea account for up to 10% of all anaerobes inhabiting the colon of a healthy person. Studies show their positive effect on human health [45], while previous studies demonstrated their proinflammatory effect via stimulating the growth of pathogenic bacteria [46]. Gut microbiota also consists of viruses. The quantitative and qualitative composition of the virome also depends on the prevailing food products in the diet, place of residence, hygiene, environmental factors, and the type of breastfeeding [47]. In a healthy person, bacteriophages persist in bacterial hosts, maintaining the constancy of the internal state of gut microbiota. Under the influence of environmental factors in genetically predisposed individuals, or following a combination of a eukaryotic virus and a genome of a macroorganism, activation of phages (in the latent period) and viruses takes place, which leads to a disturbance of the dynamic balance in the microbial composition of the gastrointestinal tract (GIT).

A number of aggression factors, such as a disturbance of the intestinal microbiome composition and the presence of aggressive intestinal metabolites, lead to dysfunction of mucosal permeability, disrupting its barrier function, which is normally determined by the state of tight junctions with the help of claudins, as well as by the content and quality of mucin that protects the epithelium [48]. When defects of the mucous membrane emerge, food and bacterial agents can penetrate into deeper layers of the intestinal wall, which then stimulate development of inflammatory and immune responses [49].

In recent years, fecal microbiota transplantation (FMT) has been performed to restore intestinal homeostasis in patients with UC. A study by P. Moayyedi et al. [50] showed that FMT induces remission in patients with active UC. A total of 70 UC patients underwent FMT or received a water enema (placebo) every week for 6 weeks. The remission rate in the FMT group was significantly higher than in the placebo group (24% versus 5%, respectively). Meta-analysis of 14 cohort and 4 randomized clinical trials (308 patients with UC) by S. Costello et al. [51] demonstrated the effectiveness of UC treatment with a clinical remission rate of 28% in patients undergoing FMT, compared to a 9% remission rate in patients receiving placebo. In addition, a clinical response was achieved

in 49% of patients treated with FMT compared to 28% of patients receiving placebo.

EPITHELIAL BARRIER

Scientists agreed that disruption of the epithelial barrier is the underlying factor in the pathogenesis of UC. Given complex organization and regulation of the intestinal mucosal barrier, it is necessary to determine which elements are most important for the pathophysiology of IBD.

The intestinal barrier function is provided by a complex of components that combine mucosal, epithelial, and immune (innate and adaptive) barriers. The mucosal barrier is a double layer. Colonic mucus contains more bacteria in the thinner outer layer than in the denser inner mucosal layer. The parietal mucosal layer contains secretory immunoglobulins A and antibacterial substances (defensins, lysozyme, and ribonuclease). The mucosal layer provides the first apical line of defense against luminal microbes and forms a sieve-like gel structure that prevents large particles and bacteria from contacting the intestinal epithelium [52].

Thanks to the almost impermeable polarized monolayer of intestinal epithelial cells, a second physical component of the intestinal barrier is formed. Enterocytes, the most represented type of colonic epithelial cells, are interconnected by intercellular junctions, represented by catenins, occludins, and claudins. Tight junctions are the main gatekeepers of the epithelial intestinal barrier, which can pass ions and small molecules up to 20 kDa. The throughput capacity of tight junctions depends on the state of proteins, mainly, claudins [53].

In addition to enterocytes, the epithelium is composed of other specialized cells with a wide range of functions, including goblet cells, which produce gel-like mucus, and Paneth cells, which secrete antimicrobial peptides that strengthen the immune barrier. M cells, which are also a part of the epithelium, capture luminal microbes and transport them to dendritic cells, which recognize the absorbed microorganisms and form an immune response [54].

In UC, dysfunction of antimicrobial peptide secretion and disruptions of tight junctions (the physical component of the barrier) are observed [55]. In active UC, key proinflammatory cytokines, such as tumor necrosis factor-α (TNF-α), interferon (IFN) -γ, and interleukin (IL)-13, have a direct pathological effect on the integrity of the epithelial barrier [56]. Genome-wide association study (GWAS) reveals UC-specific susceptibility genes that regulate the epithelial barrier, mucus production, and stability of the membrane and intercellular junctions. However, the main mechanism has not yet been fully understood.

MITOCHONDRIAL DYSFUCTION

The PROTECT study (analysis of complete genomic sequencing of 206 children with a short history of UC) is highly interesting. It demonstrated a decrease in the expression of genes encoding an oxidative phosphorylation chain in mitochondria and a polymorphism of the *PPARGC1A* gene, which affects the activation of the mitochondrial function. Thus, mitochondriopathy was determined as one of the possible mechanisms in the pathogenesis of UC [57]. The role of mitochondrial dysfunction was discussed previously in the pathogenesis of this disease [58]. For the past 10 years, researchers have identified mitochondriopathy as one of the main and most poorly understood “pieces of the puzzle” in the genesis of inflammation [59]. The data obtained over the past 3 years again revive and confirm this concept in the pathogenesis of UC [60–62].

CONCLUSION

The presented literature review summarizes current research on the etiology and pathogenesis of UC (Table).

Table

Modern concepts of etiology and pathogenesis of UC

Parameter	Description
Genetics	Most genetic factors (67% of the susceptibility loci) are common for UC and CD. Sixteen HLA allelic associations have been described for UC (mainly class II). Outside the HLA region, the *ADCY7* gene has the strongest association with UC. UC-specific genes are involved in the regulation of the intestinal epithelial barrier function. Many patients do not have a genetic predisposition, according to a polygenic risk scale that takes into account all susceptibility loci (6.3% in monozygotic twins)
Environmental factors	Westernization includes urban lifestyle, environmental pollution, dietary habits, antibiotics, improved sanitation and fewer infections. Smoking is a protective factor against UC. Giving up smoking often precedes UC. Appendectomy reduces the risk of UC development, if the surgery was performed for acute appendicitis at a young age

Table (continued)

Parameter	Description
Microbiota	A decrease in gut microbiota diversity (viruses, bacteria, and fungi). Fecal microbial transplantation is effective in treating UC. It is not known whether a disturbance in the composition of microbiota is a consequence or an initiator of inflammation. Depletion of microbes with anti-inflammatory activity (*Ruminococcaceae* and *Lachnospiraceae*) and an increase in microbes with proinflammatory activity (*Enterobacteriaceae* and *Fusobacteriaceae*)
Epithelial barrier	Disruption of the epithelial barrier is a key mechanism in the pathogenesis of UC. Barrier function of the intestine is provided by a number of components that combine mucosal, epithelial (physical), and immune (innate and adaptive) barriers
Mitochondria	Mitochondriopathy is one of the mechanisms in the pathogenesis of UC. Mitochondriopathy leads to impaired energy production, increased oxidative stress, and release of molecular patterns associated with a proinflammatory response

A modern lifestyle of people with a genetic predisposition has a significant effect on the microbial composition of the intestine and leads to a change in the diversity of the intestinal microbiota in UC, a decrease in the resident flora, and an increase in the number of opportunistic and pathogenic microorganisms. A number of aggression factors, such as disturbances of the intestinal microbiome composition and the presence of aggressive intestinal metabolites, impair mucosal permeability and disturb the barrier function, which is normally determined by the state of tight junctions, as well as by the amount and quality of mucin that protects the epithelium. Food and bacterial agents can penetrate into deeper layers of the intestinal wall through the defects in the mucous membrane, which then stimulate the development of inflammatory and immune responses [63].

REFERENCES

1. Mulder D.J., Noble A.J., Justinich C.J. et al. A tale of two diseases: the history of inflammatory bowel disease. *Journal of Crohn's and Colitis*. 2014; 8 (5): 341–348. DOI: 10.1016/j.crohns.2013.09.009.
2. Ungaro R., Mehandru S., Allen P. et al. Ulcerative colitis. *Lancet.* 2017; 389 (10080): 1756–1770. DOI: 10.1016/s0140-6736(16)32126-2.
3. Kaplan G.G. The global burden of IBD: from 2015 to 2025. *Nature Reviews Gastroenterology & Hepatology.*2015; 12 (12): 720–727. DOI: 10.1038/nrgastro.2015.150.
4. Ramos G., Papadakis K. Mechanisms of disease: inflammatory bowel diseases. *Mayo Clin. Proc.* 2019; 94 (1): 155–165. DOI: 10.1016/j.mayocp.2018.09.013.
5. Nikitin A.V., Khavkin A.I., Skvortsova T.A., Volynets G.V., Atameeva A.O. Combination of ulcerative colitis with cirrhosis of the liver in the outcome of primary sclerosing cholangitis. *Experimental and Clinical Gastroenterology*. 2020; 174 (5): 104–107 (in Russ.). DOI: 10.31146/1682-8658-ecg-177-5-104-107.
6. Shouval D., Rufo P. The role of environmental factors in the pathogenesis of inflammatory bowel diseases: are view. *JAMA Pediatrics*. 2017; 171 (10): 999–1005. DOI: 10.1001/jamapediatrics.2017.2571.
7. Ng S. Epidemiology of inflammatory bowel disease: focus on Asia. *Best Practice & Research Clinical Gastroenterology*. 2014; 28 (3): 363–372. DOI: 10.1016/j.bpg.2014.04.003.
8. Nyboe Andersen N., Gørtz S., Frisch M. et al. Reduce drisk of UC in families affected by appendicitis: a Danish national cohort study. *Gut.* 2017; 66 (8): 1398–1402. DOI: 10.1136/gutjnl-2015-311131.
9. Zhu F., Li C., Gong J. et al. The risk of Parkinson's disease in inflammatory bowel disease: A systematic review and meta-analysis. *Digestive and Liver Disease.* 2019; 51 (1): 38–42. DOI: 10.1016/j.dld.2018.09.017.
10. Liu J.Z., van Sommeren S., Huang H. et al. Association analyses identify 38 susceptibility loci for inflammatory bowel disease and highlight shared genetic riska cross populations. *Nature Genetics*. 2015; 47 (9): 979–986. DOI: 10.1038/ng.3359.
11. Luo Y., de Lange K.M., Jostins L. et al. Exploring the genetic architecture of inflammatory bowel disease by whole-genome sequencing identifies association at *ADCY7*. *Nature Genetics.* 2017; 49 (2): 186–192. DOI: 10.1038/ng.3761.
12. Huang H., Fang M., Jostins L. et al. Fine-mapping inflammatory bowel disease loci to single-variant resolution. *Nature.* 2017; 547 (7662): 173–178. DOI: 10.1038/nature22969.
13. Makeykina M.A., Livzan M.A. Genetic prognostic factors of course of nonspecific ulcerative colitis. *Practical Medicine.* 2012; 9 (65): 133–136 (in Russ.).
14. Jostins L., Ripke S., Weersma R.K. et al. Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. *Nature.* 2012; 491 (7422): 119–124. DOI: 10.1038/nature11582.
15. Goyette P., Boucher G., Mallon D. et al. High-density mapping of the MHC identifies a shared role for HLA-DRB1*01:03 in inflammatory bowel diseases and heterozygous advantage in ulcerative colitis. *Nature Genetics*. 2015; 47 (2): 172–179. DOI: 10.1038/ng.3176.
16. Cleynen I., Boucher G., Jostins L. et al. Inherited determinants of Crohn's disease and ulcerative colitis phenotypes: a genetic association study. *Lancet.* 2016; 387 (10014): 156–167. DOI: 10.1016/S0140-6736(15)00465-1.
17. Vancamelbeke M., Vanuytsel T., Farré R., Verstockt S., Ferrante M., Van Assche G., Rutgeerts P., Schuit F., Vermeire S.,

Arijs I., Cleynen I. Genetic and transcriptomic basis of intestinal epithelial barrier dysfunction in inflammatory bowel disease. *Inflammatory Bowel Diseases*. 2017; 23 (10): 1718–1729. DOI: 10.1097/MIB.0000000000001246.
18. Luo Y., de Lange K.M., Jostins L. et al. Exploring the genetic architecture of inflammatory bowel disease by whole-genome sequencing identifies association at ADCY7. *Nature Genetics.*2017; 49 (2): 186–192. DOI: 10.1038/ng.3761.
19. Chen G.B., Lee S.H., Brion M.J. et al. Estimation and partitioning of (co)heritability of inflammatory bowel disease from GWAS and immuno chip data. *Human Molecular Genetics.* 2014; 23 (17): 4710–4720. DOI: 10.1093/hmg/ddu174.
20. Lee H.S., Cleynen I. Molecular profiling of inflammatory bowel disease: is it ready for use in clinical decision-making. *Cells.* 2019; 8 (6): e535. DOI: 10.3390/cells8060535.
21. Kalla R., Ventham N.T., Kennedy N.A. MicroRNAs: new players in inflammatory bowel disease. *Gut.* 2015; 64 (6): 1008. DOI: 10.1136/gut.2014.307891corr1.
22. Ventham N.T., Kennedy N.A. Nimmo E.R. et al. Beyond gene discovery in inflammatory bowel disease: the emerging role of epigenetics. *Gastroenterology.* 2013; 145 (2): 293–308. DOI: 10.1053/j.gastro.2013.05.050.
23. Azuara D., Aussó S., Rodriguez-Moranta F. et al. New Methylation biomarker panel for early diagnosis of dysplasia or cancer in high-risk inflammatory bowel disease patients. *Inflammatory Bowel Diseases*. 2018; 24 (12): 2555–2564. DOI: 10.1093/ibd/izy255.
24. Koizumi K., Alonso S., Miyaki Y. et al. Array-based identification of common DNA methylation alterations in ulcerative colitis. *International Journal of Oncology*. 2012; 40 (4): 983–994. DOI: 10.3892/ijo.2011.1283.
25. Häsler R., Feng Z., Bäckdahl L. et al. A functional methylome map of ulcerative colitis. *Genome Research.* 2012; 22 (11): 2130–2137. DOI: 10.1101/gr.138347.112.
26. Cooke J., Zhang H., Greger L. et al. Mucosal genome-wide methylation changes in inflammatory bowel disease. *Inflammatory Bowel Diseases*. 2012; 18 (11): 2128–2137. DOI: 10.1002/ibd.22942.
27. Tahara T., Shibata T., Nakamura M. et al. Effect of MDR1 gene promoter methylation in patients with ulcerative colitis. *International Journal of Molecular Medicine.* 2009; 23 (4): 521–527. DOI: 10.3892/ijmm_00000160.
28. Tahara T., Shibata T., Nakamura M. et al. Promoter methylation of protease-activated receptor (PAR2) is associated with severe clinical phenotypes of ulcerative colitis (UC). *Clinical and Experimental Medical Sciences*. 2009; 9 (2): 125–130. DOI: 10.1007/s10238-008-0025-x.
29. Kaplan G. The global burden of IBD: From 2015 to 2025. *Nature Reviews Gastroenterology & Hepatology.* 2015; 12: 720–727. DOI: 10.1038/nrgastro.2015.150.
30. Bicbavova G.R., Livzan M.A., Sovalkin V.I., Turchaninov D.V., Lopatina O.E., Tretyakova T.V., Ahramovich A.P., Gordienko T.S. Modern lifestyle and its impact on the ulcerative colitis incidence. *The Russian Archives of Internal Medicine.* 2019;3: 188–194 (in Russ.).
31. Kaplan G., Ng S. Understanding and preventing the global increase of inflammatory bowel disease. *Gastroenterology*.2017; 152 (2): 313–321. DOI: 10.1053/j.gastro.2016.10.020.
32. Strachan D.P., Taylor E.M., Carpenter R.G. Family structure, neonatal infection, and hayfever in adolescence. *Archives of Disease in Childhood.* 1996; 74 (5): 422–426. DOI: 10.1136/adc.74.5.422.
33. Galley J.D., Nelson M.C., Yu Z., Dowd S.E., Walter J., Kumar P.S. et al. Exposure to a social stressor disrupts the community structure of the colonic mucosa-associated microbiota. *BMC Microbiology.* 2014; 14: 189. DOI: 10.1186/1471-2180-14-189.
34. Bicbavova G.R, Livzan M.A, Sovalkin V.I, Turchaninov D.V, Akhramovich A.P. Is psychological stress a risk factor for ulcerative colitis? *Russian Journal of Evidence-Based Gastroenterology.*2019; 8 (2): 37–42 (in Russ.). DOI: 10.17116/dokgastro2019802137.
35. Mukhtar K., Nawaz H., Abid S. Functional gastrointestinal disorders and gut-brain axis: what does the future hold? *World Journal of Gastroenterology.* 2019; 25 (5): 552–566. DOI: 10.3748/wjg.v25.i5.552.
36. Thomas T., Chandan J.S., Li V.S. et al. Global smoking trends in inflammatory bowel disease: A systematic review of inception cohorts. *PLoS One*. 2019; 14 (9): e0221961. DOI: 10.1371/journal.pone.0221961.
37. Chassaing B., Koren O., Goodrich J.K. et al. Dietary emulsifiers impact the mouse gut microbiota promoting colitis and metabolic syndrome. *Nature.* 2015; 519 (7541): 92–96. DOI: 10.1038/nature14232.
38. De Filippo C., Cavalieri D., Di Paola M., Ramazzotti M., Poullet J.B., Massart S., Collini S., Pieraccini G., Lionetti P. Impact of diet in shaping gutmicrobiota revealed by a comparative study in children from European drup al Africa. *Proceedings of the National Academy of Sciences.* 2010; 107 (33): 14691–14696. DOI: 10.1073/pnas.1005963107.
39. Qin J., Li R., Raes J., Arumugam M., Burgdorf K. S., Manichanh C. A human gut microbial gene catalogue established by metagenomic sequencing. *Nature.* 2010; 464 (7285): 59–65. DOI: 10.1038/nature08821.
40. Sokol H., Leducq V., Aschard H. et al. Fungal microbiota dysbiosis in IBD. *Gut.* 2017; 66 (6): 1039–1048. DOI: 10.1136/gutjnl-2015–310746.
41. Gonze D., Coyte K.Z., Lahti L., Faust K. Microbial communitiesas dynamical systems. *Current Opinion in Microbiology*. 2018; 44: 41–49. DOI: 10.1016/j. mib.2018.07.004.
42. Martinez-Medina M., Garcia-Gil L.J. *Escherichia coli* inchronic inflammatory bowel diseases: Anupdate on adherent invasive *Escherichia coli* pathogenicity. *World Journal of Gastrointestinal Pathophysiology*.2014; 5 (3): 213–227. DOI: 10.4291/wjgp.v5.i3.213.
43. Anantharaman K., Hausmann B., Jungbluth S.P., Kantor R.S., Lavy A., Warren L.A., Rappe M.S., Pester M., Loy A., Thomas B.C. et al. Expanded diversity of microbial groups that shape the dissimilatory sulfur cycle. *ISME Journal.* 2018; 12 (7): 1715–1728. DOI: 10.1038/s41396-018-0078-0.
44. Tito R.Y., Chaffron S., Caenepeel C. et al. Population-level analysis of Blastocystissubtype prevalence and variation in the human gut microbiota. *Gut.* 2019; 68 (7): 1180–1189. DOI: 10.1136/gutjnl-2018-316106.

45. Borrel G., McCann A., Deane J. et al. Genomics and metagenomics of trimethylamine-utilizing Archaea in the human gut microbiome. *ISME Journal*. 2017; 11 (9): 2059–2074. DOI: 10.1038/ismej.2017.72.
46. Vianna M.E., Conrads G., Gomes B. P.F.A., etal. Identification and quantification of archaea involved in primary endodontic infections. *Journal of Clinical Microbiology*. 2006; 44 (4): 1274–1282. DOI: 10.1128/jcm.44.4.1274-1282.2006.
47. Manrique P., Bolduc B., Walk S. T., van der Oost J., deVos W.M., Young M. J. Healthy human gut phageome. *Proceedings of the National Academy of Sciences*. 2016; 113 (37): 10400– 10405. DOI: 10.1073/pnas.1601060113.
48. Kriss M., Hazleton K.Z., Nusbacher N.M., Martin C.G., Lozupone C.A. Low diversity gut microbiota dysbiosis: drivers, functional implications and recovery. *Current Opinion in Microbiology.* 2018; 44: 34–40. DOI: 10.1016/j.mib.2018.07.003.
49. Nagao-Kitamoto H., Kitamoto S., Kuffa P., Kamada N. Pathogenic role of the gut microbiota in gastrointestinal diseases. *Intest Res.* 2016; 14 (2): 127–138. DOI: 10.5217/ir.2016.14.2.127.
50. Moayyedi P., Surette M.G., Kim P.T., Libertucci J., Wolfe M., Onischi C., Armstrong D., Marshall J.K., Kassam Z., Reinisch W., Lee C.H. Fecal microbiota transplantation induces remission in patients with active ulcerative colitis in a randomized controlled trial. *Gastroenterology.* 2015; 149 (1): e102–109. DOI: 10.1053/j.gastro.2015.04.001.
51. Costello S.P., Soo W., Bryant R.V., Jairath V., Hart A.L., Andrews J.M. Systematic review with meta-analysis: Faecal microbiota transplantation for the induction of remission for active ulcerative colitis. *Alimentary Pharmacology & Therapeutics.* 2017; 46 (3): 213–224. DOI: 10.1111/apt.14173.
52. Turner J.R. Intestinal mucosal barrier function in health and disease. *Nature Reviews Immunology.* 2009; 9 (11): 799–809. DOI: 10.1038/nri2653.
53. Vialov S.S. Mucosal permeability disturbances as a pathogenesis factor of gastrointestinal tract functional disorders: rationale and correction possibilities. *Consilium Medicum*. 2018; 20 (12): 99–104. DOI: 10.26442/20751753.2018.12.180062.
54. Mazankova L.N., Rybalchenko O.V., Nikolaeva I.V. Microdysbiosis and endogenous infections: guidelines for doctors. M.: GEOTAR-Media, 2018: 15–18 (in Russ.).
55. Mc Cauley HA, Guasch G: Three cheers for the goblet cell: maintaining homeostasis in mucosal epithelia. *Trends in Molecular Medicine.* 2015; 21 (8): 492–503. DOI: 10.1016/j.molmed.2015.06.003.
56. Heller F., Fromm A. Gitter A.H. et al. Epithelial apoptosis is a prominent feature of the epithelial barrier disturbance in intestinal inflammation: effectofpro-inflammatory interleukin-13 on epithelial cell function. *Mucosal Immunology*. 2008; 1 (Suppl.): S58–61. DOI: 10.1038/mi.2008.46.
57. Haberman Y., Karns R., Dexheimer P.J. et al. Ulcerative colitis mucosal transcriptomes reveal mitochondriopathy and personalized mechanisms underlying disease severity and treat mentresponse. *Nature Communications*. 2019; 10 (1): 38. DOI: 10.1038/s41467-018-07841-3.
58. Roediger W.E. The colonic epithelium in ulcerative colitis: an energy-deficiency disease? *Lancet*. 1980; 2 (8197): 712–715. DOI: 10.1016/s0140-6736(80)91934-0.
59. West A.P., Shadel G.S. Mitochondrial DNA in innate immune responses and inflammatory pathology. *Nature Reviews Immunology*. 2017; 17 (6): 363–375. DOI: 10.1038/nri.2017.21.
60. Novak E.A., Mollen K.P. Mitochondrial dysfunction in inflammatory bowel disease. *Frontiers in Cell and Developmental Biology*. 2015; 3: 62. DOI: 10.3389/fcell.2015.00062.
61. Ho G.T., Aird R.E., Liu B. et al. MDR1 deficiency impairs mitochondrial homeostasis and promotes intestinal inflammation. *Mucosal Immunology.* 2018; 11 (1): 120–130. DOI: 10.1038/mi.2017.31.
62. Boyapati R.K., Dorward D.A., Tamborska A. et al. Mitochondrial DNA is a pro-inflammatory damage-associated molecular pattern released during active IBD. *Inflammatory Bowel Diseases.* 2018; 24 (10): 2113–2122. DOI: 10.1093/ibd/izy095.
63. Gonze D., Coyte K.Z., Lahti L., Faust K. Microbial communities as dynamical systems. *Current Opinion in Microbiology*. 2018; 44: 41–49. DOI: 10.1016/j. mib.2018.07.004.

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Molecular mechanisms of oogenesis

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ABSTRACT

This literature review is devoted to the molecular mechanisms of oogenesis and depletion of the ovarian reserve. One of the factors in this process is constantly changing environment of the ovaries, both during intrauterine development and the postnatal period. Numerous mechanisms and factors affecting the internal environment of the female gonad are described, such as stem cell factor (SCF), which regulates migration of primordial germ cells and survival of early oocytes, insulin-like growth factor I (IGF-I), and leukocyte migration inhibitory factor (LMIF). The capabilities of the endocrine system, namely sex steroids, which can both replenish the number of germ cells and deplete the ovarian reserve through the expression of apoptotic markers, were shown. Apoptosis causes degeneration of most of the germ cells formed during oogenesis. The molecular mechanisms and factors involved in this process are numerous.

Pathways mediated by mitochondria of germ cells and external pathways mediated by receptors of the cell surface were described. A mediator between two apoptotic pathways was established – the Bid protein (BH3-interacting domain death agonist), the activation of which triggers the apoptosis mechanism of the intrafollicular microenvironment. Some other factors were identified that mediate programmed germ cell death and result in diminished ovarian reserve: eukaryotic elongation factor 2 kinase (eEF-2 K), *PUMA* and *NOXA* genes, the absence of growth factors and members of tumor necrosis factor (TNF) family. Changes in the epigenetic modification of chromatin in the follicular and germ cells, oxidative stress, decreased DNA repair, and the involvement of the genes *BRAC1, RAD51, ERCC2,* and *H2AX* associated with this process can also affect reproductive health and the ovarian reserve. A significant role of mitochondrial dysfunction of granulosa cells in depletion of the ovarian reserve is of great interest, which leads to impaired oocyte competence, deteriorates the gamete quality, and depletes the ovarian reserve. Therefore, oogenesis depends on a huge number of factors and the internal environment of the ovaries, the knowledge of which can maintain the stability of the reproductive function and preserve the quality of the ovarian reserve.

Key words: oogenesis, molecular genetic mechanisms, folliculogenesis, apoptosis, ovarian reserve.

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Молекулярные механизмы оогенеза

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РЕЗЮМЕ

Обзор литературы посвящен молекулярным механизмам оогенеза и истощения овариального резерва. Одним из аспектов данного процесса является постоянно изменяющаяся среда яичников, как во время внутриутробной закладки, так и постнатальном периоде. Описаны многочисленные механизмы и факторы, влияющие на внутреннюю среду женской гонады, такие как SCF, регулирующий миграцию первичных половых клеток и выживание ранних ооцитов; инсулиноподобный фактор роста I и фактор ингибирования лейкоцитов. Показана возможность эндокринной системы, а именно половых стероидов, которые способны как пополнять количество половых клеток, так и истощать овариальный запас через экспрессию апоптозных маркеров. Апоптоз вызывает дегенерацию большей части образующихся в процессе оогенеза половых клеток. Молекулярные механизмы, факторы, участвующие в данном процессе, многочисленны.

Описаны собственные, опосредованные митохондриями половых клеток и внешние, опосредованные рецепторами клеточной поверхности пути. Установлен посредник между двумя апоптозными путями – белок Bid, активация которого запускает механизм клеточной смерти внутрифолликулярного микроокружения. Определены и некоторые другие факторы, опосредующие запрограммированную гибель половых клеток и, как следствие, приводящие к сокращению овариального резерва: фактор элонгации киназа-2, гены *PUMA* и *NOXA*, отсутствие факторов роста и членов факторов некроза опухоли. Изменения в эпигенетической модификации хроматина в клетках гранулезы и половых клетках, окислительный стресс, снижение репаративной способности ДНК и связанное с этим процессом участие генов репарации *BRAC1, RAD51, ERCC2* и *H2AX* также могут повлиять на репродуктивное здоровье и фолликулярный запас. Особо следует отметить значительную роль в истощении запаса половых клеток митохондриальной дисфункции клеток гранулезы, что приводит к нарушению компетентности ооцитов, ухудшает качество гамет и истощает овариальный резерв. Следовательно, оогенез зависит от огромного количества факторов и внутренней среды яичников, владение которыми способно сохранить стабильность репродуктивной функции и качество овариального резерва.

Ключевые слова: оогенез, молекулярно-генетические механизмы, фолликулогенез, апоптоз, овариальный резерв.

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INTRODUCTION

The relationship between the size of the ovarian reserve and a woman's reproductive life span highlights the importance of understanding the regulatory factors and processes that determine its formation [1–4]. Studies conducted in mice describe in detail (some at the molecular level) the processes involved in determining the number of oogonia and oocytes, while our knowledge about these processes in the human body is not sufficient [3, 5].

The degree of change in the number of germ cells at every stage leading to the formation of the ovarian reserve is particularly worth noting. However, we have almost no understanding of the causes of the dynamics in folliculogenesis and germ cell death, which may be related to the nature or timing of triggers for each of the stages of oogenesis. We do not

understand why so many oocytes are formed and then lost at the pre-reproductive stage of the ovarian reserve. For example, in women, only 1 in 1,000 primordial follicles at birth will mature before ovulation and produce estradiol and progesterone necessary for fertility during the reproductive period of life [6].

It seems that mammalian fetal oocytes face a number of challenges to survive throughout all the stages of the oogenesis, especially in prophase I of meiosis up to the diplonema stage and the initial follicle assembly [7]. Depending on the period of development and experimental conditions, these oocytes can undergo various forms of programmed cell death. We assume that they require constant support of growth factors to carry out the activities necessary to overcome apoptotic death during prophase I. Before the formation of primordial follicles, a decrease in the amount of nutrients or growth factors can activate protective autophagy, but if fasting is prolonged, it can end in death.

In fact, elucidating the relationship between signaling growth factors (mainly the caspase cascade) and apoptotic and autophagic proteins that probably co-exist in fetal oocytes may be essential for understanding the causes of death of these cells. However, recent progress in molecular markers for the prophase of meiosis I, as well as for oocytes and stem cells has greatly helped in the identification and classification of developing gametes [1, 2, 7]. Such progress in apoptotic markers and many other pathways related to ovarian cellular functions contributes to our better understanding of oogenesis. However, it is important to understand how individual signals of local cell death accumulate, leading to changes in reproductive function at the level of the entire body.

MECHANISMS AFFECTING OOCYTE SURVIVAL: THE CHANGING OVARIAN ENVIRONMENT

It is still unclear to what extent the balance of individual oocyte decisions to continue development or initiate cell death may relate to the local effects of the developing ovarian somatic cell cluster in oocyte survival (for example, endocrine environment, intercellular signaling, extracellular matrix) or to the inherent properties of oocytes in meiotic transformations (for example, errors in synapsis that can delay meiotic progression or cause arrest).

Paracrine factors of oocyte survival. A number of paracrine factors identified in the human fetal ovary may affect oocyte survival at one stage or another [8–10]. For some paracrine factors, progress has been made in identifying the primordial cell type and receptor locations. However, the local environment of the ovaries is complex and constantly changes both during intrauterine development and in the future.

There are a number of so-called survival factors without which germ line cells die, for example, the stem cell factor (SCF), also known as the KIT ligand, which is essential during migration of primordial germ cells and for survival of early oocytes [11]. The KIT ligand has been noted by numerous studies as a critical regulator of primary follicle activation. By binding to its receptor, it sends signals along several pathways, including activation of the oocyte phosphotidylinositol-3-kinase (PI3K), which are particularly important for ovarian development, restoring intercompartment communication and reducing the rate of follicular atresia. SCF, along with insulin-like growth factor I (IGF-I) and leukocyte migration inhibitory factor (LMIF), support the survival of germ line cells after migration. They are overlaid with pro- and antiapoptotic mechanisms and other cell death pathways that function at certain stages [8]. For example, in mice, the absence of signaling in a complex of factors activates the Fas death pathway in pre-follicular oocytes [12], and tumor necrosis factor-α promotes apoptosis during follicle formation [13, 14].

Endocrine factors of oocyte survival. Within egg nests, inter-oocyte communication is mediated through cytoplasmic bridges, but after the collapse of this formation, interfollicular communication can continue, for example, through molecules produced by granulosa cells [14]. The developing ovary is also influenced by the embryonic endocrine system, and transcripts of some steroidogenic enzymes are present in the ovary for at least 15 weeks of gestation, while the ability to metabolize androgens into estrogens is only present for about 12 weeks of gestation. Estrogens and progesterone inhibit follicular assembly in rats, possibly by inhibiting apoptosis in oocytes [15, 16].

However, progesterone may be at least partially endocrine rather than local, since the removal of ovaries from the environment *in vivo* marked an accelerated transition from primordial to primary follicles in the absence of progesterone [14, 17]. Possible extragonadal sources of such steroids were identified in human embryonic tissues. Experiments in which ovaries of a specific genotype are transplanted in mice with severe combined immunodeficiency can be used to demonstrate the need for local effect of the gene.

Therefore, the local presence of the *Fas* gene in the ovary is necessary for normal elimination of oocytes in transplanted ovaries [9, 15].

Intraoocytic factors and intercellular interactions. Against the background of this complex local environment, internal factors of the oocyte can also influence the prospects of their survival. Some researchers noted an increase in the frequency of abnormal synapsis in genetically abnormal fetuses, as expected, and also associated some chromosomal abnormalities with increased apoptosis. However, when studying individual mouse oocytes in the prophase of meiosis I, only a slight relationship was found between apoptotic molecular markers and normal or abnormal SCF appearance in mice [7, 18, 19]. In contrast to the relative absence of an association between apoptotic markers and meiotic abnormalities in individual oocytes, significant differences were observed in the behavior of ovarian tissue samples *in vitro* in accordance with gestational age [20]. Consequently, this may affect the environment that surrounds oocytes entering meiosis at different stages of pregnancy.

It was found that cultures of ovarian fragments from fetuses at 14 weeks of gestation were prone to expansion *in vitro*, the cells moved from the original fragment and eventually covered most of the membrane with clusters and aggregates. Alkaline phosphatase staining showed that germ cells were mainly concentrated in the original tissue fragment and in clusters that were formed as a result of reproduction. The remarkable ability of oocytes to migrate during intrauterine development even during prophase I of meiosis was noted by previous authors [18].

For example, X. Wu et al. (2017) used organ culture techniques applied to adult tissues. Survival and growth of follicles *in vitro* from the tissue were observed at 16–22 and 22–23 weeks of gestation, respectively, and confirmed meiotic initiation and progression [19]. Optimization of culture methods for fetal ovaries will be a valuable tool for studying hormonal and paracrine effects on the key aspects of human ovarian development. The mechanism of movement of germ line cells is currently being studied. This ability was significantly improved by including growth factors in the culture medium (SCF 10 ng / ml and IGF-I 15 ng / ml).

In contrast, the ovarian tissue increased significantly *in vitro* at the 15th week of gestation, and the stimulating effect of growth factors was no longer significant. It was also noted that later gestational ages (up to 23 weeks) cultured under similar conditions tend to round off *in vitro* and form dense surface epithelium [21].

It is well known that mouse primordial germ cells can be successfully cultured and their number increases in cultures supplemented with growth factors, including SCF [22]. It is also known that c-kit is present on human oogonia and oocytes from 14–21 weeks of gestation and that the distribution of c-kit (SCF) in the human germ line varies depending on the status [23]. For example, S. Gkountela et al. (2013), using immunohistochemistry, found that primordial germ cells and oogonia are c-kit-positive, while free oocytes or those enclosed in primordial follicles are stained poorly or do not express these factors at all. C-kit is again found on the surface of oocytes in growing follicles [23, 24]. We know that progenitor cells remain in the fetal ovaries well after 15 weeks, so perhaps they multiply less or undergo apoptosis at later stages.

Meiosis as a survival factor. The authors also proposed to evaluate the progression of meiotic transformations of oocytes under cultured conditions using growth factors, such as SCF. The number of oocytes decreased significantly during the first week of cultivation, probably due to a drastic change in the environment [24]. After that, oogenesis was restored with an increase in the number of oocytes at the stages of leptonema and zygonema, progressing during the next week of cultivation. Recovery was more pronounced at 14 weeks than at 15 weeks, and it was evident with or without the addition of the growth factor until the second week *in vitro* [16, 21]. After that, cultures with growth factors were more likely to maintain recovery. It is not yet clear whether growth factors support progenitor cells, oocytes directly, or have an indirect effect through the somatic environment.

Therefore, in addition to expression of numerous genes and formation of growth factors described in previous works [2, 3], oogenesis also depends on the constantly changing environment of the ovaries, namely, endocrine and paracrine factors, inter-oocyte communication, meiotic transformations, and possible abnormalities of germ cell division. Further research will require the use of specific markers for differentiating oocytes and progenitor cells at different stages and using tissue samples from a wider range of gestational age.

DEPLETION OF THE OVARIAN RESERVE OF GERM CELLS

Apoptosis causes elimination of more than 99% of germ cells from the ovaries through follicular atresia

[2, 25, 26]. Less than 1% of germ line cells, following oocyte cultivation, further undergo apoptosis during the last phases of oogenesis and deplete the ovarian reserve in most mammalian species, including humans. The maximum number of germ cells in mice was determined at the time of entry of primary oocytes into the meiosis prophase (Figure). After that, up to two-thirds of the germ cells die and by the time of birth, the ovarian reserve is established, which remains for the rest of life [3, 27, 28]. The peak number of gametes in the human ovary occurs by 20 weeks of pregnancy, after which a drastic decrease in their number takes place, similar to that observed in mice. The degree of germ cell loss during this time (about 20 weeks of gestation) ultimately affects the size of the ovarian reserve [1, 2, 26, 29]. The molecular mechanisms via which oocytes are eliminated and the factors involved in this process remain largely unknown.

According to various authors, the death of mammalian ovarian somatic cells can occur in various ways: apoptosis, necrosis, autophagy, or necroptosis [29–31]. These processes differ death mode, as well as in morphological, biochemical, and molecular characteristics. However, there are currently no published data on which of these pathways is primary and responsible for germ cell death before the formation of the ovarian reserve [32, 33]. Some scientists report a decrease in the number of primordial follicles in prepubertal mice, not associated with the pronounced expression of classical markers of apoptosis and cleaving caspase-3, which results in authors' suggesting an alternative mechanism of oocyte death [34–36]. Consequently, multiple perinatal mechanisms affect the primary follicular reserve.

There are several factors that induce apoptosis directly or indirectly in oocytes at various stages of the cell cycle and meiosis. Premature removal of surrounding granulosa cells from immature oocytes, decreased levels of adenosine 3',5'-cyclic monophosphate and guanosine 3',5'-cyclic monophosphate, increased levels of calcium and oxidants, sustained decreased levels of maturation-promoting factors, depletion of survival factors, nutrients, and cell cycle proteins, decreased meiotic competence, and increased levels of proapoptotic and apoptotic factors lead to oocyte apoptosis [27–30].

Both internal (mediated by mitochondria) and external (mediated by cell surface receptors) pathways are involved in programmed germ cell death. An intermediary between two apoptosis pathways was found – the Bid protein, which is present in an inactive form in the cytosol. In response to the stimulus of the external apoptotic pathway, the N-terminal part of the protein is cleaved off to form the active form tBid. The activated protein moves to the mitochondria and, interacting with the proapoptotic proteins Bak and Bax, permeabilizes the mitochondria with the release of apoptogenic factors, such as cytochrome C [37, 38]. One of the eight helices (H3) of the Bid protein contains the BH3 domain, which activates the mechanism of cell death in the intrafollicular microenvironment. Oocyte apoptosis leads to depletion of the ovarian reserve, directly affecting the reproductive outcome of various mammals, including humans [2, 14, 28].

The role of programmed cell death has been well studied in the ovaries during the transition from mitosis to meiosis and degeneration of follicular clusters in mice [16, 30], when the number of germ cells decreases dramatically (Figure). Apoptotic death at this stage was also demonstrated in the human ovary [8, 26]. Apoptosis is probably of great biological significance for elimination of defective or "low-quality" oocytes with damaged nuclear or mitochondrial DNA [27, 37, 39]. However, there is no direct evidence that the quality of germ cells is maintained by apoptosis during oogenesis. It was suggested that eukaryotic elongation factor 2 kinase (eEF-2 K) is involved in this process by inhibiting antiapoptotic proteins in response to oxidative stress, which makes germ cells more susceptible to apoptosis and elimination [40, 41].

There is evidence that certain proteins mediate apoptosis in somatic cells and affect the number of ovarian germ cells [3, 26, 42]. M. Myers et al. (2014) reported that in mice that are genetically deficient in the *PUMA* gene, increased expression of apoptotic factors and reduction of germ cells entering meiosis by half are observed; therefore, the size of the ovarian reserve is significantly reduced [14, 43].

This effect cannot be associated with altered proliferative activity of germ cells. Data indicate that the *PUMA* gene affects only oogonia granulosa cells before the formation of egg nests, but not during the subsequent decrease in the number of germ cells during degeneration of the latter [44]. Conversely, the antiapoptotic Bcl-2-like protein, MCL-1, is expressed in oocytes relatively late, just before the formation of primordial follicles, and, therefore, may indirectly be involved in preserving the ovarian reserve during oogenesis and at the end of pregnancy [44]. Inactivation of antiapoptotic Bcl-x led to increased apoptosis in granulosa cells of embryonic follicles [36, 39, 40].

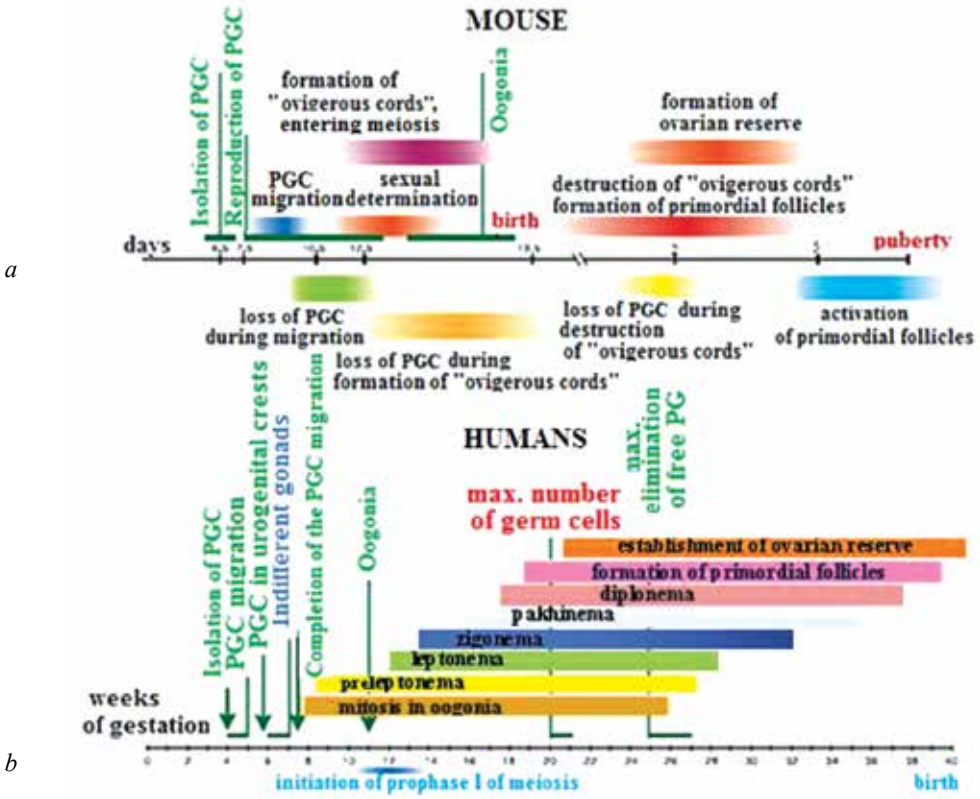

Figure. Comparative characteristics of the ovarian reserve in mice (*a*) and humans (*b*), the number of primordial germ cells (PGC) in different gestation periods

Apoptosis requires activation of either an internal or an external pathway and physiological or stress-related triggers responsible for the activation of these pathways, which have not yet been completely elucidated in granulosa cells. In somatic cells [30, 40] and postpartum mouse oocytes [43, 45], failure to repair DNA damage was shown to cause apoptotic death via *PUMA* and *NOXA*. Other factors responsible for apoptosis in somatic cells include the absence of growth factors (internal pathway) and the absence of tumor necrosis factors (TNF) (external pathway). TNF/TNFR1 and FasL/Fas are expressed in the neonatal ovaries of rodents [3, 13, 46]. In addition, TNF contributes to oocyte death *in vitro*, and deletion of TNFa or Fas in mice increases the initial number of follicles at birth [9, 47]. These data indicate a crucial role of apoptosis for death receptor signaling in female germ cells, particularly during the period of egg nest breakdown and primordial follicle formation.

We know very little about how epigenetic modifications of chromatin in granulosa cells and germ cells can affect reproductive health and follicular supply. Epigenetic modification of chromatin occurs mainly due to DNA methylation, modification of histones, or non-coding RNA, but it is unknown to what extent these processes affect the final number of oocytes in the reserve [34, 36, 48]. Oxidative stress causes oocyte apoptosis with activation of the Fas/FasL system, and oocyte competence correlates more closely with histone modification than with chromatin configuration [36, 40].

The age-related decline in reproductive function in women is not poorly understood, and apoptosis is considered in this process as one of the reasons for the decrease in the primary follicular reserve. One of the papers described a decrease in the DNA repair capacity in age-related rats and, as a result, demonstrated a fall in the mRNA level of the *BRCA1* and *H2AX* repair genes [40, 49]. This study identified 13 differentially expressed proteins involved in a wide range of biological functions, including apoptosis, DNA repair, and the immune system. The differentially expressed FIGNL1 proteins responsible for DNA repair and BOK, a apoptotic protein found in primary follicles, were described for the first time and are associated, according to the authors, with some common features of ovarian aging, loss of follicular reserve, and genome integrity [14, 49].

Another similar study measured the mRNA levels of DNA repair genes in aging animals compared to young ones. The results showed a significant decrease in the mRNA levels of the *BRAC1, RAD51, ERCC2*, and *H2AX* genes for DNA repair and the levels of BRAC1 and H2AX phosphoproteins in the primordial follicles of elderly rats [49]. Therefore, the impairment of DNA repair is confirmed as a mechanism of oocyte aging.

More and more studies are devoted to finding new methods that identify the size of the ovarian reserve. Of course, ovarian biopsy and histological examination provide the most accurate representation of the follicular reserve compared to ultrasound or other indirect laboratory tests. Currently, more data appear on other, non-invasive, but reliable methods for diagnosing the ovarian reserve. In one of these studies, it was determined that mitochondrial biogenesis in granulosa cells may be associated with impaired oocyte competence in patients with reduced ovarian reserve [38, 50].

Mitochondria, which contribute to the quality of oocytes, may be involved in the pathogenesis of follicular depletion. The study of granulosa cells offers a non-invasive approach to assessment of the quality of oocytes and the metabolic processes affecting it. If mitochondrial dysfunction is involved in depletion of the ovarian reserve, it is likely to affect the functioning of cumulus cells. The content of mitochondria in oocytes and cumulus cells was evaluated by quantitative determination of mitochondrial DNA by PCR and expression of 13 genes involved in mitochondrial functions, such as apoptosis and antioxidant protection [38, 40, 50].

Therefore, we can state that follicular cells can regulate mitochondrial biogenesis, creating an adequate pool of mitochondria in oocytes for further development. Changes in this process in patients with diminished ovarian reserve may explain the deterioration of the quality of germ cells. Consequently, some characteristics of the mitochondria in cumulus cells can serve as indicators of oocyte competence, and the quality of germ cells can be improved by products that enhance mitochondrial biogenenesis.

CONCLUSION

Even after decades of study, oogenesis is still not known completely. It is obvious that the internal environment of a pregnant woman at key stages of fetal ovarian development can directly affect both fertility of her future daughter (by controlling the size of the ovarian reserve) and the quality of her oocytes (by influencing the degree of selection and apoptosis). Oogenesis is an integral process of ovarian reserve formation, and the preservation and depletion of the latter depends on a huge number of factors of intraovarian and extragonadal origin, proapoptotic and apoptotic agents, mitochondrial dysfunction, expression of certain genes at all stages of oogenesis and follicle formation, epigenetic modification of chromatin, the ability to repair DNA, and many other, still unknown, markers.

The study of follicular dynamics is gaining momentum due to the use of modern methods that allow to determine the factors of germ cell survival and ovarian reserve formation, as well as to search for possible regulators in preventing pathological germ cell death.

REFERENCES

1. Denisenko M.V., Kurcer M.A., Kurilo L.F. Dynamics of ovarian follicular reserve formation. *Andrology and Genital Surgery*. 2016; 17: 20–28 (in Russ.). DOI: 10.17650/2070-9781-2016-17-2-20-28.
2. Zenkina V.G. Formation of ovarian follicular reserve. *Bulletin of Siberian Medicine*. 2018; 17 (3): 197–206 (in Russ.). DOI: 10.20538/1682-0363-2018-3-197–206.
3. Zenkina V.G., Solodkova O.A. Molecular and genetic mechanisms of ovarian organization and development. *Bulletin of Siberian Medicine*. 2018; 17 (2): 133–142 (in Russ.). DOI: 10.20538/1682-0363-2018-2-133–142.
4. Turdybekova Ya.G. Folliculogenesis and ovarian follicular reserve in norm and pathology: aspects (stages) of clinical and morphological study. *Bulletin of KazNMU*. 2019; 1: 41–45 (in Russ.).
5. Hsueh A.J.W., Kawamura K., Cheng Y., Fauser B.C.J.M. Intraovarian control of early folliculogenesis. *Endocr. Rev.* 2015; 36 (1): 1–24. DOI: 10.1210/er.2014-1020.
6. Sato E., Kimura N., Yokoo M., Miyake Y., Ikeda J.E. Morphodynamics of ovarian follicles during oogenesis in mice. *Microsc. Res. and Techn.* 2006; 69 (6): 427–435. DOI: 10.1002/jemt.20302.
7. Pan B., Li J. The art of oocyte meiotic arrest regulation. *Reprod. Biol. Endocrinol*. 2019; 17 (1): 8. DOI: 10.1186/s12958-018-0445-8.
8. Fulton N., Martins da Silva S.J., Bayne R.A.L., Anderson R.A. Germ cell proliferation and apoptosis in the developing human ovary. *J. Clin. Endocrinol. Metab*. 2005; 90: 4664–4670.
9. Moniruzaman M., Sakamaki K., Akazawa Y., Miyano T. Oocyte growth and follicular development in KIT-deficient Fas-knockout mice. *Reproduction*. 2007; 133: 117–125. DOI: 10.1530/REP-06-0161.
10. Hansen K.R., Hodnett G.M., Knowlton N., Craig LB. Correlation of ovarian reserve tests with histologically determined primordial follicle number. *Fertil. Steril*. 2011; 95: 170–175. DOI: 10.1016/j.fertnstert.2010.04.006.
11. Thuwanut P., Comizzoli P., Wildt D.E., Keefer C.L., Songsasen N. Stem cell factor promotes in vitro ovarian follicle development in the domestic cat by upregulating c-kit mRNA

expression and stimulating the phosphatidylinositol 3-kinase/AKT pathway. *Reprod. Fertil. Dev.* 2017; 29 (7): 1356–1368. DOI: 10.1071/RD16071.
12. Nilsson E., Detzel C., Skinner M. Platelet-derived growth factor modulates the primordial to primary follicle transition. *Reproduction.* 2006; 6: 1007–1015. DOI: 10.1530/rep.1.00978.
13. Greenfeld C.R., Roby K.F., Pepling M.E., Babus J.K., Terranova P.F., Flaws J.A. Tumor necrosis factor (TNF) receptor type 2 is an important mediator of TNF alpha function in the mouse ovary. *Biol. Reprod*. 2007; 76: 224–231. DOI: 10.1095/biolreprod.106.055509.
14. Monget P., Bobe J., Gougeon A., Fabre S., Monniaux D., Dalbies-Tran R. The ovarian reserve in mammals: a functional and evolutionary perspective. *Mol. Cell Endocrinol.* 2012; 356 (1-2): 2–12. DOI: 10.1016/j.mce.2011.07.046.
15. Rimon-Dahari N., Yerushalmi-Heinemann L., Alyagor L., Dekel N. Ovarian folliculogenesis. *Results Probl. Cell Differ.* 2016; 58: 167–190. DOI: 10.1007/978-3-319-31973-5_7.
16. Wear H.M., McPike M.J., Watanabe K.H. From primordial germ cells to primordial follicles: a review and visual representation of early ovarian development in mice. *J. Ovarian Res.* 2016; 9: 36. DOI: 10.1186/s13048-016-0246-7.
17. Dutta S., Mark-Kappeler C.J., Hoyer P.B., Pepling M.E. The steroid hormone environment during primordial follicle formation in perinatal mouse ovaries. *Biol. Reprod.* 2014; 91 (3): 68. DOI: 10.1095/biolreprod.114.119214.
18. Ghafari F., Gutierrez C.G., Hartshorne G.M. Apoptosis in mouse fetal and neonatal oocytes during meiotic prophase one. *BMC Dev. Biol.* 2007; 7: 87. DOI: 10.1186/1471-213X-7-87.
19. Wu X., Fu Y., Sun X., Liu C., Chai M., Chen C., Dai L., Gao Y., Jiang H., Zhang J. The possible FAT1-mediated apoptotic pathways in porcine cumulus cells. *Cell Biol. Int.* 2017; 41 (1): 24–32. DOI: 10.1002/cbin.10695.
20. Childs A.J., Kinnell H.L., He J., Anderson R.A. LIN28 is selectively expressed by primordial and pre-meiotic germ cells in the human fetal ovary. *Stem Cells Dev*. 2012; 21 (13): 2343–2349. DOI: 10.1089/scd.2011.0730.
21. Hartshorne G.M., Lyrakou S., Hamoda H., Oloto E., Ghafari F. Oogenesis and cell death in human prenatal ovaries: what are the criteria for oocyte selection? *Mol. Hum. Reprod.* 2009; 15 (12): 805–819. DOI: 10.1093/molehr/gap055.
22. DeFelici M., Lobascio A.M., Klinger F.G. Cell death in fetal oocytes: many players for multiple pathways. *Autophagy.* 2008; 4: 240–242. DOI: 10.4161/auto.5410.
23. Gkountela S., Li Z., Vincent J.J., Zhang K.X., Chen A., Pellegrini M., Clark A.T. The ontogeny of cKIT+ human primordial germ cells proves to be a resource for human germ line reprogramming, imprint erasure and *in vitro* differentiation. *Nat. Cell* Biol. 2013; 15 (1): 113–122. DOI: 10.1038/ncb2638.
24. Fabbri R., Zamboni C., Vicenti R., Macciocca M., Paradisi R., eracchioli R. Update on oogenesis *in vitro. Minerva Ginecol.* 2018; 70 (5): 588–608. DOI: 10.23736/S0026-4784.18.04273-9.
25. Zenkina V.G., Karedina V.S., Solodkova O.A., Sluckaya T.N., Yufereva A.L. Morphology of ovaries of androgenized rats while receiving an extract from cucumaria. *Pacific Medical Journal*. 2007; 4: 70–72 (in Russ.).
26. Banerjee S., Banerjee S., Saraswat G., Bandyopadhyay S.A., Kabir S.N. Female reproductive aging is master-planned at the level of ovary. *PLoS One.* 2014; 9 (5): e96210. DOI: 10.1371/journal.pone.0096210.
27. Zenkina V.G. Factors of angiogenesis in the development of physiological and pathological processes of the female gonad. *Bulletin of Siberian Medicine.* 2016; 15 (4): 111–119 (in Russ.). DOI 10.20538/1682-0363-2016-4-111–119.
28. Tiwari M., Prasad S., Tripathi A., Pandey A.N., Ali I., Singh A.K., Shrivastav T.G., Chaube S.K. Apoptosis in mammalian oocytes: a review. *Apoptosis.* 2015; 20 (8): 1019–1025. DOI: 10.1007/s10495-015-1136-y.
29. Gartner A., Boag P.R., Blackwell T.K. Germline survival and apoptosis. *WormBook.* 2008; 4: 1–20. DOI: 10.1895/wormbook.1.145.1.
30. Klinger F.G., Rossi V., De Felici M. Multifaceted programmed cell death in the mammalian fetal ovary. *Int. J. Dev. Biol.* 2015; 59 (1-3): 51–54. DOI: 10.1387/ijdb.150063fk.
31. Linkermann A., Green D.R. Necroptosis. *N. Engl. J. Med*. 2014; 370 (5): 455–465. DOI: 10.1056/NEJMra1310050.
32. Rodrigues P., Limback D., McGinnis L.K., Plancha C.E., Albertini D.F. Multiple mechanisms of germ cell loss in the perinatal mouse ovary. *Reproduction.* 2009; 137: 709–720. DOI: 10.1530/REP-08-0203.
33. Gawriluk T.R., Hale A.N., Flaws J.A., Dillon C.P., Green D.R., Rucker E.B. Ankophagy is a cell survival program for female germ cells in the murine ovary. *Reproduction*. 2011; 141 (6): 759–765. DOI: 10.1530/REP-10-0489.
34. Kimura T., Kaga Y., Sekita Y., Fujikawa K., Nakatani T., Odamoto M., Funaki S., Ikawa M., Abe K., Nakano T. Pluripotent stem cells derived from mouse primordial germ cells by small molecule compounds. Stem Cells. 2015; 33 (1): 45–55. DOI: 10.1002/stem.1838.
35. Tingen C., Kim A., Woodruff T.K. The primordial pool of follicles and nest breakdown in mammalian ovaries. *Mol. Hum. Reprod*. 2009; 15 (12): 795–803. DOI: 10.1093/molehr/gap073.
36. Lin J., Chen F., Sun M.J., Zhu J., Li Y.W., Pan L.Z., Zhang J., Tan J.H. The relationship between apoptosis, chromatin configuration, histone modification and competence of oocytes: A study using the mouse ovary-holding stress model. *Sci. Rep.* 2016; 6: 28347. DOI: 10.1038/srep28347.
37. Seidler E.A., Moley K.H. Metabolic determinants of mitochondrial function in oocytes. *Semin. Reprod. Med.* 2015; 33 (6): 396–400. DOI: 10.1055/s-0035-1567822.
38. Boucret L., Chao de la Barca J.M., Morinière C., Desquiret V., Ferré-L'Hôtellier V., Descamps P., Marcaillou C, Reynier P., Procaccio V., May-Panloup P. Relationship between diminished ovarian reserve and mitochondrial biogenesis in cumulus cells. *Hum. Reprod.* 2015; 30 (7): 1653–1664. DOI: 10.1093/humrep/dev114.
39. Wang C., Zhou B., Xia G. Mechanisms controlling germline cyst breakdown and primordial follicle formation. *Cell Mol. Life Sci.* 2017; 74 (14): 2547–2566. DOI: 10.1007/s00018-017-2480-6.
40. Zhang J.Q., Gao B.W., Wang J., Ren Q.L., Chen J.F., Ma Q., Zhang Z.J., Xing B.S. Critical role of foxo1 in granulosa cell apoptosis caused by oxidative stress and protective effects of grape seed procyanidin B2. *Oxid. Med. Cell Longev.* 2016; 2016: 6147345. DOI: 10.1155/2016/6147345.

41. Chu H.P., Liao Y., Novak J.S., Hu Z., Merkin J.J., Shymkiv Y., Braeckman B.P., Dorovkov M.V., Nguyen A., Clifford P.M., Nagele R.G., Harrison D.E., Ellis R.E., Ryazanov A.G. Germline quality control: eFK2K stands guard to eliminate defective oocytes. *Dev. Cell.* 2014; 28: 561–572. DOI: 10.1016/j.devcel.2014.01.027.
42. Hutt K.J. The role of BH3-only proteins in apoptosis within the ovary. *Reproduction*. 2015; 149 (2): R81–R89. DOI: 10.1530/REP-14-0422.
43. Myers M., Morgan F.H., Liew S.H., Zerafa N., Gamage T.U., Sarraj M., Cook M., Kapic I., Sutherland A., Scott C.L., Strasser A., Findlay J.K., Kerr J.B., Hutt K.J. PUMA regulates germ cell loss and primordial follicle endowment in mice. *Reproduction*. 2014; 148 (2): 211–219. DOI: 10.1530/REP-13-0666.
44. Omari S., Waters M., Naranian T., Kim K., Perumalsamy A.L., Chi M., Greenblatt E., Moley K.H., Opferman J.T., Jurisicova A. Mcl-1 is a key regulator of the ovarian reserve. *Cell Death Dis.* 2015; 6 (5): e1755. DOI: 10.1038/cddis.2015.95.
45. Kerr J.B., Hutt K.J., Michalak E.M., Cook M., Vandenberg C.J., Liew S.H., Bouillet P., Mills A., Scott C.L., Findlay J.K., Strasser A. et al. DNA damage-induced primordial follicle oocyte apoptosis and loss of fertility require Tap63-mediated maturation of Puma and Noxa. *Mol. Cell*. 2012; 48 (3): 343–352. DOI: 10.1016/j.molcel.2012.08.017.
46. Sui X.X., Luo L.L., Xu J.J., Fu Y.C. Evidence that FOXO3 is involved in oocyte apoptosis in the neonatal rat ovary. *Biochem. Cell Biol.* 2010; 88 (4): 621–628. DOI: 10.1139/O10-001.
47. Cui L.L., Yang G., Pan J., Zhang C. Tumor necrosis factor alpha knockout increases fertility of mice. *Theriogenology*. 2011; 75 (5): 867–876. DOI: 10.1016/j.theriogenology.2010.10.029.
48. Jung D., Kee K. insights into female germ cell biology: from *in vivo* development to *in vitro* derivations. *Asian J. Androl.* 2015; 17 (3): 415–420. DOI: 10.4103/1008-682X.148077.
49. Govindaraj V., Rao A.J. Comparative proteomic analysis of primordial follicles from ovaries of immature and aged rats. *Syst. Biol. Reprod. Med*. 2015; 61 (6): 367–375. DOI: 10.3109/19396368.
50. May-Panloup P., Boucret L., Chao de la Barca J.M., Desquiret-Dumas V., Ferré-L'Hotellier V., Morinière C., Descamps P., Procaccio V., Reynier P. Ovarian ageing: the role of mitochondria in oocytes and follicles. *Hum. Reprod. Update*. 2016; 22 (6): 725–743. DOI: 10.1093/humupd/dmw028.

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Potential biochemical markers of chronic bronchitis

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ABSTRACT

The review systematizes modern data on the biochemical markers that can clarify the nature and the course of chronic bronchitis. The article describes the markers associated with bronchopulmonary pathology, such as tumor necrosis factor alpha (TNFα), interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-10 (IL-10), tissue factor, type 1 plasminogen activator inhibitor (PAI-1), and monocyte chemoattractant protein-1 (MCP-1). For each biomolecule, its properties, functions, direct role in body processes, and associations with bronchopulmonary pathology are described. The use of these markers for early diagnosis of bronchopulmonary pathology and monitoring of the treatment effectiveness is promising and requires further in-depth study.

Key words: chronic obstructive pulmonary disease, chronic bronchitis, biochemical markers, tumor necrosis factor alpha, interleukin-1, interleukin-6, interleukin-8, interleukin-10, tissue factor, type 1 plasminogen activator inhibitor, monocyte chemoattractant protein 1.

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Потенциальные биохимические маркеры хронического бронхита

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РЕЗЮМЕ

В обзоре систематизируются современные данные о биохимических маркерах, которые расширяют наше понимание о закономерностях развития хронического бронхита. В статье приведены маркеры, ассоциированные с патологией бронхолегочной системы: фактор некроза опухоли альфа; интерлейкин (ИЛ) 1, 6, 8, 10; тканевой фактор; ингибитор активатора плазминогена 1-го типа; моноцитарно-хемоаттрактантный протеин 1. Для каждой представленной биомолекулы описаны ее свойства, функции, непосредственная роль в организме, взаимосвязи с патологией бронхолегочной системы. Использование

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данных маркеров целесообразно для ранней диагностики, контроля лечения и требует более глубокого изучения.

Ключевые слова: хронический бронхит, хроническая обструктивная болезнь легких, биохимические маркеры, фактор некроза опухоли альфа, интерлейкин 1, интерлейкин 6, интерлейкин 8, интерлейкин 10, тканевой фактор, ингибитор активатора плазминогена 1-го типа, моноцитарно-хемоаттрактантный протеин 1.

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

At the moment, diseases of the bronchopulmonary system have gained enormous prevalence not only in Russia but throughout the world. Chronic bronchitis (CB) occupies a leading position among chronic non-specific lung diseases. According to various estimates, the number of patients with CB in Russia is about 33 million people. According to the WHO recommendation and Russian clinical guidelines, CB is a chronic diffuse progressive inflammation of the bronchi, manifested by productive cough lasting at least three months a year for two consecutive years, with the exception of other diseases of the upper respiratory tract, bronchi, and lungs, which could cause these symptoms. Chronic obstructive pulmonary disease is not a single, specific disease, but a collective term used to describe chronic lung diseases that restrict airflow to the lungs. A combination of chronic bronchitis CB with emphysema is defined as chronic obstructive pulmonary disease (COPD).

However, at the moment, CB occurs as an independent disease, which may not be associated with obstruction. According to the WHO, today, respiratory diseases are the third leading cause of death in the world with about 2.8 million people dying every year, which accounts for 4.8% of all causes of death. The prevalence of CB varies throughout the world, ranging from 3.4 to 22.0% in the general population up to 74.1% in patients with COPD [1–3].

In the largest study of current or former smokers without airflow obstruction (4,900 participants), 12.2% of people had CB using the classical definition [4]. A recent European study showed that the prevalence of CB was 18% in 972 patients with COPD [5]. A Chinese study of 1,668 patients with COPD showed that 30% of participants met the diagnostic criteria for CB [6]. Therefore, it is important to understand the need for earlier diagnosis of these diseases and the search for possible predictors and ways of influencing the pathogenesis of respiratory pathology. The data below describe promising biochemical markers which, according to available literature, may have diagnostic benefits in examination of pulmonary pathology.

TUMOR NECROSIS FACTOR

Tumor necrosis factor (TNF, cachexin or cachectin, TNFα) is a cellular acute-phase signaling protein involved in systemic inflammation and one of the representatives of the cytokine family. It is produced by macrophages, lymphocytes, natural killer cells, neutrophils, mast cells, eosinophils, neurons, etc. [7]. TNF is synthesized as a type II membrane protein, with a molecular weight of 26 kDa (233 amino acids). It is released via proteolytic cleavage by the TNF-converting enzyme (a disintegrin and metalloprotease 17 (ADAM17)), soluble TNF with a molecular weight of 17 kDa (157 amino acids) is cleaved from the membrane-binding fragment.

The TNF family includes TNF-alpha, TNF-beta, CD40 ligand (CD40L), Fas ligand (FasL), TNF-related apoptosis-inducing ligand (TRAIL), and LIGHT (homologous to lymphotoxins) [8].

TNF has many important physiological and pathological effects. TNF causes necrosis of tumor cells (a process that includes swelling of cells, destruction of organelles, and lysis of cells) and apoptosis (a process that involves contraction of cells, formation of condensed bodies, and DNA fragmentation).

In addition, TNF is a key mediator of both acute and chronic systemic inflammatory responses. TNF not only induces its own secretion, but also stimulates

the production of other inflammatory cytokines and chemokines. TNF plays a central role in autoimmune diseases, such as rheumatoid arthritis (RA), inflammatory bowel diseases (IBD), including Crohn's disease and ulcerative colitis, multiple sclerosis, systemic lupus erythematosus, and systemic sclerosis [9–11].

There is a rather large and extensive knowledge base on the association between TNF and CB. Over the past 20 years, several extensive fundamental meta-analyses on this topic have been published, which did not come to a single conclusion. Thus, according to W. Gan et al., TNF has a pronounced correlation between its serum level and the severity of CB [12].

A number of centers stated that there was no significant correlation between TNF and CB, however, in a detailed review of the findings, it was concluded that at early stages of CB, the correlation between markers of inflammation and the degree of impaired respiratory function was poorly significant [13].

Recent studies by Y. Mosrane et al. demonstrated a higher correlation with TNF in smoking CB patients than in the group of non-smokers [14].

When assessing biochemical markers during treatment, a significant direct correlation was found between the response to COPD treatment and the TNF level in the blood [15].

Therefore, at present, TNF in patients with CB is a promising biochemical parameter that requires a more in-depth analysis as a biomarker and a target during treatment in COPD patients.

INTERLEUKIN-1

Interleukin-1 (IL-1) is one of the most important cytokines of innate immunity and inflammation. The IL-1 family includes 7 ligands with proinflammatory activity: IL-1α and β, IL-18, IL-33, IL-36α, β, γ, three receptor antagonists (IL-1Ra, IL-36Ra, IL-38), and an anti-inflammatory cytokine (IL -37). The IL-1 receptor (IL-1R) family includes 6 receptor chains forming 4 receptor complexes, two decoy receptors (IL-1R2, IL-18BP), and two negative regulatory receptors (TIR 8, IL-1RAcPb). Strict regulation by receptor antagonists, decoy receptors, and signal transduction inhibitors provides a balance between enhanced innate immunity and uncontrolled inflammation [16].

The most studied representatives of this family at the moment are IL-1α and IL-1β. The precursor IL-1α is constantly present in the epithelial layers of the entire gastrointestinal tract, lungs, liver, kidneys, endothelial cells, and astrocytes. In cell death from necrosis, as occurs in diseases associated with local or global ischemia, the precursor IL-1α is released. Thus, IL-1α mediates early phases of sterile inflammation by rapidly initiating a cascade of inflammatory cytokines and chemokines and functions as an alarmin [17].

In contrast, IL-1β is produced by hematopoietic cells, such as blood monocytes, tissue macrophages, dendritic skin cells, and brain microglia, in response to Toll-like receptors (TLR), activated complement components, and other cytokines (Table) [18].

Table

Members of the IL-1 family		
Interleukin	Receptor	Function
IL-1α, IL-1β	IL-1R1	Proinflammatory
IL-1β	IL-1R2	Anti-inflammatory
IL-1ra	IL-1R1	Anti-inflammatory
IL-18	IL-1R5	Proinflammatory
IL-33	IL-1R4	Proinflammatory
IL-36α, β, γ	IL-1R6	Proinflammatory
IL-36Ra	IL-1R6	Anti-inflammatory
IL-37	IL-1R5	Anti-inflammatory
IL-38	IL-1R6	Anti-inflammatory

Members of the IL-1 family regulate most cells of the innate immunity, including macrophages, neutrophils, eosinophils, basophils, and mast cells. Based on this, control over IL-1 in patients with CB is considered justified. Although the pathogenesis of COPD has not been fully studied and is still under discussion, it is known that chronic inflammation caused by constant exposure of the respiratory tract and lung parenchyma to cigarette smoke is a leading cause of COPD [19]. In a mouse model, N.S. Pauwels et al. showed an increase in the level of IL-1 in mice with long-term exposure to cigarette smoke compared to the control group. Later, the study in humans confirmed the data obtained in mouse models in lung tissue samples, as well as in the induced sputum of patients with COPD: the IL-1 level was significantly increased compared to the healthy controls [20].

The serum levels of IL-1β were also higher in patients with COPD than in the healthy controls. The level of the inflammatory mediator in the serum correlated with important clinical parameters for controlling the course of the disease, such as airflow limitation, smoking status, C-reactive protein (CRP), serum neutrophilia, etc. [21].

The levels of inflammatory markers, such as procalcitonin, CRP, CCL17, TNF and IL-1β, were analyzed depending on the type of CB exacerbation. A pronounced correlation was found between the severity of the exacerbation and the level of IL-1β. The

authors also came to the conclusion about a more significant correlation with bacterial inflammation and ventilator-associated pneumonia (VAP), which complicated the course of CB exacerbation [22].

A very promising research area is phenotyping of the *NLRP* gene depending on the level of IL-1β. So, studies by P. Ozretić not only proved an increase in IL-1β in the group of patients with CB compared to the healthy controls, but also traced the IL-1β level depending on the *NLRP* gene polymorphism. It was found that homozygosity for the main alleles was associated with a lower concentration of IL-1 [23].

Phenotyping of *NLRP* is of great scientific interest, since it makes it possible to detect various risk groups for developing bronchopulmonary pathology, on the whole, and CB, in particular.

INTERLEUKIN-6

Interleukin-6 (IL-6) is a member of the cytokine family which has proinflammatory and anti-inflammatory properties. IL-6 is encoded by the *IL-6* gene. Human IL-6 consists of 212 amino acids, including a signal peptide with 28 amino acids. Its gene is mapped to the chromosome 7, locus 7p15-21-q21. A segment of DNA in the regulatory region of this gene at position –572, where guanine (G) is replaced by cytosine (C), is called a genetic marker G (-572) C. The *IL-6* gene can exist in the form of two allelic variants, designated as the G-allele and the C-allele [24].

The cytokine is produced primarily by cells of the immune system, such as monocytes, lymphocytes, macrophages, endotheliocytes, microglia, and a number of non-immune cells, such as osteoblasts, myocytes, keratinocytes, synovial cells, chondrocytes, epithelial cells, folliculo-stellate cells of the pituitary gland, trophoblasts, vascular smooth muscle cells, etc.

IL-6 transmits signals through a complex of type I cytokine receptors on the cell membrane, consisting of a ligand-binding chain of IL-6Rα (CD126) and a signal-transmitting component, gp130 (also called CD130) [25].

IL-6 is responsible for stimulation of protein synthesis in the acute phase, as well as for neutrophilia. It supports the growth of B cells and is an antagonist of regulatory T cells. IL-6 can be secreted by macrophages in response to specific microbial molecules called pathogen-associated molecular pattern molecules (PAMPs). These PAMPs bind to an important group of detecting molecules of the innate immune system called pattern recognition receptors (PRRs), including Toll-like receptors (TLRs).

They are present on the cell surface and in intracellular compartments and induce intracellular signaling cascades that cause production of inflammatory cytokines [26]. In the light of recent data on the structure and functions of IL-6, studies have been conducted to identify the relationship between chronic obstructive and non-obstructive bronchitis. So, a recent longitudinal study, which investigated 1,843 participants for three years, demonstrated that an increased level of IL-6 was a prognostic factor in increasing mortality in chronic obstructive bronchitis [27]. Serum IL-6 level was significantly increased in the COPD groups compared to the healthy control [28, 29].

A. Agusti et al. demonstrated in the sample of 2,254 people that an increase in IL-6 associated with persistent inflammation was characterized by worse prognosis for CB [30].

A meta-analysis conducted by J. Wei et al. including at least 6,837 patients showed that serum IL-6 levels increased even in mild COPD, which may be the best marker for early inflammation and associated comorbidities. IL-6 is directly involved in inflammation and can be considered as a marker of mild systemic inflammation and an additional parameter for risk assessment along with smoking, the number of exacerbations, the frequency of hospitalization, and mortality [31]. Some authors point out contradictions with some studies that did not find significant differences in the level of IL-6 and the severity of the disease; however, most studies have a small sample [32, 33].

INTERLEUKIN-8

Interleukin-8 (IL-8) is a member of the CXC chemokine subfamily. It is an important activator and chemoattractant for neutrophils and is involved in various inflammatory diseases. Numerous reports show that various cells express IL-8 mRNA and produce IL-8 protein, including monocytes, T-lymphocytes, neutrophils, fibroblasts, endothelial cells, and epithelial cells [34]. The human *IL-8* gene has a length of 5191 bp and contains four exons separated by three introns. It is located on the human chromosome 4, locus 4q12-q21. There are at least two different types of IL-8 receptors (CXCR1 and CXCR2). The activity of IL-8 is not species-specific. IL-8 affects adhesion of neutrophils to the endothelium and induces transendothelial migration of neutrophils. IL-8 also exhibits *in vitro* chemotactic activity against T-lymphocytes and basophils [35].

Since IL-8 is responsible for induction and maintenance of the inflammatory state, there is a high

probability of a correlation between exacerbations of CB and the serum IL-8 level. W.I. de Boer et al. demonstrated that the IL-8 level in the bronchoalveolar lavage (BAL) was 1.4 times higher compared to the control group, but did not determine a significant correlation between the level of IL-8 in the epithelial tissue and the severity of exacerbation [36]. These findings allow to suppose that the IL-8 level can be a parameter of a local neutrophil response before manifestations of CB exacerbation, which will allow to take preventive measures.

In the experimental model, a relationship between high levels of IL-8 and airway remodeling in diseases associated with chronic inflammation of the lung tissue was revealed, acting directly on smooth muscle cells reducing their length and increasing their sensitivity to inflammation [37]. The work by J. Zhang and C. Bai demonstrated a correlation between the IL-8 level in an exacerbation of chronic obstructive bronchitis and the level of inflammatory markers. In people with COPD without an exacerbation, IL-8 was significantly higher compared to the healthy controls, which may again indirectly indicate a relationship between the level of the cytokine and airway wall remodeling [38].

IL-8 is a promising marker, an increase in which may signal more pronounced airway remodeling in people with persistent COPD. However, this hypothesis is always secondary in the studies mentioned above, and no targeted long-term studies have been carried out on this topic.

INTERLEUKIN-10

Interleukin-10 (IL-10) is a powerful anti-inflammatory cytokine that reduces inflammation in some disease models [39]. Being an anti-inflammatory cytokine, IL-10 serves to counteract the proinflammatory effects of other cytokines, and thus can strike the balance between pro- and anti-inflammatory systems. IL-10 inhibits expression of cytokines, such as TNFα, IL-1β, and IL-8. It can inhibit expression of adhesion molecules [40]. It has immunoregulatory and pleiotropic effects. It is mainly secreted by macrophages, Th1 and Th2 lymphocytes, dendritic cells, cytotoxic T cells, B lymphocytes, monocytes, and mast cells [41]. It inhibits expression of Th1 cytokines, MHC class II molecules, and co-stimulatory molecules on macrophages. IL-10 increases B-cell survival and proliferation and production of antibodies. IL-10 can also block the activity of NF-κB and is involved in the regulation of the JAK-STAT signaling pathway [42]. Further studies showed that IL-10 mainly inhibits the proinflammatory cytokines TNFα, IL-1β, IL-12, and IFNγ from TLR induced by lipopolysaccharide (LPS) and bacterial production and activates myeloid cells [43].

In terms of pulmonary inflammation, there are currently extensive data on direct involvement of IL-10 in regulation of inflammation in the lungs. According to recent observations, a decreased level of IL-10 was associated with more frequent development of exacerbations in people with CB, and IL-10 levels were significantly lower compared to the healthy controls [44, 45]. In addition, it was demonstrated that serum and sputum levels of IL-10 were higher in healthy, non-smoking patients compared to patients with COPD and healthy smokers [46]. Moreover, the levels of IL-10 in healthy smokers were suppressed in the BAL [47, 48]. Currently, there is some inconsistency among the authors regarding a correlation between IL-10 and other factors that activate inflammation, which is most likely associated with its polymorphism and requires a more detailed and in-depth study [49–51]. Therefore, the IL-10 level may be useful for prognosing the patient's condition in terms of development of bronchopulmonary pathologies and determination of risk groups, as well as for taking more effective preventivemeasures.

MONOCYTE CHEMOATTRACTANT PROTEIN-1

Monocyte chemoattractant protein-1 (MCP-1, CCL2) appears to be a member of the cytokine group belonging to the CC chemokine family, also known as CCL2. MCP-1 is a monomeric polypeptide with a molecular weight of about 13–15 kDa, depending on the level of glycosylation [52]. CCL2 is mainly secreted by monocytes, macrophages, dendritic cells, epithelial cells, astrocytes, fibroblasts, and endotheliocytes.

The *MCP-1* gene located on the chromosome 17 consists of three exons and two introns; the gene length is 1927 bp [53]. CCL2 is fixed in the plasma membrane of endothelial cells with glycosaminoglycan side chains of proteoglycans. Enhanced production of MCP-1 can occur under the influence of many factors, such as TNF, LPS of bacterial agents, interleukin-1, interferons, platelet growth factor, etc. [54].

Induction of MCP-1 initially attracts monocytes and basophils to the site of inflammation. After deletion of the N-terminal residue, MCP-1 loses its specificity for basophils and becomes an eosinophil chemoattractant. After exposure to MCP-1, basophils and mast cells release their granules into the intercellular space. This effect can also be enhanced by pretreat-

ment with IL-3 or other cytokines [55]. CCL2 is involved in the pathogenesis of several diseases characterized by monocytic infiltrates.

So, in the studies by A. Di Stefano et al., an increase in serum MCP-1 was observed in patients with COPD with an exacerbation compared to patients without COPD [56]. The same trend was demonstrated by S. Traves et al. for the levels of MCP-1 in BAL and sputum: the content of MCP-1 in sputum was elevated in patients with CB compared to the control group and the group of healthy smokers. There was a direct correlation between the level of neutrophils in the sputum and the level of MCP-1 and a negative correlation between the MCP-1 level and FEV1, which suggests that MCP-1 can participate in the inflammatory load during an exacerbation of CB and directly indicates clinical manifestations [57]. This work confirmed the findings of earlier studies on direct involvement of MCP-1 in the inflammatory process and monocyte macrophage infiltration of the bronchiole walls during an exacerbation and without it in patients with CB [58, 59].

TYPE 1 PLASMINOGEN ACTIVATOR INHIBITOR

Type 1 plasminogen activator inhibitor (PAI-1), also known as an endothelial plasminogen inhibitor, is a serine protease inhibitor that functions as an antagonist of tissue plasminogen activator and inhibits fibrinolysis [60]. It is located on the chromosome 7, locus 7q21.3-Q2 in the gene called SERPINE1, in the promoter region of which there is a 5G \ 4G polymorphism [61].

PAI-1 is mainly produced by the endothelium (cells lining the blood vessels). High expression of PAI-1 in cultured endothelial cells suggests that these cells make a significant contribution to the PAI-1 pool. However, *in vitro* studies show that PAI-1 is synthesized by various cells, and its biosynthesis can be caused by growth factors, cytokines, hormones, and other compounds [62].

In pathological conditions, a big amount of PAI-1 is secreted by other tissues: tumor cells, endothelial cells in response to inflammatory cytokines, and other cells activated by inflammation. High plasma PAI-1 levels are constantly detected in patients with severe sepsis, tumor processes, and other acute or chronic inflammatory diseases, such as atherosclerosis. PAI-1 is activated by inflammatory cytokines and, therefore, can be considered as a marker for the ongoing inflammatory process. However, it is very important that no classical elements of the inflammatory response were found in the promoter region of PAI-1, and it is still unclear through what mechanism PAI-1 expression is activated during inflammation [63].

For example, in the analysis of CB and COPD with and without metabolic syndrome (MS) in the ethnic group, it was shown that the polymorphism of alleles can directly predispose to the development of both variants. The 4G \ 4G genotype was more common in the group with MS and in the group with COPD and MS [61], as evidenced by worldwide studies of other ethnic groups [64, 65, 67].

A direct correlation between the clinical data on manifestations of CB and laboratory data was proved in the work by H. Wang et al. Serum PAI-1 levels were significantly increased in patients with COPD, especially in smokers with COPD, and serum PAI-1 levels were associated with parameters of lung function, such as FEV 1 / Pre, FEV 1 / FVC, and CRP [66]. However, neither comorbid COPD nor airflow limitation (from mild to very severe stages) was considered.

According to a similar design, the same results were obtained in the study by B. Waschki et al. The level of PAI-1 increased regardless of the concomitant pathology, and the highest levels of PAI-1 were observed in patients with stage II and III COPD according to Global Initiative for Obstructive Lung Disease (GOLD) [68].

TISSUE FACTOR

Tissue factor (TF) is a transmembrane protein that is present on the surface of subendothelial tissue and leukocytes and directly involved in the cascade of the coagulation system, both in the external and internal pathways [69]. The tissue factor is a 47 kDa glycoprotein consisting of three domains: the cytoplasmic domain, which is involved in the signaling function of the tissue factor, the hydrophobic transmembrane domain, which passes directly through the membrane, and the extracellular domain, which consists of two fibronectin filaments and a hydrophobic nucleus and has three N-terminal binding sites with carbohydrates. The main function is performed by the last two domains; without the cytoplasmic end, the tissue factor is functional [70].

TF signaling plays a role in the angiogenesis and apoptosis [71]. In the context of coagulation, TF can be found in the pool of circulating TF in the soluble form or bound to the membrane [72, 73].

Monocytes are some of the main sources of TF [74, 75] which is involved in formation of blood clots in pa-

tients with myocardial infarction [76, 77], as well as in other thrombotic diseases [78]. *In vitro* platelet particle generation from differentiated human megakaryocytes showed that platelets can carry both TF and its mRNA [79]. TF is also expressed by neutrophils, triggering thrombin generation and clot formation. Neutrophil activation is necessary for the effect of TF on the cell membrane [80]. Platelets, neutrophils, and, as recently reported, even T-lymphocytes can be an important source of TF in patients [81].

Based on the research data, a hypothesis was put forward on the role of TF not only in coagulation, but also in other pathological processes. So, in a number of studies, it was shown that the level of TF can be increased not only in patients with severe or moderately severe COPD [82], but also in stable CB [83]. Not only did the pool of TF associated with the occurrence of chronic inflammation in the airways increase, but also a decrease in tissue factor pathway inhibitor (TFPI) was recorded, aimed at restraining the procoagulant ability of TF [84]. A direct correlation with other procoagulants and inflammatory markers was also observed [85].

COMPLEMENT SYSTEM

Complement factors are a part of the immune system, a set of circulating and membrane-bound proteins in human blood, the main function of which is to fight foreign agents [86]. Most of them belong to β-globulins [87]. According to the nomenclature, individual components of the complement system are denoted by the symbols Cl, C2, C3, C4, C5, C6, C7, C8, C9 or capital letters (D, B, P) and are called factors. [88]. There are also regulators of complement activity (RCA), whose main function is to inhibit activation of the complement system and protect cells [89].

There are three main ways to activate the complement system: the classical pathway, the lectin pathway, and the alternative pathway.

Activation of the classical pathway requires the presence of an antigen-antibody pattern. Activation occurs when C1q binds to IgM or IgG in complex with antigens. After that, a cascade of reactions takes place during which the activation of the C3 component occurs [91].

The lectin pathway is homologous to the classical pathway, but instead of C1q, there are opsonin, mannose-binding lectin (MBL), and ficolins. This pathway is activated by binding MBL to mannose residues on the surface of the pathogen, which activates MBL-related serine proteases, MASP-1 and MASP-2, which can then cleave C4 and C2. Their products bind together to form a classical C3 convertase, as in the classical pathway. Further, the pathway continues homologously according to the classical pattern [92].

The alternative pathway is associated with constant hydrolysis of a small amount of the C3 complement molecule due to the presence of a thioester bond in the given molecule. The process is called tickover and its speed is approximately 0.3–1% of C3 molecules per hour. This process has an internal positive loop, due to which, in theory, it should have an avalanche-like nature following support of factors B and D. However, due to factors H and I, this does not happen, as they inhibit this loop by breaking the C3 complex [93].

It is generally accepted that the main site for synthesis of complement system proteins is the liver, however, pulmonary alveolar type 2 epithelial cells synthesize and secrete complement proteins C2, C3, C4, C5 and factor B [94], while human bronchial epithelial cells can generate C3 [95]. Local complement synthesis provides understanding of the interaction between complement factors and lung disease. Inflammatory cytokines, such as IL-6, IL-1, TNFα, and IFNγ, can initiate complement synthesis in cells, such as resident polymorphonuclear leukocytes, epithelial cells, and fibroblasts [96].

Complement anaphylatoxins (C3a, C5a) are powerful inflammatory mediators involved in the exaggerated inflammatory response observed in CB. Recent studies have revealed elevated levels of circulating C3a and C5a in patients with COPD, which indirectly suggests that complement proteins may contribute to the pathogenesis of the disease [97]. Moreover, when assessing the levels of C3 and C4, which account for approximately ⅔ of the total pool of complement proteins, it was found that they were initially lower in patients with chronic obstructive bronchitis; and the more severe the course of the disease, the lower the levels [98, 99].

There is also a large number of studies that suggest that exposure to cigarette smoke leads to chronic activation of the complement system according to the alternative pathway [97, 100, 101]. S. Grumelli et al. obtained data showing that a decrease in CD46 expression correlated with a loss of lung function in COPD, which may help explain the principles of inflammation and excessive complement activation in this group of patients [102]. However, most authors agree that due to the sufficiently large pool of complement proteins and heterogeneity of the studied groups, there is currently no clear understanding of the state

of complement factors in CB, therefore, this topic requires further research [97, 98, 102].

CONCLUSION

The above-described biochemical markers are involved in the pathological processes in CB. Among these biochemical markers, IL-6 is especially worth noting as a marker that can help in early detection of a disease exacerbation, which allows to start more well-thought treatment. The same can be concluded about MCP-1, however, its evidence base is somewhat smaller and requires more detailed consideration.

These markers are useful not only in the field of scientific knowledge about the pathogenesis of CB, but also in clinical use and as potential targets for targeted therapy for this disease. A more detailed study of these biomarkers may help to construct a model of disease development and develop ways of clinical control and programs for prevention and control of disease sanogenesis.

REFERENCES

1. Kim V., Criner G.J. The chronic bronchitis phenotype in chronic obstructive pulmonary disease: Features and implications. *Curr. Opin. Pulm. Med*. 2015; 21 (2): 133–141. DOI: 10.1097/MCP.0000000000000145
2. Burgel P.R., Nesme-Meyer P., Chanez P. et al. Cough and sputum production are associated with frequent exacerbations and hospitalizations in COPD subjects. *Chest*. 2009; 135 (4): 975–982. DOI: 10.1378/CHEST.08-2062.
3. De Oca M. M., Halbert R.J., Lopez M.V. et al. The chronic bronchitis phenotype in subjects with and without COPD: the PLATINO study. *Eur. Respir. J*. 2012; 40 (1): 28–36. DOI: 10.1183/09031936.00141611.
4. Martinez C.H., Kim V., Chen Y. et al. The clinical impact of non-obstructive chronic bronchitis in current and former smokers. *Respir. Med*. 2014; 108 (3): 491–499. DOI: 10.1016/J.RMED.2013.11.003.
5. Lahousse L., Seys L.J.M., Joos G.F., Franco O.H., Stricker B.H., Brusselle G.G. Epidemiology and impact of chronic bronchitis in chronic obstructive pulmonary disease. *Eur. Respir. J*. 2017; 50 (2): 1–4. DOI: 10.1183/13993003.02470-2016.
6. Lu M., Yao W., Zhong N. et al. Chronic obstructive pulmonary disease in the absence of chronic bronchitis in China. *Respirology*. 2010; 15 (7): 1072–1078. DOI: 10.1111/J.1440-1843.2010.01817.X.
7. Carbone M., Ly B., Dodson R., Pagano I., Morris P., Dogan U., Gazdar A., Pass H., Yang H. Malignant mesothelioma: facts, myths, and hypotheses. *J. Cell Physiol.* 2012; 227 (1): 44–58. DOI: 10.1002/jcp.22724.
8. Chu W.M. Tumor necrosis factor. *Cancer Lett.* 2013; 328 (2): 222–225. DOI: 10.1016/j.canlet.2012.10.014.
9. Swardfager W., Lanctôt K., Rothenburg L., Wong A., Cappell J., Herrmann H. A meta-analysis of cytokines in Alzheimer's disease. *Biol. Psychiatry.* 2010; 68 (10): 930–941. DOI: 10.1016/j.biopsych.2010.06.012.
10. Dowlati Y., Herrmann N., Swardfager W., Liu H., Sham L., Reim E.K., Lanctôt K.L. A meta-analysis of cytokines in major depression. *Biol. Psychiatry.* 2010; 67 (5): 446–457. DOI: 10.1016/j.biopsych.2009.09.033.
11. Kim E.Y., Moudgil K.D. Immunomodulation of autoimmune arthritis by pro-inflammatory cytokines. *Cytokine.* 2017; 98: 87–96. DOI: 10.1016/j.cyto.2017.04.012.
12. Gan W.Q., Man S.P., Senthilselvan A. et al. Association between chronic obstructive pulmonary disease and systemic inflammation: a systematic review and a meta-analysis. *Thorax.* 2004; 59 (7): 547–580. DOI: 10,1136 / thx.2003.019588.
13. Yang Y., Jing Z., Xin D., Wang S. Association between tumor necrosis factor-α and chronic obstructive pulmonary disease: a systematic review and meta-analysis. *Ther. Adv. Respir. Dis*. 2019; 13: 1–4. DOI: 10.1177/1753466619866096.
14. Mosrane Y., Bougrida M., Alloui A.S., Martani M., Rouabah L. Systemic inflammatory profile of smokers with and without COPD. *Rev. Pneumol. Clin.* 2017; 73 (4): 188–198. DOI: 10.1016/j.pneumo.2017.07.003.
15. Jiang D.H., Wang X., Liu L.S. et al. The effect of ventilator mask atomization inhalation of ipratropium bromide and budesonide suspension liquid in the treatment of COPD in acute exacerbation period on circulating levels of inflammation and prognosis. *Eur. Rev. Med. Pharmacol. Sci.* 2017; 21 (22): 5211–5216. DOI: 10.26355 / eurrev_201711_13843.
16. Boraschi D., Tagliabue A. The interleukin-1 receptor family. *Semin. Immunol*. 2013; 25 (6): 394–407. DOI: 10.1016/j.smim.2013.10.023.
17. Rider P., Carmi Y., Guttman O., Braiman A., Cohen I., Voronov E., White M.R. et al. IL-1α and IL-1β recruit different myeloid cells and promote different stages of sterile inflammation. *J. Immunol.* 2011; 187 (9): 4835–4843. DOI: 10.4049/jimmunol.1102048.
18. Dinarello C.A. Interleukin-1 in the pathogenesis and treatment of inflammatory diseases. *Blood.* 2011; 117 (14): 3720–3732. DOI: 10.1182 / blood-2010-07-273417.
19. Cosio M.G., Majo J., Cosio M.G. Inflammation of the airways and lung parenchyma in COPD: role of T cells. *Chest.* 2002; 121 (5): 160–165. DOI: 10.1378/chest.121.5_suppl.160s.
20. Pauwels N.S., Bracke K.R., Dupont L.L. et al. Role of IL-1alpha and the Nlrp3/caspase-1/IL-1beta axis in cigarette smoke-induced pulmonary inflammation and COPD. *Eur. Respir. J.* 2011; 38 (5): 1019–1028. DOI: 10.1183 / 09031936.00158110.
21. Bafadhel M., McKenna S., Terry S. et al. Acute exacerbations of chronic obstructive pulmonary disease: identification of biologic clusters and their biomarkers. *Am. J. Respir. Crit. Care Med.* 2011; 184 (6): 662–671. DOI: 10.1164/rccm.201104-0597OC.
22. Zou Y., Chen X., Liu J., Zhou D.B., Kuang X., Xiao J., Yu Q. et al. Serum IL-1β and IL-17 levels in patients with COPD: Associations with clinical parameters. *Int.*

J. Chronic Obstr. Pulm. Dis. 2017; 12: 1247–1254. DOI: 10.2147/COPD.S131877.
23. Ozretić P., Filho P., Catalano C., Sokolović I., Vukić-Dugac A., Šutić M. Association of NLRP1 coding polymorphism with lung function and serum IL-1β concentration in patients diagnosed with chronic obstructive pulmonary disease (COPD). 2019; 10 (10): 783. DOI: 10.3390/genes10100783.
24. Fragoso J.M., Delgadillo H., Juárez-Cedillo T., Rodríguez-Pérez J.M., Vallejo M., Pérez-Méndez O. et al. The interleukin 6 -572 G>C (rs1800796) polymorphism is associated with the risk of developing acute coronary syndrome. *Genet. Test Mol. Biomarkers.* 2010; 14 (6): 759–763. DOI: 10.1089/gtmb.2010.0001.
25. Heinrich P.C., Behrmann I., Müller-Newen G., Schaper F., Graeve L. Interleukin-6-type cytokine signalling through the gp130/Jak/STAT pathway. *The Biochemical Journal.* 1998; 334 (2): 297–314. DOI: 10.1042/bj3340297.
26. Tanaka T., Narazaki M., Kishimoto T. Il-6 in inflammation, immunity, and disease. *Cold Spring Harb. Perspect. Biol.* 2014; 6 (10): 1–4. DOI: 10.1101/cshperspect.a016295.
27. Celli B.R., Locantore N., Yates J., Tal-Singer R., Miller B.E., Bakke P., Calverley P., Coxson H., Crim C. et al. ECLIPSE Investigators. Inflammatory biomarkers improve clinical prediction of mortality in chronic obstructive pulmonary disease. *Am. J. Respir. Crit. Care Med.* 2012; 185 (10): 1065–1072. DOI: 10.1164/rccm.201110-1792OC
28. Donaldson et al. Airway and systemic inflammation and decline in lung function in patients with COPD. *Chest.* 2005;128 (4):1995–2004. DOI: 10.1378/chest.128.4.1995.
29. Garcia-Rio F., Miravitlles M., Soriano J.B., Munoz L., Duran-Tauleria E., Sanchez G., Sobradillo V., Ancochea J. Systemic inflammation in chronic obstructive pulmonary disease: a population-based study. *Respiratory Research.* 2010; 11 (1): 63–77. DOI: 10.1186/1465-9921-11-63.
30. Agusti A., Edwards L.D., Rennard S.I., MacNee W., Tal-Singer R., Miller B.E., Vestbo J. et al. Persistent systemic inflammation is associated with poor clinical outcomes in COPD: a novel phenotype. *PLoS ONE.* 2012; 7 (5): e37483. DOI: 10.1371/journal.pone.0037483.
31. Wei J., Xiong X., Lin Y., Zheng B., Cheng D. Association between serum interleukin-6 concentrations and chronic obstructive pulmonary disease: a systematic review and meta-analysis. *Peer J.* 2015; 3: 1199. DOI: 10.7717 / peerj.1199.
32. Sabit R., Bolton C.E., Edwards P.H., Pettit R.J., Evans W.D., McEniery C.M. et al. Arterial stiffness and osteoporosis in chronic obstructive pulmonary disease. *American Journal of Respiratory and Critical Care Medicine.* 2007; 175 (12): 1259–1265. DOI: 10.1164/rccm.200701-067OC.
33. Van Helvoort H.A., Heijdra Y.F., Thijs H.M., Vina J., Wanten G.J., Dekhuijzen P.N. Exercise-induced systemic effects in muscle-wasted patients with COPD. *Medicine and Science in Sports and Exercise.* 2006; 38 (9): 1543–1552. DOI: 10.1249/01.mss.0000228331.13123.53.
34. Zhang W., Chen H. The study on the interleukin-8. *Sheng Wu Yi Xue Gong Cheng Xue Za Zhi.* 2002; 19 (4): 697–702.
35. Hébert C.A., Baker J.B. Interleukin-8: a review. *Cancer Invest.* 1993; 11 (6): 743–750. DOI: 10.3109/07357909309046949.
36. De Boer W.I., Sont J.K., van Schadewijk A., Stolk J., van Krieken J.H., Hiemstra P.S. Monocyte chemoattractant protein 1, interleukin 8, and chronic airways inflammation in COPD. *J. Pathol.* 2000; 190 (5): 619–626. DOI: 10.1002/(SICI)1096-9896(200004)190:5<619::AID-PATH555>3.0.CO;2-6.
37. Govindaraju V., Michoud M.C., Al-Chalabi M., Ferraro P., Powell W.S., Martin J.G. Interleukin-8: novel roles in human airway smooth muscle cell contraction and migration. *Am. J. Physiol. Cell Physiol.* 2006; 291 (5): 957–965. DOI: 10.1152/ajpcell.00451.2005.
38. Zhang J., Bai C. The significance of serum interleukin-8 in acute exacerbations of chronic obstructive pulmonary disease. *Tanaffos.* 2018; 17 (1): 13–21.
39. Samuel D., López-Vales R., Wee Yong V. Harmful and beneficial effects of inflammation after spinal cord injury: potential therapeutic implications. *Handbook of Clinical Neurology.* 2012; 109: 485–502. DOI: 10.1016/B978-0-444-52137-8.00030-9.
40. Hector R., Wong J.E., Nowak-Stephen W., de Oliveira C.F. *Sepsis. Pediatric Critical Care.* 2011;4: 1413–1429. DOI: 10.1016/B978-0-323-07307-3.10103-X.
41. Pestka S., Krause C.D., Sarkar D., Walter M.R., Shi Y., Fisher P.B. Interleukin-10 and Related Cytokines and Receptors. *Ann. Rev. Immunol.* 2004; 22: 929–979. DOI: 10.1146/annurev.immunol.22.012703.104622.
42. McGeachy M.J., Bak-Jensen K.S., Chen Y., Tato C.M., Blumenschein W., Cua D.J. TGF-β and IL-6 drive the production of IL-17 and IL-10 by T cells and restrain TH-17 cell-mediated pathology. *Nat. Immunol*. 2007; 8 (12): 1390–1397. DOI: 10.1038/ni1539.
43. Spits H., De Waal M.R. Functional characterization of human IL-10. *Int. Arch. Allergy Immunol*. 1992; 99 (1): 8–15. DOI: 10.1159/000236329.
44. Couper K.N., Blount D.G., Riley E.M. IL-10: The Master Regulator of Immunity to Infection. *J. Immunol*. 2008; 180 (9): 5771–5777. DOI: 10.4049/jimmunol.180.9.5771.
45. LeVan T.D., Romberger D.J., Siahpush M., Grimm B.L. Relationship of systemic IL-10 levels with proinflammatory cytokine responsiveness and lung function in agriculture workers. *Respir. Res*. 2018; 19 (1): 166. DOI: 10.1186/s12931-018-0875-z.
46. Zhang L., Cheng Z., Liu W., Wu K. Expression of interleukin (IL)-10, IL-17A and IL-22 in serum and sputum of stable chronic obstructive pulmonary disease patients. *COPD*. 2013; 10 (4): 459–465. DOI: 10.3109/15412555.2013.770456.
47. Takanashi S., Hasegawa Y., Kanehira Y., Yamamoto K., Fujimoto K., Satoh K., Okamura K. Interleukin-10 level in sputum is reduced in bronchial asthma, COPD and in smokers. *Eur. Respir. J*. 1999; 14 (2): 309–314. DOI: 10.1034/j.1399-3003.1999.14b12.x.
48. Moermans C., Heinen V., Nguyen M., Henket M., Sele J., Manise M., Corhay J.L., Louis R. Local and systemic cellular inflammation and cytokine release in chronic

obstructive pulmonary disease. *Cytokine*. 2011; 56 (2): 298–304. DOI: 10.1016/j.cyto.2011.07.010.
49. Figueiredo C.A., Barreto M.L., Alcantara-Neves N.M., Rodrigues L.C., Cooper P.J., Cruz A.A. et al. Coassociations between IL10 polymorphisms, IL-10 production, helminth infection, and asthma/wheeze in an urban tropical population in Brazil. *J. Allergy Clin. Immunol*. 2013; 131 (6): 1683–1690. DOI: 10.1016/j.jaci.2012.10.043.
50. Bradford E., Jacobson S., Varasteh J., Comellas A.P., Woodruff P., O'Neal W., DeMeo D.L., Li X., Kim V., Cho M. et al. The value of blood cytokines and chemokines in assessing COPD. *Respir. Res*. 2017; 18 (1): 180. DOI: 10.1186/s12931-017-0662-2.
51. Demeo D.L., Campbell E.J., Barker A.F., Brantly M.L., Eden E., McElvaney N.G. et al. IL10 polymorphisms are associated with airflow obstruction in severe alpha1-antitrypsin deficiency. *Am. J. Respir. Cell Mol. Biol*. 2008; 38 (1): 114–120. DOI: 10.1165/rcmb.2007-0107OC.
52. Yoshimura T. The chemokine MCP-1 (CCL2) in the host interaction with cancer: a foe or ally? *Cellular & MolecularImmunology.* 2018; 15 (4): 335–345. DOI: 10.1038/cmi.2017.135.
53. Batiushin M., Gadaborsheva K. Monocyte chemoattractant protein-1: its role in the development of tubulointerstitial fibrosis in nephropathies. *Medical News of the North Caucasus.* 2017; 2: 1–3.
54. Panee J. Monocyte Chemoattractant Protein 1 (MCP-1) in obesity and diabetes. *Cytokine.* 2012; 60 (1): 1–12. DOI: 10.1016/j.cyto.2012.06.018.
55. Dean R.A., Cox J.H., Bellac C.L., Doucet A., Starr A.E., Overall C.M. Macrophage -specific metalloelastase (MMP-12) truncates and inactivates ELR+ CXC chemokines and generates CCL2, -7, -8, and -13 antagonists: potential role of the macrophage in terminating polymorphonuclear leukocyte influx. *Blood.* 2008; 112 (8): 3455–3464. DOI: 10.1182/blood-2007-12-129080.
56. Di Stefano A., Coccini T., Roda E., Signorini C., Balbi B., Brunetti G., Ceriana P. Blood MCP-1 levels are increased in chronic obstructive pulmonary disease patients with prevalent emphysema. *Int. J. Chron. Obstruct. Pulmon. Dis*. 2018; 13: 1691–1700. DOI: 10.2147/COPD.S159915.
57. Traves S., Culpitt S., Russell R., Barnes P., Donnelly L. Increased levels of the chemokines GROα and MCP-1 in sputum samples from patients with COPD. *Thorax*. 2002; 57 (7): 590–595. DOI: 10.1136/thorax.57.7.590.
58. De Boer W.I., Sont J.K., van Schadewijk A., Stolk J., van Krieken H., Hiemstra P.S. Monocyte chemoattractant protein 1, interleukin 8, and chronic airways in ammation in COPD. *J. Pathol*. 2000; 190 (5): 619–626. DOI:10.1002/(SICI)1096-9896(200004)190:5<619::AID-PATH555>3.0.CO;2-6.
59. Aldonyte R., Jansson L., Piitulainen E. Circulating monocytes from healthy individuals and COPD patients. *Respir. Res*. 2003; 4 (1): 11. DOI: 10.1186/1465-9921-4-11.
60. Mimuro J. Type 1 plasminogen activator inhibitor: its role in biological reactions. *The Japanese Journal of Clinical Hematology*. 1991; 32 (5): 487–489.
61. Borisova E.P., Kylbanova E.S., Asekritova A.S. Clinical and genetic features of chronic bronchitis with comorbid COPD with metabolic syndrome in Yakuts. *Bulletin of North-Eastern Federal University named after M.K.Ammosov*. 2014; 11 (4): 1 (in Russ.).
62. Lijnen H.R. Pleiotropic functions of plasminogen activator inhibitor-1. *Journal of Trombosis and Hemostasis.* 2005; 3 (1): 35–45. DOI: 10.1111 / j.1538-7836.2004.00827.x.
63. Binder B.R., Christ G., Gruber F., Grubic N., Hufnagl P., Krebs M., Mihaly J., Prager G.W. Plasminogen activator inhibitor 1: physiological and pathophysiological roles. *News in Physiological Sciences*. 2002; 17: 56–61. DOI: 10.1152/nips.01369.2001.
64. Berberoglu M., Evliyaoglu O., Adiyaman P. et al. Plasminogen activator inhibitor-1 (PAI-1) gene polymorphism (-675 4G/5G) associated with obesity and vascular risk in children. *Pediatr. Endocrinol. Metab.* 2006; 19 (5): 741–748. DOI: 10.1515/jpem.2006.19.5.741.
65. Khavinson V.Kh., Strekalov D.L., Lyshchev A.A. et al. Association analysis of some genetic risk factors for coronary heart disease with indicators of lipid metabolism and arterial pressure. *Kliniko-Laboratornyi Konsilium*. 2010; 4: 52–53.
66. Wang H., Yang T., Li D. et al. Elevated circulating PAI-1 levels are related to lung function decline, systemic inflammation, and small airway obstruction in chronic obstructive pulmonary disease. *Int. J. Chron. Obstruct. Pulmon. Dis.* 2016; 11: 2369–2376. DOI: 10.2147 / COPD.S107409.
67. Essa E.S., Wahsh R.A. Association between plasminogen activator inhibitor-1-675 4G/5G insertion/deletion polymorphism and chronic obstructive pulmonary disease. *COPD.* 2016; 13 (6): 756–775. DOI: 10.3109/15412555.2016.1168392.
68. Waschki B., Watz H., Holz O., Magnussen H., Olejnicka B., Welte T., Rabe K.F., Janciauskiene S. Plasminogen activator inhibitor-1 is elevated in patients with COPD independent of metabolic and cardiovascular function. *Int. J. Chron. Obstruct. Pulmon. Dis*. 2017; 12: 981–987. DOI: 10.2147/COPD.S128689.
69. Muller Y.A., Ultsch M.H., de Vos A.M. The crystal structure of the extracellular domain of human tissue factor refined to 1.7. *Journal of Molecular Biology*. 1996; 256 (1): 144–459. DOI: 10.1006/jmbi.1996.0073.
70. Zhang E., Charles R., Tulinsky A. Structure of extracellular tissue factor complexed with factor VIIa inhibited with a BPTI mutant. *Journal of Molecular Biology*.1999; 285 (5): 2089–2104. DOI: 10.1006/jmbi.1998.2452.
71. Ruf W., Disse J., Carneiro-Lobo T.C., Yokota N., Schaffner F. Tissue factor and cell signalling in cancer progression and thrombosis. *Journal of Thrombosis and Haemostasis*. 2011; 9 (1): 306–315. DOI: 10.1111/j.1538-7836.2011.04318.x.
72. Szotowski B., Antoniak S., Poller W. et al. Procoagulant soluble tissue factor is released from endothelial cells in response to inflammatory cytokines. *Circ. Res.* 2005; 96 (12): 1233–1239. DOI: 10.1161/01.RES.0000171805.24799.fa.

73. Davizon P., Lopez J.A. Microparticles and thrombotic disease. *Curr Opin Hematol.* 2009; 16(5): 334–341. DOI: 10.1097/MOH.0b013e32832ea49c.
74. Osterud B., Bjorklid E. Sources of tissue factor. *Semin. Thromb. Hemost.* 2006; 32 (1): 11–23. DOI: 10.1055/s-2006-933336.
75. Peshkova A.D., Le Minh G., Tutwiler V. et al. Activated monocytes enhance platelet-driven contraction of blood clots via tissue factor expression. *Sci. Rep*. 2017; 7 (1): 5149. DOI: 10.1038/s41598-017-05601-9.
76. Chiva-Blanch G., Laake K., Myhre P. et al. Platelet-, monocyte- derived and tissue factor-carrying circulating microparticles are related to acute myocardial infarction severity. *PLoS One*. 2017; 12 (2): 1–3. DOI: 10.1371/journal.pone.0172558.
77. Leatham E.W., Bath P.M., Tooze J.A. et al. Increased monocyte tissue factor expression in coronary disease. *Br. Heart J.* 1995; 73 (1): 10–13. DOI: 10.1136/hrt.73.1.10.
78. Shantsila E., Lip G.Y. The role of monocytes in thrombotic disorders. Insights from tissue factor, monocyte-platelet aggregates and novel mechanisms. *Thromb. Haemost.* 2009; 102(5): 916–924. DOI: 10.1160/TH09-01-0023
79. Brambilla M., Facchinetti L., Canzano P. et al. Human megakaryocytes confer tissue factor to a subset of shed platelets to stimulate thrombin generation. *Thromb. Haemost*. 2015; 114 (3): 579–592. DOI: 10.1160/TH14-10-0830
80. Darbousset R., Thomas G.M., Mezouar S. et al. Tissue factor-positive neutrophils bind to injured endothelial wall and initiate thrombus formation. *Blood.* 2012; 120 (10): 2133–2143. DOI: 10.1182/blood-2012-06-437772
81. De Palma R., Cirillo P., Ciccarelli G. et al. Expression of functional tissue factor in activated T-lymphocytes in vitro and in vivo: A possible contribution of immunity to thrombosis? *Int. J. Cardiol*. 2016; 218: 188–195. DOI: 10.1016/j.ijcard.2016.04.177
82. Vaidyula V.R., Criner G.J., Grabianowski C., Rao A.K. Circulating tissue factor procoagulant activity is elevated in stable moderate to severe chronic obstructive pulmonary disease. *Thromb. Res*. 2009; 124 (3): 259–261 DOI: 10.1016/j.thromres.2008.12.030
83. Jankowski M., Undas A., Kaczmarek P., Butenas S. Activated factor XI and tissue factor in chronic obstructive pulmonary disease: links with inflammation and thrombin generation. *Thromb. Res*. 2011; 127 (3): 242–246. DOI: 10.1016/j.thromres.2010.11.005.
84. Undas A., Jankowski M., Kaczmarek P., Sladek K., Brummel-Ziedins K. Thrombin generation in chronic obstructive pulmonary disease: dependence on plasma factor composition. *Thromb. Res*. 2011; 128 (4): 24–28. DOI: 10.1016/j.thromres.2011.05.004.
85. Szczypiorska A., Czajkowska-Malinowska M., Góralczyk B. Tissue factor and tissue factor pathway inhibitor in chronic obstructive pulmonary disease. *Folia Medica Copernicana*. 2015; 3 (1): 32–37.
86. Go A.S., Chertow G.M., Fan D. et al. Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. *N. Engl. J. Med*. 2004; 351 (13): 1296–1305. DOI: 10.1056/NEJMoa041031.
87. Drannik G.N., Maidannyk V.G. The role of the complement system in physiological and pathological body responses. *Medical Practice.* 1989; (4): 69–73 (in Russ.).
88. Maidannyk V.G., Bohomolets A.A. The complement system and complement-mediated injury of kidney disease in children. *International Journal of Pediatric, Obstetric and Gynecology.* 2013; 49 (1): 119–134.
89. Holers V.M. Complement and its receptors: new insights into human disease. *Annu. Re.v Immunol*. 2014; 32: 433–459. DOI: 10.1146/annurev-immunol-032713-120154.
90. Abbas A.K., Lichtman A.H., Pillai S. Cellular and molecular. *Immunology*. 2010; 6: 272–288.
91. Serna M., Giles J.L., Morgan B.P., Bubeck D. Structural basis of complement membrane attack complex formation. *Nature Communications Pediatric Hematology/Oncology and Immunopathology*. 2016; 7: 10587. DOI: 10.1038/ncomms10587.
92. Héja D., Kocsis A., Dobó J., Szilágyi K., Szász R., Závodszky P., et al. Revised mechanism of complement lectin-pathway activation revealing the role of serine protease MASP-1 as the exclusive activator of MASP-2. *PNAS USA.* 2012; 109(26): 10498–10503. DOI: 10.1073/pnas.1202588109.
93. Ferreira V.P., Pangburn M.K., Cortés C. Complement control protein factor H: The good, the bad, and the inadequate. *Mol. Immunol*. 2010; 47(13): 2187–97. DOI: 10.1016/j.molimm.2010.05.007.
94. Strunk R.C., Eidlen D.M., Mason R.J. Pulmonary alveolar type ii epithelial cells synthesize and secrete proteins of the classical and alternative complement pathways. *J. Clin. Invest*. 1988; 81: 1419–1426. DOI: 10.1172/JCI113472.
95. Varsano S., Kaminsky M., Kaiser M., Rashkovsky L. Generation of complement c3 and expression of cell membrane complement inhibitory proteins by human bronchial epithelium cell line. *Thorax*. 2000; 55 (5): 364–369. DOI: 10.1136/thorax.55.5.364.
96. Volanakis J.E. Transcriptional regulation of complement genes. *Annu. Rev. Immunol*. 1995; 13: 277–305. DOI: 10.1146/annurev.iy.13.040195.001425.
97. Westwood J.P., Mackay A., Donaldson G., Machin S., Wedzicha J.A., Scully M. The role of complement activation in COPD exacerbation recovery. *ERJ Open Res*. 2016; 2 (4): 27. DOI: 10.1183/23120541.00027-2016.
98. Chauhan S., Gupta M.K., Goyal A., Dasgupta D.J. Alterations in immunoglobulin and complement levels in chronic obstructive pulmonary disease. *Indian. J. Med. Res*. 1990; 92: 241-245.
99. Mahesh M., Yalamudi M., Lokesh S. Complement levels in chronic obstructive pulmonary disease: correlation with pulmonary function and radiological emphysema score. *International Journal of Scientific Study*. 2016; 3 (12): 284.
100. Kew R.R., Ghebrehiwet B., Janoff A. Cigarette smoke can activate the alternative pathway of complement in vitro by modifying the third component of complement.

J. Clin. Invest. 1985; 75 (3): 1000–1007. DOI: 10.1172/JCI111760.

101. Floreani A.A., Wyatt T.A., Stoner J., Sanderson S.D., Thompson E.G., Allen-Gipson D., Heires A.J. Smoke and c5a induce airway epithelial intercellular adhesion molecule-1 and cell adhesion. *Am. J. Respir. Cell Mol. Biol*. 2003; 29 (4): 472–448. DOI: 10.1165/rcmb.2002-0143OC

102. Grumelli S., Lu B., Peterson L., Maeno T., Gerard C. Cd46 protects against chronic obstructive pulmonary disease. *PLoS ONE*. 2011; 6(5): 18785. DOI: 10.1371/journal.pone.0018785.

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Role of the ubiquitin-proteasome system in the progression of oral squamous cell carcinoma

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ABSTRACT

The ubiquitin-proteasome system (UPS) controls the activity, subcellular localization, and stability of many cellular proteins that affect cellular homeostasis by regulating multiple signaling cascades. The activity of this system is associated with the emergence and progression of oral squamous cell carcinoma, since specific proteolysis of most intracellular proteins involved in the pathogenesis of cancer is implemented by this system.

The review article presents data on the characteristics of proteasomes and the process of substrate protein ubiquitination. The role of the ubiquitin-proteasome system in the pathogenesis of oral squamous cell carcinoma is shown, and the prospects of its use in precancerous diseases are described. The literature search was carried out in the search engines Medline, eLIBRARY, Scopus, The Cochrane Library, and RSCI.

Key words: ubiquitin-proteasome system, oral squamous cell carcinoma, proteasome, pathogenesis.

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Роль убиквитин-протеасомной системы в развитии плоскоклеточного рака полости рта

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РЕЗЮМЕ

Убиквитин-протеасомная система контролирует активность, субклеточную локализацию и стабильность множества клеточных белков, которые влияют на клеточный гомеостаз посредством регуляции сигнальных каскадов. Активность данной системы связана с возникновением и прогрессированием плоскоклеточного рака полости рта, так как специфический протеолиз большинства внутриклеточных протеинов, участвующих в патогенезе рака, происходит с помощью вышеупомянутой системы.

В обзорной статье представлены данные о характеристике протеасом и процессе убиквинтирования белков-субстратов. Показана роль убиквитин-протеасомной системы в патогенезе плоскоклеточного рака полости рта, приведены сведения о перспективах использования ее при предраке. Поиск литературы осуществлялся в поисковых системах Medline, Elibrary, Scopus, The Cochrane Library, РИНЦ.

Ключевые слова: убиквитин-протеасомная система, плоскоклеточный рак полости рта, протеасома, патогенез.

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

Oral squamous cell carcinoma (OSCC) is characterized by high mortality, early metastases, relapses, and a delay in seeking medical care in specialized institutions. The leading causes of death in patients with OSCC are metastases to the regional cervical lymph nodes and disease relapses. Doctors usually look at the prevalence of the tumor process to predict the course of the disease and choose an approach to treatment, however, the relationship between the prevalence of the tumor and the outcome of the disease and the treatment effectiveness is not always traced [1].

In some cases, clinical and morphological criteria are not very informative. According to statistics, about 25% of patients have latent metastases to regional lymph nodes at initial stages of the malignant process. The search for informative and reliable markers of squamous cell carcinoma is important for improving the prognosis and treatment of OSCC patients. [2]. Currently, the scientific literature describes many proteins that are involved in the pathogenesis of OSCC and control induction of angiogenesis, apoptosis, and metastasis. The ubiquitin-proteasome system (UBS) carries out specific proteolysis of most of these peptides [3].

CHARACTERISTICS OF THE UBIQUITIN-PROTEASOME SYSTEM

The ubiquitin-proteasome system (UPS) generates regulatory peptides, activates precursor proteins, provides intracellular protein hydrolysis, and is involved in preparing peptides for the class I major histocompatibility complex (MHC-1) [4]. The main components of UPS are proteasomes, ubiquitin molecules, and enzymes that activate and transport ubiquitin. The functional unit of this system is the proteasome.

Proteasomes are the main non-lysosomal multi-subunit proteases of eukaryotes; they hydrolyze up to 90% of cellular proteins. Proteasomes are multicatalytic complexes containing a cylinder-shaped 20S core particle, which consists of four heteroheptameric rings [5]. The two inner β-rings contain six proteolytic centers where substrates are cleaved; each ring has caspase-like (β1), trypsin-like (β2), and chymotrypsin-like (β5) activity [6].

The β-subunits (β1, β2, and β5) of the 20S proteasome particle can be completely or partially replaced by the immunosubunits LMP7 (β5i), LMP2 (β1i), and MECL1 (β2i), resulting in the immunoproteasome formation [7]. The main role of the immunoproteasome is to process antigens for presentation on MHC-

1 molecules. The immunoproteasome has higher chymotrypsin and trypsin activity and lower caspase activity than the standard 20S proteasome, which leads to alternative protein cleavage [8].

The two outer rings are composed of α-subunits that act as gatekeepers, controlling the access of substrates to the catalytically active β-chamber.

Proteasomes are not static complexes, and their activity can be modulated by binding of various proteasome activators (PAs), such as 19S, PA28, and PA200.

These proteasome regulators can symmetrically and asymmetrically bind to the α-rings of the 20S nucleus, forming proteasomes with a single or double cap. The binding of α-rings to regulatory particles leads to an increase in the proteasome activity by many times. Nevertheless, the free 20S proteasome unit remains a very common conformation in cells [9–12].

There are two types of proteasomes: 26S and 20S. The main hydrolyzing 26S proteasome consists of two subcomplexes: a catalytic 20S core particle and one or two 19S regulatory particles, which act as proteasome activators with a molecular weight of approximately 700 kDa (PA700). The 19S subcomplex recognizes ubiquitinated proteins, unfolds them, and moves inside the 20S core particle [13–15]. The immune forms of the 26S proteasome perform an important function: they produce immunogenic proteins for their further presentation by MHC-1 [15]. Regulatory particles implement specific substrate degradation. For example, if the PA28 protein complex acts as a regulatory particle, then the activated 20S proteasome will expose abnormal, small, and short-lived proteins to proteolysis [14].

UBIQUITINATION

The entry into the 20S core particle is usually closed by a regulatory particle acting as a gatekeeper. For penetration into the proteasome, the substrate protein must undergo polyubiquitination – attachment of a polyubiquitin chain (polyUb), which contains at least four monomers of ubiquitin (Ub). Then ATP-dependent activation of ubiquitin by a ubiquitin-activating enzyme (E1) and transfer of activated ubiquitin to a ubiquitin-conjugating enzyme (E2) take place, followed by formation of a peptide bond between ubiquitin and a substrate protein, catalyzed by a ubiquitin ligase (E3). The process is repeated several times in order to create a polyubiquitin chain through inter-ubiquitin bonds. During several cycles of protein ubiquitination, a build-up of a ubiquitin tag occurs, which is recognized by the 26S proteasome.

The substrate recognition by 26S proteasomes and their transfer to the proteolytic chamber occur due to the multi-subunit structure of the PA700 activator. After binding to the proteasome PA700 regulator, the ubiquitin chain is cleaved from the ubiquitinated substrate protein, the protein is unfolded and then transferred to the central chamber of the 20S proteasome, where it is degraded to short peptides, which then exit at the opposite pole of the proteasome.

When the proteolysis of the tagged molecule is complete, ubiquitin is released and tags another target. The proteasome is able to regulate both the amount and function of proteins: in some cases, the protein undergoes limited proteolysis (processing), which contributes to a significant change in the protein function (Fig. 1). Kinases, phosphatases, transcription and translation factors, cyclins, and inhibitors of cyclin-dependent kinases are processed or eliminated by the proteasome. This essential biological role of the UPS suggests that it is involved in the pathophysiology of inflammatory, viral, neurodegenerative, autoimmune, and oncological diseases [6].

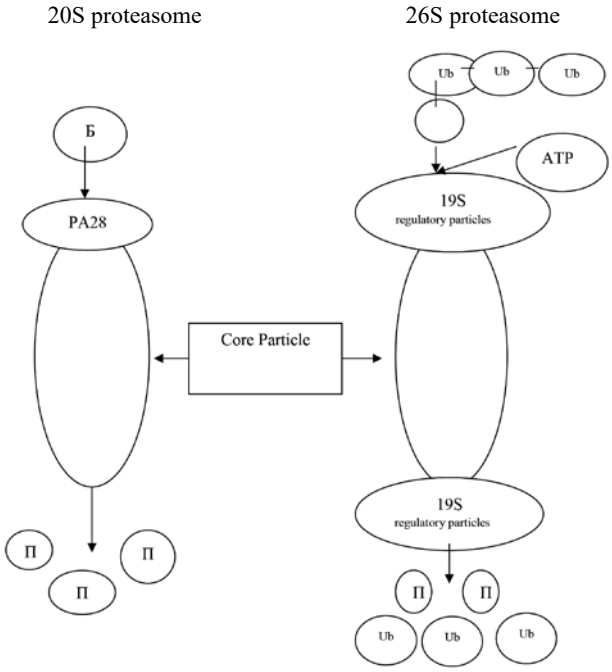

Fig. 1. Ubiquitination of proteins

CIRCULATING PROTEASOMES

Currently, circulating proteasomes are being actively investigated: the pathogenetic and prognostic value of these proteasomes, their biological signifi-

cance, and routes of exit into the extracellular space are being discussed.

Using the enzyme-linked immunosorbent assay (ELISA), 20S proteasomes were found in human serum. These proteasomes are now called circulating proteasomes or c-proteasomes. It was established that c-proteasomes are detected in the extracellular fluid of healthy people and patients with pathology [16].

According to the findings of quantitative iTRAQ-based proteomic analyses, it was revealed that the composition of the extracellular proteasome population included 19S regulatory particles and 20S core particles [17]. In addition, c-proteasomes obtained from blood plasma of healthy patients were similar in size, shape, subunit composition, and proteolytic activity to intracellular 20S proteasomes isolated from erythrocytes [18, 19]. Electron microscopy showed that purified c-proteasomes are intact 20S proteasome particles that are capable of hydrolyzing fluorogenic peptides [18, 20].

Taking into account the important role of the proteasome system in the pathogenesis of malignant neoplasms, it can be assumed that during tumor processes, proteasomes are capable of being secreted by cancer cells into the extracellular space or released into the circulation during breakdown of tumor cells [21].

Moreover, c-proteasomes can appear when the microparticles formed as a result of membrane blebbing are destroyed. This process is characterized by transfer of the contents of the plasma membrane to the membrane protrusions and subsequent formation of vesicles from the activated cells, which are microparticles of heterogeneous size (0.1–1 μm) with the corresponding content. The above-described structures, transporting various molecules, can act as messengers between cells [22, 23].

C-proteasomes can exist in a free, non-vesicular form. They are able to exist in the extracellular space, leaving exosomes. Exosomes are microscopic extracellular vesicles with a diameter of 30–100 nm, secreted by various cells and capable of carrying genetic information and protein markers, thus, being involved in intercellular communication [24]. It is believed that exosomes are involved in antigen presentation, non-classical secretion of proteins, and the pathogenesis of diseases associated with metabolic disorders, facilitate the immune response, and play a fundamental role in development of malignant tumors [25–27].

THE ROLE OF THE UPS IN THE MOLECULAR PATHOGENESIS OF OSCC

The 26S proteasomes play a significant role in the pathogenesis of malignant tumors, in particular, in the regulation of proliferation. Cyclins regulate progression of the cell through the cell cycle by sequential activation of cyclin-dependent kinases (CDK). These intracellular proteins are very unstable and exist for a short time. The number and presence of cyclins in the cell are regulated by proteasome-dependent degradation and transcription factors. UPS is involved in regulation of the stability of CDK inhibitors, as well as in hydrolysis of cyclins and their complexes [28].

The general scheme of interaction between cyclin and the 26S proteasome is the following: cyclin is polyubiquitinated and hydrolyzed by the proteasome after implementing its function, due to which the corresponding CDK becomes inactive, and the next cell cycle phase begins. For example, proteasome destruction of cyclin B leads to exit from mitosis [29]. When the cell passes through the restriction point located between the G1 phase and the S phase, proteasome-mediated destruction of cyclin A occurs. The anaphase stimulation complex (APC), which is a E3 ubiquitin ligase, ubiquitinates this cyclin [30]. The Skp1-Cul1-F-box(SCF)-containing and APC complexes are the key factors in cyclin degradation. At the same time, the SCF complex itself is regulated by APC through ubiquitination of the adapter protein Skp2, which suppresses SCF activity before the transition from the G1 phase to the S phase [31].

Studies reported that cyclin D1 is overexpressed in some primary human cancers, which confirms its role as an oncogene. In many tumors, genetic changes related to the cyclin *D1* gene often result in overexpression of the cyclin D1 protein. It was found that cyclin D1 also acts as a transcription modulator and regulates the activity of several transcription factors and histone deacetylase. The cyclin D1 protein is unstable with a short half-life of about 24 minutes. It is cleaved mainly by the 26S proteasome following the ubiquitin-dependent pathway. At the same time, cyclin D1 is an important proto-oncogene. Overexpression of cyclin D1 leads to shortening of the G1 phase and lower dependence on exogenous mitogens, which leads to abnormal cell proliferation, which, in turn, may contribute to additional genetic damage [32].

UPS plays an important role in maintaining the functional activity of cells, namely, in the regulation of the signaling systems, which are activated by inter-

action of growth factors with the corresponding receptors [33]. It was shown that proteasomes regulate the level of the transcription factor NF-κB, which is important for activation of gene expression in innate and adaptive immunity, inflammation, and stress responses. In cancer cells, NF-κB is involved in expression of the anti-apoptotic *IAP* gene family, as well as the pro-survival *BCL-2* genes [34]. Studies showed that proteasome activity in patients with head and neck cancers was higher than in the surrounding, relatively healthy tissue.

There is evidence that in patients with squamous cell carcinoma of the head and neck, the involvement of regional lymph nodes was accompanied by increased intracellular proteolysis. An increase in the total activity of proteasomes occurred along with an increase in the stage of cancer; however, a decrease in the expression of the LMP-2 proteasome subunit was observed. Changes in the expression of the transcription factor NFkappaBp50 and regression dependencies of the expression of the nuclear factor NF-kappaBp65 on the total activity of proteasomes were found [35].

The mechanisms of UPS involvement in the carcinogenesis include inhibition of vascular endothelial growth factor (VEGF)- and platelet-derived growth factor (PDGF)-mediated angiogenesis through degradation of platelet-derived growth factor receptor (PDGFR) and ubiquitination of vascular endothelial growth factor receptor (VEGFR) signaling pathway components, as well as proteasomal destruction of the α-subunit of the transcription factor HIF-1, which is impaired under hypoxic conditions. It subsequently leads to accumulation of HIF-1 in tumor cells and activation of transcription of genes involved in the angiogenesis [36, 37]. There are studies proving that proteasomes are involved in the post-translational modification of the p105 polypeptide, which is the precursor of NF-kappaBp50, which results in emergence of active forms of the transcription factor NF-kappaB. Moreover, a relationship between the level of HIF-1 production and the content of the transcription factor NF-kappaB was demonstrated. Most likely, it provides indirect involvement of NF-kappaBp50 in regulation of the VEGF level and neoangiogenesis in the tissue in head and neck squamous cell carcinomas [35]. This study showed that proteasome degradation of HEF-1 with the participation of PP-2A led to disruption of adhesive contacts with the extracellular matrix *in vitro* [37].

The UPS can play an important role in acquisition of immunity to antigrowth signals by transformed cells, degrading, along with caspases, the retinoblastoma (pRb) protein with the participation of a mouse double minute 2 (Mdm2) E3 ubiquitin ligase and destroying many components of the signaling pathway mediated by TGF-β [38]. In addition, the UPS is involved in regulation of apoptosis. Many nuclear proteins that implement programmed cell death are substrates for proteasomes: p53 tumor suppressor, transcription factors (c-Fos, c-Myc, AP-1), NFkB / IkB inhibitor, cell cycle regulators, caspase activity regulators (inhibitors of apoptosis (IAPs)), and proteins of the Bcl-2 family involved in proapoptotic signal transduction (cFLIP) [39].

UBIQUITIN-PROTEASOME SYSTEM IN OSCC

The accumulated data confirm that the UPS plays a key role in metabolism of proteins involved in regulation of many biological processes, such as cell cycle control, proliferation, apoptosis, neoangiogenesis, tumor progression, and metastasis [40].

Analysis of systematic literature reviews on PubMed (Ovid), EMBASE (Ovid), EBM (Ovid), and Web of Science (ISI) platforms by A. Villa et al. in 2018, aimed at identifying predictive biomarkers for stratification and long-term follow-up of progression of oral leukoplakia as an obligate precancer of OSCC, showed a correlation between the increased expression of genes associated with the proteasome system and a high risk of developing OSCC [41].

A study by J. Li et al. indicated that overexpression of the proteasome activator PA28γ was associated with a poor prognosis in patients with OSCC and promoted tumor progression. In addition, as a result of a study on a mouse xenograft model, it was found that the absence of PA28γ expression dramatically inhibited the growth and proliferation of cells in OSCC and slowed down tumor growth [42].

A proteomic study conducted by Z. Wang et al. to identify potential pathways for malignant transformation of oral leukoplakia into OSCC showed an increase in the expression of proteasome activators PA28a and PA28b, which confirms the clinical significance of proteasomes as a marker of early malignancy. This study demonstrated the role of proteasome degradation of proteins in the processing of intracellular antigens into peptides, which subsequently bound to MHC-1 molecules [43].

PA28γ-mediated mechanisms are of great importance for cancer therapy, especially in light of profoundly elevated levels of PA28γ in the tumor tissue. A significant increase in the level of PA28γ was ob-

served mainly in breast tumors, especially with a poor prognosis [44, 45], colorectal cancer [46], hepatocellular carcinoma [47], and OSCC [48].

The results of the studies by X. Feng et al. in 2016 showed that the activity of the proteasome activator PA28α was significantly higher in OSCC tissues compared to its activity in the healthy tissue. This study demonstrated that in immunohistochemistry, PA28α expression increased with the progression of dysplasia in the epithelium of the oral mucosa. It was found that after surgical treatment of moderately differentiated squamous cell carcinoma, no relapses occurred in the first two years. However, after radical treatment of a well-differentiated tumor, metastases to the cervical lymph nodes were diagnosed after two years, and the survival rate was four years.

The authors used reverse genetic approaches, which revealed that in oral squamous cells, along with a decrease in the PA28α expression, a consistent and statistically significant decrease in the ability to invade and migrate was observed. Invasion was reduced to 52% and migration – to 44%. Suppression of the PA28α expression led to a decrease in tumor growth of oral squamous cells *in vivo*. The volume of tumors decreased by 56% compared to tumors from the control group, while angiogenesis and apoptosis were not affected [49].

CONCLUSION

The UPS plays a key role in the pathogenesis of OSCC at the stages of malignancy onset and subsequent tumor progression. Recent studies on proteasome functioning in OSCC have demonstrated their key role in the molecular mechanisms of this disease. Further study of the proteasome system in the pathogenesis of OSCC will make it possible to find reliable markers for predicting the development of oral cancer from a precancer pathology and to assess the course of the disease.

REFERENCES

1. Choinzonov E.L., Spirina L.V., Kondakova I.V., Chizhevskaya S.Yu., Shishkin D.A., Kulbakin D.E. Prognostic value of determining the proteasome activity in the tissues affected by head and neck squamous cell carcinoma. *Bulletin of the Siberian Branch of RAS.* 2014; 34 (4): 103–108 (in Russ.).
2. Kakurina G.V., Kondakova I.V., Choinzonov E.L. Prognosis for head and neck squamous cell carcinomas. *Issues of Oncology*. 2012; 58 (1): 26–32 (in Russ.).
3. Voutsadakis I.A. Ubiquitination and the ubiquitin – proteasome system in the pathogenesis and treatment of squamous head and neck carcinoma. *Anticancer Research*. 2013; 33 (9): 3527–3542.
4. Lub S., Maes K., Menu E., Bruyne E., Vanderkerken K., Valckenborgh E. Novel strategies to target the ubiquitin proteasome system in multiple myeloma. *Oncotarget.* 2016; 7 (6): 6521–6537. DOI: 10.18632/oncotarget.6658.
5. Harshbarger W., Miller C., Diedrich C., Sacchettini J. Crystal structure of the human 20S proteasome in complex with carfilzomib. *Structure.* 2015; 23 (2): 418–424. DOI: 10.1016/j.str.2014.11.017.
6. Sorokin A.V., Kim E.R., Ovchinnikov L.P. Proteasome system of protein degradation and processing. *Advances in Biological Chemistry*. 2009; 49: 3–76 (in Russ.).
7. Sijts E.J., Kloetzel P.M. The role of the proteasome in the generation of MHC class I ligands and immune responses. *Cellular and Molecular Life Sciences.* 2011; 68 (9): 1491–1502. DOI: 10.1007/s00018-011-0657-y.
8. Guimaraes G., Gomes M., Campos P.C., Marinho F.V., de Assis N., Silveira T.N., Oliveira S.C. Immunoproteasome subunits are required for CD8+ T cell function and host resistance to brucella abortus infection in mice. *Infection and Immunity*. 2018; 86 (3): e00615–00617. DOI: 10.1128/IAI.00615-17.
9. Mao I., Liu J., Li X., Luo H. REGγ, a proteasome activator and beyond? *Cellular and Molecular Life Sciences.* 2008; 65 (24): 3971–3980. DOI: 10.1007/s00018-008-8291-z.
10. Savulescu A.F., Glickman M.H. Proteasome activator 200: the heat is on. *Molecular and Cellular Proteomics*. 2011; 10 (5): R110.006890. DOI: 10.1074/mcp.R110.006890.
11. Liu C.W., Jacobson A.D. Functions of the 19S complex in proteasomal degradation. *Trends in Biochemical Sciences.* 2013; 38 (2): 103–110. DOI: 10.1016/j.tibs.2012.11.009.
12. Cascio P. PA28αβ: the enigmatic magic ring of the proteasome? *Biomolecules.* 2014; 4 (2): 566–584. DOI: 10.3390/biom4020566.
13. Sharova N., Zakharova L. Multiple forms of proteasomes and their role in tumor fate. *Recent Patents on Endocrine, Metabolic Immune Drug Discovery*. 2008; 2: 152–161. DOI: 10.2174/187221408786241847.
14. Tanaka K. The proteasome: overview of structure and function. *The Proceedings of the Japan Academy, Series B*. 2009; 85: 12–36. DOI: 10.2183/pjab.85.12.
15. Sharova N.P., Astakhova T.M., Karpova Ya.D., Abaturova S.B., Lyupina Yu.V., Bogomyakova Yu.V., Abramova E.B., Erokhov P.A. Multiple forms of proteasomes as targets for development of new anti-cancer drugs. *Oncosurgery.* 2011; 3 (2): 37–42 (in Russ.).
16. Schliessmann S.S., Cicko S., Thomassen J., Anlasik T., Müller T., Idzko M., Zissel G., Sixt S.U. Acute smoke exposure decreases bronchial extracellular proteasome concentration. *The European Respiratory Journal*. 2011; 38: 737.
17. Zaykova Yu.Ya., Kulichkova V.A., Ermolayeva Yu.B., Bottrill A., Barlev N.A., Tsimokha A.S. Characteristics of extracellular proteasomes and proteins associated with them by iTRAQ-based mass spectrometry. *Cytology.* 2013; 55 (2): 111–122 (in Russ.).
18. Sixt S.U., Beiderlinden M., Jennissen H.P., Peters J. Extracellular proteasome in the human alveolar space: a new housekeeping enzyme? *American Journal of Physiology-Lung Cellular and Molecular Physiology*. 2007; 292 (2): 1280–1288. DOI: 10.1152/ajplung.00140.2006.

19. Bochmann I., Ebstein F., Lehmann A., Wohlschlaeger J., Sixt S.U., Kloetzel P.M., Dahlmann B. T lymphocytes export proteasomes by way of microparticles: a possible mechanism for generation of extracellular proteasomes. *Journal of Cellular and Molecular Medicine.* 2014; 18 (1): 59–68. DOI: 10.1111/jcmm.12160.
20. Zaykova Yu.Ya., Kulichkova V.A., Ermolayeva Yu.B., Gauze L.N., Tsimokha A.S. Comparative analysis of extra- and intracellular proteasomes in human K562 cell line. *Cytology*. 2011; 53 (6): 459–465 (in Russ.).
21. Van Zijl F., Krupitza G., Mikulits W. Initial steps of metastasis: cell invasion and endothelial transmigration. *Mutation Research*. 2011; 728 (1-2): 23–34. DOI: 10.1016/J.MRREV.2011.05.002.
22. Tang K., Liu J., Yang Z., Zhang B., Zhang H., Huang C., Ma J., Shen G.X., Ye D., Huang B. Microparticles mediate enzyme transfer from platelets to mast cells: a new pathway for lipoxin A4 biosynthesis. *Biochemical and Biophysical Research Communications.* 2010; 400 (3): 432–436. DOI: 10.1016/j.bbrc.2010.08.095.
23. Yuan A., Farber E.L., Rapoport A.L., Tejada D., Deniskin R., Akhmedov N.B., Farber D.B. Transfer of microRNAs by embryonic stem cell microvesicles. *PLoS One*. 2009; 4 (3): 4722. DOI: 10.1371/ journal.pone.0004722.
24. Yunusova N.V., Kondakova I.V., Kolomiets L.A., Molchanov S.V. Proteasomes and exosomes in ovarian cancer: association with the clinical course and prognosis. *Siberian Journal of Oncology.* 2014; (4): 53–59 (in Russ.).
25. Stamm T.A., Naryzhniy S.N., Landa S.B., Burdakov V.S., Artamonova T.O., Filatov M.V. Isolation and analysis of exosomes secreted by human malignant cells *in vitro*. *Cytology*. 2012; 54 (5): 430–438 (in Russ.).
26. Keller S., Konig A.-K., Marme F., Runz S., Wolterink S., Koensgen D., Mustea A., Sehouli J., Altevogt P. Systemic presence and tumor-growth promoting effect of ovarian carcinoma released exosomes. *Cancer Letters*. 2009; 278 (1): 73–81. DOI: 10.1016/j.canlet.2008.12.028.
27. Rupp A.-K., Rupp C., Keller S., Brase J.C., Ehehalt R., Fogel M., Moldenhauer G., Marmé F., Sültmann H., Altevogt P. Loss of EpCAM expression in breast cancer derived serum exosomes: role of proteolytic cleavage. *Gynecologic Oncology*. 2011; 122 (2): 437–446. DOI: 10.1016/j. ygyno.2011.04.035.
28. Tu Y., Chen C., Pan J., Xu J., Zhou Z-G., Wang C-Y. The ubiquitin proteasome pathway (UPP) in the regulation of cell cycle control and DNA damage repair and its implication in tumorigenesis. *International Journal of Clinical and Experimental Pathology*. 2012; 5 (8): 726–738.
29. Bassermann F., Eichner R., Pagano M. The ubiquitin proteasome system – implications for cell cycle control and the targeted treatment of cancer. *Biochimica et Biophysica Acta*. 2014; 1843 (1): 150–162. DOI: 10.1016/j.bbamcr.2013.02.028.
30. Borg N.A., Vishva M.D. Ubiquitin in cell-cycle regulation and dysregulation in cancer. *Annual Review of Cancer Biology.* 2017; 1: 59–77. DOI: 10.1146/annurev-cancerbio-040716-075607.
31. Skaar J.R., Pagano M. Control of cell growth by the SCF and APC/C ubiquitin ligases. *Current Opinion in Cell Biology*. 2009; 21 (6): 816–824. DOI: 10.1016/j.ceb.2009.08.004.
32. Alao J.P. The regulation of cyclin D1 degradation: roles in cancer development and the potential for therapeutic invention. *Molecular Cancer*. 2007; 6: 24. DOI: 10.1186/1476-4598-6-24.
33. Huang Q., Figueiredo-Pereira M.E. Ubiquitin/proteasome pathway impairment in neurodegeneration: therapeutic implications. *Apoptosis: an International Journal on Programmed Cell Death.* 2010; 15 (11): 1292–1311. DOI: 10.1007/s10495-010-0466-z.
34. Thibaudeau T.A., Smith D.M. A practical review of proteasome pharmacology. *Pharmacological Reviews*. 2019; 71 (2): 170–197. DOI: 10.1124/pr.117.015370.
35. Spirina L.V., Kondakova I.V., Choynzonov E.L., Chigevskaya S.Y., Shishkin D.A., Kulbakin, D.Y. Expression of vascular endothelial growth factor and transcription factors HIF-1, NF-kB expression in squamous cell carcinoma of head and neck; Association with proteasome and calpain activities. *Journal of Cancer Research and Clinical Oncology* 2013; 139 (4): 625–633 (in Russ.). DOI: 10.1007/s00432-012-1366-0.
36. Spirina L.V., Yunusova N.V., Kondakova I.V., Kolomiets L.A., Koval V.D., Chernyshova A.L., Shpileva O.V. Association of growth factors, HIF1 and NF-κB expression with proteasomes in endometrial cancer. *Molecular Biology Reports*. 2012; 39 (9): 8655–8662. DOI: 10.1007/ s11033-012-1720-y.
37. Rahimi N. The ubiquitin-proteasome system meets angiogenesis. *Molecular Cancer Therapeutics.* 2012; 11 (3): 538–548. DOI: 10.1158/1535-7163.MCT-11-0555.
38. Glasgow E., Mishra L. Transforming growth factor-beta signaling and ubiquitinators in cancer. *Endocrine-Related Cancer*. 2008; 15 (1): 59–72. DOI: 10.1677/ERC-07-0168.
39. Tsimokha A.S. Proteasomes: role in extracellular processes. *Cytology.* 2010; 52 (4): 277–300 (in Russ.).
40. Jang H.H. Regulation of protein degradation by proteasomes in cancer. *Journal of Cancer Prevention.* 2018; 23 (4): 153–161. DOI: 10.15430/JCP.2018.23.4.153.
41. Villa A., Celentano A., Glurich I., Borgnakke W.S., Jensen S.B., Peterson D.E., Delli K., Ojeda D. Vissink A., Farah C.S. World workshop on oral medicine vii: prognostic biomarkers in oral leukoplakia: a systematic review of longitudinal studies. *Oral Diseases*. 2019; 25 (Suppl. 1): 64–78. DOI: 10.1111/odi.13087.
42. Li J., Feng X., Sun C., Zeng X., Xie L., Xu H., Li T., Wang R., Xu X., Zhou X., Zhou M., Zhou Y., Dan H., Wang Z., Ji N., Deng P., Liao G., Geng N., Wang Y., Zhang D., Lin Y., Ye L., Liang X, Li L., Luo G., Jiang L., Wang Z., Chena Q. Associations between proteasomal activator PA28γ and outcome of oral squamous cell carcinoma: Evidence from cohort studies and functional analyses. *EBioMedicine*. 2015; 2 (8): 851–858. DOI: 10.1016/j.ebiom.2015.07.004.
43. Wang Z., Feng X., Liu X., Jiang L., Zeng X., Ji N., Li J., Li L., Chen Q. Involvement of potential pathways in malignant transformation from oral leukoplakia to oral squamous cell carcinoma revealed by proteomic analysis. *BMC Genomics*. 2009; 10: 383. DOI: 10.1186/1471-2164-10-383.
44. Wang X., Tu S., Tan J., Tian T., Ran L., Rodier J.F., Ren G. REG gamma: a potential marker in breast cancer and effect on

cell cycle and proliferation of breast cancer cell. *Medical Oncology*. 2011; 28 (1): 31–41. DOI: 10.1007/s12032-010-9546-8.
45. Chai F., Liang Y., Bi J., Chen L., Zhang F., Cui Y., Bian X., Jiang J. High expression of REGgamma is associated with metastasis and poor prognosis of patients with breast cancer. *International Journal of Clinical and Experimental Pathology*. 2014; 7(1): 7834–7843.
46. Arlt A., Bauer I., Schafmayer C., Tepel J., Sebens Muerkoster S., Brosch M., Roder C., Kalthoff H., Hampe J., Moyer M.P., Folsch U.R., Schäfer H. Increased proteasome subunit protein expression and proteasome activity in colon cancer relate to an enhanced activation of nuclear factor E2-related factor 2 (Nrf2). *Oncogene.* 2009; 28 (45): 3983–3996. DOI: 10.1038/onc.2009.264.
47. Kondo M., Moriishi K., Wada H., Noda T., Marubashi S., Wakasa K., Matsuura Y., Doki Y., Mori M., Nagano H. Up-regulation of nuclear PA28gamma expression in cirrhosis and hepatocellular carcinoma. *Experimental and Therapeutic Medicine*. 2012; 3 (3): 379–385. DOI: 10.3892/etm.2011.415.
48. Kontos C.K. Surrogate prognostic biomarkers in OSCC: The paradigm of PA28gamma overexpression. *EBioMedicine.* 2015; 2 (8): 784–785. DOI: 10.1016/j.ebiom.2015.07.032.
49. Xiaodong F., Yuchen J., Liang X., Lu J., Jing L., Chongkui S., Hao X., Ruinan W., Min Z., Yu Z., Dan H., Zhiyong W., Ning J., Peng D., Ga L., Ning G., Yun W., Dunfang Z., Yunfeng L., Qianming C. Overexpression of proteasomal activator PA28α serves as a prognostic factor in oral squamous cell carcinoma. *Journal of Experimental and Clinical Cancer Research*. 2016; 35: 35. DOI: 10.1186/s13046-016-0309-z.

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Mikhalev D.E. – conception and design, drafting of the manuscript. Baydik O.D. – conception and design, analysis of the article, critical revision of the manuscript for important intellectual content. Kondakova I.V., Sidenko E.A., Mukhamedov M.R., Sysolyatin P.G. – analysis of the article, critical revision of the manuscript for important intellectual content.

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Pathomorphological and molecular genetic features of diffuse gastric cancer

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ABSTRACT

Gastric cancer (GC) is the fifth most common type of cancer in the world and the third leading cause of death from cancer. GC is a multi-factorial and morphologically heterogeneous disease. Currently, several morphological classifications of GC are used, however, for diagnosis, it is necessary to take into account not only the morphological type of the tumor, but also its molecular subtype. According to the literature, the intestinal type of GC is most often associated with effects of environmental factors and is usually found in older age groups in men, while diffuse gastric cancer (DGC) is a genetically determined disease which is more common in younger patients, with the same frequency among men and women.

This review covers in detail GC, its classification by P.A. Lauren (1965), and its molecular subtypes characterized during the Cancer Genome Atlas project and examines the impact of certain risk factors on the pathogenesis of the disease, such as *H. pylori* infection or Epstein – Barr virus. A separate section in this analytical work is dedicated to expression of the PD-L1 marker by tumor cells and the use of this parameter for prognosis and therapy of this disease. An essential part of the work is discussion of the features of intestinal and diffuse types of gastric cancer, which reflect not only the differences in classifications used in modern diagnosis, but also the relationship between the pathological pattern and the molecular subtype of gastric cancer.

Key words: diffuse gastric cancer, epidemiology, molecular genetic diagnosis, classification, immunotherapy.

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Патоморфологические и молекулярно-генетические особенности диффузного типа рака желудка

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РЕЗЮМЕ

Рак желудка (РЖ) занимает пятое место в мире по распространенности среди всех злокачественных новообразований и является третьей по значимости причиной смертности от онкологических заболеваний.

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РЖ является мультифакториальным, морфологически неоднородным заболеванием. В настоящее время используется несколько морфологических классификаций РЖ, однако для постановки диагноза требуется учитывать не только морфологический тип опухоли, но и ее молекулярный подтип. По данным литературы, РЖ интестинального типа чаще всего ассоциирован с действием факторов окружающей среды и, как правило, встречается в старших возрастных группах у мужчин. Диффузный тип рака желудка (ДТРЖ) является в большей степени генетически детерминированным заболеванием и чаще встречается у более молодых пациентов, при этом с одинаковой частотой среди мужчин и женщин.

В данном обзоре подробно освещается тема РЖ, его классификация по P.A. Lauren (1965), его молекулярным подтипы, охарактеризованные в Атласе ракового генома (The Cancer Genome Atlas), а также рассматривается влияние определенных факторов риска на патогенез заболевания, таких как инфицирование *H. pylori* или вирусом Эпштейна – Барр. Отдельную роль в данной аналитической работе занимает вопрос экспрессии опухолевыми клетками маркера PD-L1 и использование данного параметра для прогнозирования и терапии этого заболевания. Немаловажной частью работы является обсуждение особенностей интестинального и диффузного типов рака желудка, которые отражают не только различия используемых в современной диагностике классификаций, но и взаимосвязь патоморфологической картины с молекулярным подтипом рака желудка.

Ключевые слова: рак желудка диффузного типа, эпидемиология, молекулярно-генетическая диагностика, классификация, иммунотерапия.

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INTRODUCTION

Gastric cancer (GC) is the fifth most common type of cancer in the world and the third leading cause of death from cancer. Morbidity and mortality differ depending on the geographical region. In countries like Japan, China, Korea, and Chile, GC ranks first in morbidity and mortality [1]. In Russia in 2018, 21,279 men and 15,662 women were diagnosed with GC. The average age of patients upon diagnosis was 67.5 years, and the incidence rate was 25.4 cases per 100,000 population. In 2017, 16,572 people died from gastric cancer in Russia. The average age of patients was 68.7 years, and the mortality rate per 100,000 population was 18.97 [2].

DEFINITION

In 1965, P.A. Lauren proposed a classification of GC, which contained intestinal, diffuse, and mixed histotypes [3]. GC is a multi-factorial and histologically heterogeneous disease. For example, intestinal GC is most often associated with environmental factors (diet, smoking, obesity, alcohol consumption) and *Helicobacter pylori* (*H. pylori*) infection [4], which, in turn, leads to development of chronic gastritis, followed by atrophic gastritis, intestinal metaplasia, dysplasia, and carcinoma through the Correa cascade [5].

The intestinal type occurs more frequently in older men, is usually macroscopically represented by a tumor with an exophytic type of growth and a tendency to ulceration, and microscopically consists of cells that form glands. Diffuse gastric cancer (DGC) is less associated with environmental factors and inflammatory diseases: the role of *H. pylori* in the pathogenesis of DGC is still debatable. However, a number of studies conducted in the UK and Japan showed the presence of *H. pylori* outside the tumor tissue in approximately 32% of cases in patients younger than 40 years with morphologically confirmed DGC [6].

DGC is considered to be a more genetically determined disease associated with loss of heterozygosity on p17 chromosome, p53 mutation or loss of heterozygosity, and mutation or loss of E-cadherin [7]. DGC is more common in younger patients with the same frequency among men and women. 1–3% of all cases of DGC are related to a proven heredi-

tary genetic syndrome that causes a mutation in the E-cadherin gene (*CDH1*) located on chromosome 16 — hereditary diffuse gastric cancer (HDGC). For this category of patients, gastrectomy is recommended as a method of preventive treatment [8].

MOLECULAR CLASSIFICATION OF GASTRIC CANCER

According to the Cancer Genome Atlas (TCGA) project, there are four molecular subtypes of GC [9]:

1. Associated with the Epstein – Barr virus (EBV-associated subtype), which accounts for about 9% of cancer cases.

2. Caused by microsatellite instability (MSI subtype): this subtype accounts for about 22% of cancer cases.

3. With genome stability (GS subtype): this subtype accounts for about 20% of cancer cases.

4. Caused by chromosomal instability (CIN subtype): this subtype accounts for about 50% of cancer cases.

EBV-associated molecular subtype of GC (Epstein – Barr Virus-Positive Gastric Cancer). Epstein – Barr virus (EBV) is a human γ-herpesvirus that is characterized by pronounced tropicity to the lymphatic system and has the ability to persist in the human body. Given the lymphotropicity of EBV after penetration into B-lymphocytes, the virus causes their uncontrolled proliferation. EBV also has pronounced tropicity to the gastric mucosa, which has a developed lymphatic system [10–12].

The International Agency for Research on Cancer (IARC) classifies EBV as a Group 1 carcinogen. According to a number of researchers, only latent EBV infection can be associated with various types of human neoplasms and hemoblastosis.

EBV-associated gastric adenocarcinoma was first described by A.P. Burke in 1990. It is known that about 90,000 people annually develop EBV-associated gastric carcinoma, which is about 10% of all cases of gastric cancer [13]. The association between *H. pylori* and EBV is not fully understood, but there is no doubt that in a group of patients with no history of *H. pylori* infection or who have undergone successful eradication of the pathogen, EBV may be a leading factor in chronic inflammation in the gastric mucosa and cause a risk of malignant neoplasms [14–16].

GC caused by microsatellite instability (MSI). Microsatellite instability (MSI) is a phenotype characterized by an increased probability of mutations occurring as a result of impaired repair system of incorrectly paired DNA bases. Following the impaired repair in cells, errors during DNA replication accumulate, which leads to emergence of new microsatellites.

When using a standard panel that includes BAT26 and BAT27 mononucleotides and D2S123, D5S346, and D17S250 dinucleotide repeats [17], microsatellite instability can be divided into 3 levels: high MSI (MSI-H), low MSI (MSI-L), and microsatellite stability (MSS). According to the literature, MSI-H-associated carcinoma, depending on the ethnic group, occurred in 5–50% of cases, and MSI-L- and MSS-associated carcinomas are known to be localized in the gastric antrum. According to the histological classification proposed by Lauren, they belong to the intestinal type of GC and have a good prognosis with rare metastasis, compared to MSI-H [18].

GC with genome stability (Genomically Stable, GS). GC with a stable genome has a lower mutation load compared to other molecular subtypes and occurs at a relatively young age. This molecular subgroup is characterized by a diffuse histological type according to Lauren, as well as a large number of mutations in RhoA and CDH1 (as mentioned earlier, CDH1 is associated with inherited DGC).

GC caused by chromosomal instability (CIN). Chromosomal instability is a type of genome instability in which non-clonal karyotype changes are observed in the daughter generations of dividing cells, namely, loss or acquisition of chromosomes and their sections. Cancer with chromosomal instability is characterized by extremely high frequency of chromosomal abnormalities and their high diversity [19]. In GC, a high CIN level is always associated with a poor prognosis.

In a study conducted by A. J. Bass et al. [9], the molecular subtype of GC was correlated with its localization. Each molecular subtype of GC could have any localization, but CIN-associated tumors were more common in the gastrointestinal tract and gastric cardia (65%); most EBV-associated tumors were found in the bottom or body of the stomach (62%); MSI-associated tumors were less often detected in the gastrointestinal tract and cardia, but

with approximately the same frequency were found in other parts of the stomach; GS-tumors were found with approximately the same frequency in all parts of the stomach, and this subtype was mostly presented by DGC (according to Lauren). At the same time, GS-tumors were diagnosed at an earlier age (the average age was 59 years), while MSI-associated tumors were diagnosed at a relatively older age (the average age was 72 years). Patients with MSI-associated tumors were usually women (56%), and most cases of EBV-associated tumors were detected in men (81%).

PD-L1 AND GASTRIC CANCER

Programmed cell death-1 (PD-1) is a membrane protein of the immunoglobulin superfamily involved in differentiation of immune cells. PD-1 plays an important role in negative regulation of the immune system by preventing activation of T-lymphocytes, which reduces autoimmunity and increases self-tolerance [20]. The protein has two ligands: PD-L1 and PD-L2. In tumor cells that express PD-L1, this ligand is involved in mechanisms of tumor escape from immune control. Determining the expression of PD-L1 makes it possible to identify a group of patients who are most likely to benefit from therapy by immune checkpoint inhibitors. A fairly good result of anti-PD-L1 therapy was achieved for non-small cell lung cancer.

A relationship between PD-L1 expression and prognosis in GC is still the subject of debate. According to a study by S. Boger et al. [21], patients with PD-L1-positive tumor cells had improved disease prognosis. However, according to H. Chang et al. [22], high PD-L1 expression was an unfavorable prognostic factor, and A. Kawazoe et al. [23] did not consider PD-L1 as a prognostic factor at all. It is important to keep in mind that all of the above-presented research data were obtained from patients of the Asian ethnic group, and, therefore, the results cannot be applied to the general population, since the response to therapy / drugs in different ethnic groups may not be the same.

According to the experiments by C. Ma. et al. [24] and S. Derks et al. [25], EBV- and MSI-associated GCs most often showed greatly positive PD-L1 expression or overexpression of PD-L1, which was a significant adverse prognostic factor. Another study [9] showed that in the molecular EBV- and MSI-associated subgroups of GC, pronounced lymphocytic infiltration in the tumor stroma was found. Therefore, these subtypes can be classified as GCs with severe lymphoid stroma (medullary carcinoma). The lymphoid stroma in these tumors has a large number of CD8+ T cells that can cause a strong anti-tumor inflammatory response. In addition, positive PD-L1 expression was associated with a significant increase in the number of CD8+ T cells at the edge of the invasive tumor front.

S. Derks et al. [25] observed a difference in the nature of infiltration of PD-L1-positive cells depending on the molecular subtype of the tumor. In EBV- and MSI-associated gastric carcinomas, PD-L1 positive cells had the ability to penetrate into the center of the tumor in contrast to MSS-associated gastric carcinomas, in which PD-L1 positive cells remained mainly at the edge of the tumor invasion area. Based on this result, patients with EBV- and MSI-associated gastric carcinomas may be the main candidates for therapy with PD-1 inhibitors.

According to H. Saito et al. [26], five-year survival in patients with and without positive PD-L1 expression differed by 48.9% and 80.7%, respectively. In addition, PD-L1 positive expression was observed in the older age group of patients, and by the histological type, positive expression was more often detected in undifferentiated gastric carcinomas.

L. Wang et al. [27] studied the correlation between HER2 status and PD-L1 expression. According to this research, HER2-positive patients had positive PD-L1 expression in 24.2% of cases, while HER2-negative patients had positive PD-L1 expression in 39.0% of cases. Based on the data obtained, HER2-negative patients may be the best candidates for targeted anti-PD-1 therapy. However, the authors drew attention to false negative results that were associated with the biopsy technique: prominent PD-L1 expression was detected at the edge of the invasive front of the tumor and not in its center.

According to H. Fukamachi et al. [28], following the study of DGC by gene expression profiling, the authors distinguished two DGC clusters. The first cluster is represented by DGC with a stable genome (GS), which can be interpreted as “primary” DGC.

The second cluster is represented by DGC with microsatellite instability (MSI) and chromosomal instability (CIN), which was defined by the authors as DGC developed from the intestinal type. In addition, an analysis of the expression of mTOR and PD-L1 in each individual cluster was performed, which showed more pronounced expression in the second cluster (MSI and CIN). Based on these data, it can be assumed that in a group of patients with DGC, which has developed from the intestinal type of GC, it is possible to use mTOR and PD1 inhibitors for treatment.

HELICOBACTER PYLORI AND DIFFUSE GASTRIC CANCER

The International Agency for Research on Cancer (IARC) classified *H. pylori* as a Group I carcinogen (strong carcinogen) in 1994 [29, 30]. Initially, *H. pylori* infection was thought to be associated mainly with the intestinal type of GC, while for DGC, the underlying factors in the pathogenesis were genetic abnormalities. However, if we do not consider cases of inherited DGC, numerous studies report a significant role of *H. pylori* and Epstein – Barr virus in the occurrence of sporadic DGC [31–33]. Serological studies also confirmed that *H. pylori* is associated with both histological types of GC.

A number of studies showed that patients with a low *H. pylori* IgG titer are more likely to develop an intestinal type of GC, while patients with a high *H. pylori* IgG titer have a high risk of developing DGC [34–36]. There is evidence that *H. pylori* is able to inhibit factors responsible for cell adhesion and, thus, participate in the pathogenesis of DGC. Y. Yang et al. demonstrated disintegration of E-cadherin by *H. pylori* SS1 and 26695 strains. It was found that the SS1 strain more effectively disintegrated E-cadherin after 12 and 24 hours [37]. After penetration of *H. pylori* into the gastric epithelium, nonphosphorylated binding of CagA to E-cadherin occurs, which leads to separation of the E-cadherin / β-catenin complex and causes accumulation of β-catenin in the cytoplasm and nucleus, ultimately activating a β-catenin-dependent gene involved in cancer progression [38]. Aberrant activation of β-catenin disrupts normal apical junctional complexes, which leads to loss of cell polarity [39].

MORPHOLOGY OF DIFFUSE GASTRIC CANCER

In a study by H.E. Lee et al., the differences in the morphology of hereditary and sporadic DGC were identified. Based on the material presented by 11 cases of gastrectomy in patients with inherited DGC and a genetically confirmed mutation in the CDH1 gene, the tumor cells were morphologically divided into three groups.

Group 1 included well-differentiated, large signet ring cells with a large amount of cytoplasm, a small nuclear-cytoplasmic ratio, and flattened and eccentrically located nuclei with moderate atypia. They were located under the surface epithelium and had positive expression to mucicarmine and pCEA and negative expression to p16 and CDX2.

Group 2 contained well-differentiated, small signet ring cells with a smaller amount of cytoplasm, with more rounded and hyperchromic nuclei with pronounced signs of atypia, and a high nuclear-cytoplasmic ratio. These cells were located in their own mucosa and had negative expression to mucicarmine, pCEA, p16, and CDX2.

Group 3 included poorly differentiated, small signet ring cells with positive expression to p16 and negative expression to CDX2. As a control group, material from 20 cases of gastrectomy in sporadic DGC was used. No morphological features were identified for them, but positive expression to p16 and CDX2 was noted [40].

In the work by H.H. Wong and P. Chu [41], the authors considered the features of immunohistochemical diagnosis in the group of gastrointestinal cancers. For DGC, positive expression to CDX-2, CK7, and HepPar-1 in approximately 70% of cases was reported. About half of the cases had positive expression to CK20, MUC2, and MUC5AC. Moreover, negative expression to MUC1 and E-cadherin was detected. According to the authors, morphological cases of low-grade adenocarcinoma with pronounced lymphoplasmacytic infiltration may be positive for EBV.

CONCLUSION

Currently, it is important not only to timely diagnose GC, but also to find a personalized approach to each patient. An extremely important aspect of diagnosis (verification) is a comprehensive

approach to the study of surgical material using traditional optical research methods with mandatory periodic acid Schiff (PAS) and Alcian Blue staining of the material and counting of the number of signet ring cells, expressed as a percentage, as well as detection of *H. pylori*, and immunohistochemistry using a panel of antibodies (CDX-2, CK7, CK20, HepPar-1, MUC1, MUC2, MUC5AC, HER2, mTOR, PD-L1, and E-cadherin).

A molecular genetic study of the material is required to determine the molecular subtype of GC and correlate the data obtained with the expression of such antibodies as HER2, PD-L1, and mTOR for more accurate determination of the cohort of patients who can benefit from therapy with mTOR and PD1 inhibitors. Only this comprehensive approach to diagnosis of GC can give specialists more clear understanding of the disease and help in choosing a treatment strategy for such patients.

REFERENCES

1. Cancer incidence in five continents. IARC Press, 1996.
2. Malignant neoplasms in Russia in 2018; ed. Kaprin A.D., Starinsky V.V., Petrova G.V. Moscow: P. Hertsen Moscow Oncology Research Institute – Branch of the National Medical Research Radiological Centre of the Ministry of Health of the Russian Federation, 2019: 250 (in Russ.).
3. Laurén P. The two histological main types of gastric carcinoma: diffuse and so-called intestinal-type carcinoma. *Acta Pathol. Microbiol. Scand.* 1965; 64: 31–49. DOI: 10.1111/apm.1965.64.1.31.
4. Binh T.T., Tuan V.P., Dung H.D.Q., Tung P.H., Tri T.D., Thuan N.P.M., Khien V.V., Hoan P.Q., Suzuki R., Uchida T. et al. Advanced non-cardia gastric cancer and *Helicobacter pylori* infection in Vietnam. *Gut. Pathog.* 2017; 9: 46. DOI: 10.1186/s13099-017-0195-8.
5. Correa P. Human gastric carcinogenesis: A multistep and multifactorial process-first American Cancer Society award lecture on cancer epidemiology and prevention. *Cancer Res.* 1992; 52 (24): 6735–6740.
6. Fischbach W., Chan A., Wong B. *Helicobacter pylori* and gastric malignancy. *Helicobacter.* 2005; 10: 34–39. DOI: 10.1111/j.1523-5378.2005.00338.x.
7. Belkovets A.V., Reshetnikov O.V., Kurilovich S.A., Maksimov V.N. Gastric cancer: molecular and genetic data (literature review). *Siberian Journal of Oncology.* 2014; 2 (62): 56–64 (in Russ.).
8. World cancer report 2014. Geneva, Switzerland: World Health Organization, International Agency for Research on Cancer, WHO Press, 2015.
9. Bass A., Thorsson V., Shmulevich I., Reynolds S.M., Miller M., Bernard B. Comprehensive molecular characterization of gastric adenocarcinoma. *Nature*. 2014; 513 (7517): 202–209. DOI: 10.1038/nature13480.
10. Grinstein S., Preciado M.V., Gattuso P., Chabay P.A., Warren W.H., De Matteo E., Gould V.E. Demonstration of Epstein-Barr virus in carcinomas of various sites. *Cancer Res*. 2002. 1; 62 (17): 4876–4878.
11. Tajima M., Komuro M., Okinaga K. Establishment of Epstein-Barr virus-positive human gastric epithelial cell lines. *Jpn. J. Cancer Res*. 1998; 89 (3): 262–268. DOI: 10.1111/j.1349-7006.1998.tb00557.x .
12. Zhang Y., Molot R. Severe gastritis secondary to Epstein-Barr viral infection. Unusual presentation of infectious mononucleosis and associated diffuse lymphoid hyperplasia in gastric mucosa. *Arch. Pathol. Lab. Med*. 2003; 127 (4): 478–480. DOI: 10.1043/0003-9985(2003)127.
13. Uozaki H., Fukayama M. Epstein-Barr virus and gastric carcinoma – viral carcinogenesis through epigenetic mechanisms. *Int. J. Clin. Exp. Pathol*. 2008; 1 (3): 198–216.
14. Kononov A.V. Heterogeneity of the inflammatory response in the population with *Helicobacter pylori* infection. *Pediatria*. 2002; 2: 124–130 (in Russ.).
15. Kononov A.V. Molecular cell basis of interaction between *Helicobacter pylori* and the host. Infectious matter eradicated - what's next? *Omsk Scientific Bulletin*. 2002; 21: 17–28 (in Russ.).
16. Kononov A.V., Mozgovoy S.I., Livzan M.A., Predvechnaya I.K., Novikova L.D. Reverse development of morphological signs in the main variants of chronic gastritis in conditions of *Helicobacter pylori* eradication. *Archives of Pathology*. 2005; 67 (3):17–21 (in Russ.).
17. Boland C.R., Thibodeau S.N., Hamilton S.R., Sidransky D., Eshleman J.R., Burt R.W., Meltzer S.J., Rodriguez-Bigas M.A., Fodde R., Ranzani G.N., Srivastava S. A National Cancer Institute Workshop on microsatellite instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. *Cancer Res*. 1998; 58 (2): 5248–5257.
18. Corso G., Pedrazzani C., Marrelli D., Pascale V., Pinto E., Roviello F. Correlation of microsatellite instability at multiple loci with long-term survival in advanced gastric carcinoma. *Arch. Surg*. 2009; 144 (8): 722–727. DOI: 10.1001/archsurg.2009.42.
19. Geigl J., Obenauf A.C., Schwarzbraun T., Speicher M.R. Defining 'chromosomal instability. *Trends Genet*. 2008; 24 (2): 64–69. DOI: 10.1016/j.tig.2007.11.006.
20. Mamalis A., Garcha M., Jagdeo J. Targeting the PD-1 pathway: a promising future for the treatment of melanoma. *Arch. Dermatol. Res.* 2014; 306 (6): 511–519. DOI: 10.1007/s00403-014-1457-7.
21. Boger C., Behrens H.M., Mathiak M., Kruger S., Kalthoff H., Rocken C. PD-L1 is an independent prognostic predictor in gastric cancer of Western patients. *Oncotarget*. 2016; 7 (17): 24269–24283. DOI: 10.18632/oncotarget.8169.

22. Chang H., Jung W.Y., Kang Y., Lee H., Kim A., Kim H.K., Shin B.K., Kim B.H. Programmed death-ligand 1 expression in gastric adenocarcinoma is a poor prognostic factor in a high CD8+ tumor infiltrating lymphocytes group. *Oncotarget*. 2016; 7 (49): 80426–80434. DOI: 10.18632/oncotarget.12603.
23. Kawazoe A., Kuwata T., Kuboki Y., Shitara K., Nagatsuma A.K., Aizawa M., Yoshino T., Doi T., Ohtsu A., Ochiai A. Clinicopathological features of programmed death ligand 1 expression with tumor-infiltrating lymphocyte, mismatch repair, and Epstein – Barr virus status in a large cohort of gastric cancer patients. *Gastric Cancer*. 2017; 20 (3): 407–415. DOI: 10.1007/s10120-016-0631-3.
24. Ma C., Patel K., Singhi A.D., Ren B., Zhu B., Shaikh F., Sun W. Programmed death-ligand 1 expression is common in gastric cancer associated with Epstein – Barr virus or microsatellite instability. *Am. J. Surg. Pathol.* 2016; 40 (11): 1496–1506. DOI: 10.1097/PAS.0000000000000698.
25. Derks S., Liao X., Chiaravalli A.M., Xu X., Camargo M.C., Solcia E., Sessa F., Fleitas T., Freeman G.J., Rodig S.J., Rabkin C.S., Bass A.J. Abundant PD-L1 expression in Epstein – Barr virus-infected gastric cancers. *Oncotarget*. 2016; 7 (22): 32925–32932. DOI: 10.18632/oncotarget.9076.
26. Saito H., Kono Y., Murakami Y., Shishido Y., Kuroda H., Matsunaga T., Fukumoto Y., Osaki T., Ashida K., Fujiwara Y. Highly activated PD-1/PD-L1 pathway in gastric cancer with PD-L1 expression. *Anticancer Res*. 2018; 38 (1): 107–112. DOI: 10.21873/anticanres.12197.
27. Wang L., Zhang Q., Ni S., Tan C., Cai X., Huang D., Sheng W. Programmed death-ligand 1 expression in gastric cancer: correlation with mismatch repair deficiency and HER2-negative status *Cancer Med*. 2018; 7 (6): 2612–2620. DOI: 10.1002/cam4.1502.
28. Fukamachi H., Kim S.K., Koh J., Lee H.S., Sasaki Y., Yamashita K. et al. A subset of diffuse-type gastric cancer is susceptible to mTOR inhibitors and checkpoint inhibitors. *J. Exp. Clin. Cancer. Res.* 2019; 38 (1): 127. DOI: 10.1186/s13046-019-1121-3.
29. Schistosomes, liver flukes and *Helicobacter pylori.* IARC working group on the evaluation of carcinogenic risks to humans. Lyon, 7–14 June 1994. *IARC Monogr. Eval. Carcinog. Risks Hum.* 1994; 61: 1–241.
30. IARC *Helicobacter pylori* working group. *Helicobacter pylori* eradication as a strategy for preventing gastric cancer. Lyon, France 2014: IARC working group reports, No. 8.
31. Uemura N., Okamoto S., Yamamoto S., Matsumura N., Yamaguchi S., Yamakido M., Taniyama K., Sasaki N., Schlemper R.J. *Helicobacter pylori* infection and the development of gastric cancer. *N. Engl. J. Med.* 2001; 345 (11): 784–789. DOI: 10.1056/NEJMoa001999.
32. Komoto K., Haruma K., Kamada T., Tanaka S., Yoshihara M., Sumii K., Kajiyama G., Talley N.J. *Helicobacter pylori* infection and gastric neoplasia: correlations with histological gastritis and tumor histology. *Am. J. Gastroenterol*. 1998; 93 (8): 1271–1276. DOI: 10.1111/j.1572-0241.1998.00408.x.
33. Nishibayashi H., Kanayama S., Kiyohara T., Yamamoto K., Miyazaki Y., Yasunaga Y., Shinomura Y., Takeshita T., Takeuchi T., Morimoto K., Matsuzawa Y. *Helicobacter pylori*-induced enlarged-fold gastritis is associated with increased mutagenicity of gastric juice, increased oxidative DNA damage, and an increased risk of gastric carcinoma. *J. Gastroenterol. Hepatol*. 2003; 18 (12): 1384–13891. DOI: 10.1046/j.1440-1746.2003.03192.x.
34. Watanabe M., Kato J., Inoue I., Yoshimura N., Yoshida T., Mukoubayashi C., Deguchi H., Enomoto S., Ueda K., Maekita T., Iguchi M., Tamai H., Utsunomiya H., Yamamichi N., Fujishiro M., Iwane M., Tekeshita T., Mohara O., Ushijima T., Ichinose M. Development of gastric cancer in nonatrophic stomach with highly active inflammation identified by serum levels of pepsinogen and *Helicobacter pylori* antibody together with endoscopic rugal hyperplastic gastritis. *Int. J. Cancer*. 2012 Dec. 1; 131 (11): 2632–2642. DOI: 10.1002/ijc.27514.
35. Tatemichi M., Sasazuki S., Inoue M., Tsugane S. Clinical significance of IgG antibody titer against *Helicobacter pylori*. *Helicobacter*. 2009; 14 (3): 231–236. DOI: 10.1111/j.1523-5378.2009.00681.x.
36. Lee J.Y., Gong E.J., Chung E.J., Park H.W., Bae S.E., Kim E.H., Kim J., Do Y.S., Kim T.H., Chang H.S., Song H.J., Choe J., Jung H.Y. The characteristics and prognosis of diffuse-type early gastric cancer diagnosed during health check-ups. *Gut and Liver*. 2017; 11 (6): 807–812. DOI: 10.5009/gnl17033.
37. Yang Y., Du J., Liu F., Wang X., Li X., Li Y. Role of caspase-3/E-cadherin in *Helicobacter pylori*-induced apoptosis of gastric epithelial cells. *Oncotarget*. 2017; 8 (35): 59204–59216. DOI: 10.18632/oncotarget.19471.
38. Oliveira M.J., Costa A.M., Costa A.C., Ferreira R.M., Sampaio P., Machado J.C., Seruca R., Mareel M., Figueiredo C. CagA associates with c-Met, E-cadherin, and p120-catenin in a multiproteic complex that suppresses *Helicobacter pylori*-induced cell-invasive phenotype. *J. Infect. Dis*. 2009; 200 (5): 745–755. DOI: 10.1086/604727.
39. Bagnoli F., Buti L., Tompkins L., Covacci A., Amieva M.R. *Helicobacter pylori* CagA induces a transition from polarized to invasive phenotypes in MDCK cells. *Proc. Natl. Acad. Sci USA*. 2005; 102 (45): 16339–16344. DOI: 10.1073/pnas.0502598102.
40. Lee H.E., Smyrk T.C., Zhang L. Histologic and immunohistochemical differences between hereditary and sporadic diffuse gastric carcinoma. *Human Pathology.* 2018; 74: 64–72. DOI: 10.1016/j.humpath.2017.12.023.
41. Wong H.H., Chu P. Immunohistochemical features of the gastrointestinal tract tumors. *J. Gastrointest. Oncol*. 2012; 3 (3): 262–284. DOI: 10.3978/j.issn.2078-6891.2012.019.

Authors contribution

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Features of apoptosis and blebbing of the lymphocyte plasma membrane in bronchial asthma

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ABSTRACT

Given a persistent global trend towards an increase in the number of patients with bronchial asthma (BA) over the past decades, researchers are facing challenges related to a comprehensive study of the pathogenesis of BA. Numerous studies have shown that BA is associated with long-term persistence of leukocytes (lymphocytes, macrophages, and eosinophils) in the bronchial tissues. However, the causes of this phenomenon remain understudied. The article provides an overview of modern research on the mechanisms of disorders of lymphocyte apoptosis in patients with BA.

Our study considers the main mechanisms of molecular regulation of lymphocyte apoptosis, including transcription factors, the Fas/FasL system, and bcl-2/bcl-XL factors. We presented the data on the role of reduced lymphocyte apoptosis in the formation of a severe BA phenotype. Taking into account high prevalence of obesity among patients with BA, we analyzed a few existing articles on apoptosis of immunocompetent cells in obesity. In addition, the article highlights the key mechanisms of development of lymphocyte plasma membrane blebbing (PMB) with formation of microvesicles, as well as their influence on the course of pathological processes in BA.

The authors believe that further in-depth study of apoptosis, lymphocyte necrosis, and plasma membrane blebbing can help improve the principles of diagnosis and treatment of BA.

Key words: bronchial asthma, obesity, lymphocyte apoptosis, programmed cell death, caspase, plasma membrane blebbing.

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Особенности апоптоза и блеббинга цитоплазматической мембраны лимфоцитов при бронхиальной астме

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РЕЗЮМЕ

С учетом стойкой общемировой тенденции к нарастанию численности больных бронхиальной астмой (БА) за последние десятилетия перед исследователями встает задача всестороннего изучения патогенеза бронхиальной астмы. В многочисленных исследованиях доказано, что течение БА сопряжено с длительной персистенцией лейкоцитов (лимфоцитов, макрофагов, эозинофилов) в тканях бронхов. Однако остается открытым вопрос о причинах данного явления. В данной статье представлен обзор современных научных исследований, посвященных изучению механизмов нарушения апоптоза лимфоцитов у больных бронхиальной астмой.

Рассматриваются основные механизмы молекулярной регуляции апоптоза лимфоцитов, например транскрипционные факторы, система Fas/FasL, факторы bcl-2/bcl-XL и др. Приводятся данные об участии снижения апоптоза лимфоцитов в формировании фенотипа с тяжелым течением бронхиальной астмы. Учитывая высокую распространенность ожирения среди больных бронхиальной астмой, проанализированы немногочисленные статьи, касающиеся апоптоза иммунокомпетентных клеток при ожирении. Кроме того, в статье освещаются ключевые механизмы развития блеббинга цитоплазматической мембраны (ЦПМ) с формированием лимфоцитарных микровезикул, а также их влияние на течение патологических процессов при астме.

Авторы считают, что дальнейшее углубленное изучение процессов апоптоза и некроза лимфоцитов, а также блеббинга ЦПМ сможет помочь в улучшении принципов диагностики и лечения бронхиальной астмы.

Ключевые слова: бронхиальная астма, ожирение, апоптоз лимфоцитов, программируемая клеточная гибель, каспазы, блеббинг цитоплазматической мембраны.

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

Bronchial asthma (BA) is a chronic respiratory disease that places a serious social and economic burden on a patient and society as a whole [1, 2]. Experts of the World Health Organization (WHO) note that the number of patients with BA tends to increase [3].

Russia has also seen a continuing increase in the overall incidence of BA in recent years [4]. According to statistical data, the prevalence of BA among adult population of Russia accounts for up to 10.6% [5].

It is known that BA reduces the average life expectancy of women by 13.5 years and men by 6.6 years; it causes 1.4% of all hospitalizations and 1.5% of all disability cases. A financial burden associated with diagnosis and treatment of BA has a significant impact on the country's economy [6].

An equally important health and social problem worldwide is obesity. WHO sees this problem now as an epidemic which has affected millions of people. Since 1980, the number of obese people in the world has more than doubled. Excess body mass index (BMI) is observed in about 1 billion people among adult population of the world, and 475 million people are obese [7, 8].

Obesity is the most common comorbidity of BA. It was shown that obesity increases the prevalence and incidence of BA and raises the risk of exacerbations, aggravation of respiratory symptoms, and poor disease control. Precise mechanisms of the mutual effects of BA and obesity remain unclear. They are probably multifactorial and mediated by mechanical changes in the airways, systemic inflammatory response, and metabolic dysregulation [9, 10].

Quite recent studies emphasize the role of programmed cell death (PCD) and autoimmunity as

potentially important factors in the pathogenesis of chronic obstructive airway diseases. Currently, there are a few methods available for registering different PCD manifestations and analyzing molecular mechanisms [11], that are closely associated with mechanisms of other important phenomena (such as cell activation and biological signal transduction).

It is the study of apoptosis that is considered effective and useful for understanding certain significant processes, including immune homeostasis.

THE ROLE OF LYMPHOCYTES IN THE DEVELOPMENT OF BA

The course of BA is characterized by progressive chronic airway inflammation, which is based on production of proinflammatory cytokines (interleukin (IL) -4, -5, -9, -13, granulocyte-macrophage colony-stimulating factor (GM-CSF), etc.) [12], as well as long-term persistence of leukocytes (lymphocytes, macrophages, and eosinophils) in the bronchial tissues [13].

Lymphocytes are essential regulatory and effector cells of adaptive immunity. Together with antigen-presenting cells (APCs), T- and B-lymphocytes provide an immune response to pathogens and form long-term immunological memory [14].

The number of lymphocytes is regulated by a constant balance between production, proliferation, and death of cells. The equilibrium of these processes is characterized as lymphocyte homeostasis [15].

The number of effector T-lymphocytes in the immune response can increase by 1,000 times [16], but programmed cell death regulates accumulation of the total number of lymphocytes, also in BA [17].

Therefore, the constant presence of regulatory and effector immune cells in BA may be due not only to their accelerated migration to tissues, but also to restricted cellular elimination following PCD dysregulation.

TYPES OF PCD

Based on the physiological, morphological, and biochemical criteria, researchers distinguish three types of PCD: apoptosis (type I PCD), autophagy (type II PCD), and necrosis (type III PCD) [18, 19].

Autophagy and apoptosis

Autophagy is a process of *in vivo* elimination of the cytoplasmic contents changed by metabolites, that involves cell self-renewal. Extensive autophagy can lead to cell death [20, 21].

At the same time, natural, physiological, and programmed cell death (50–500 billion cells daily) mainly occurs through apoptosis: after plasma membrane blebbing, cell reduction, chromatin condensation, and DNA fragmentation, apoptotic cells are rapidly absorbed by phagocytic cells without any inflammatory response [22, 23].

Mechanisms of apoptosis

Type I PCD (apoptosis) occurs in several ways via the mechanisms that depend on both cellular characteristics and the impact of internal and external signals [24]. After the effects of toxic agents or unrepaired DNA damage, the mitochondrial (internal) signaling pathway is implemented in apoptosis. The main regulators of this process are the *p53* and *Bcl-2* genes (the main suppressor of apoptosis) with corresponding proteins [25, 26].

More often, the external or receptor-mediated pathway of apoptosis is implemented. It is initiated by a variety of extracellular triggers and is carried out with the participation of tumor necrosis factor (TNF) receptors. The most studied activator is Fas (APO-l or CD95), which stimulates cell apoptosis after the formation of a complex compound with its ligand Fas / FasL [27, 28].

The effector phase of apoptosis begins after transmission of inducing signals from triggers through adaptive proteins. The main participants in this phase are cysteine proteases (caspases), which quickly switch from the inactive (procaspase) to the active form, when apoptosis is initiated. This ensures cleavage of protein molecules at aspartic acid bases [29, 30].

The growing interest of experts in PCD issues [31] has led to an increase in the number of studies related to apoptosis. Methods for detecting apoptosis and analyzing its molecular and genetic mechanisms have emerged. It allowed researchers to identify the significant role of apoptosis in maintaining homeostasis of constantly regenerating tissues and understand the effect of PCD on the pathogenesis of many pathological processes [32].

The terminal deoxynucleotidyl transferase dUTP-biotin nick end labeling (TUNEL) method, or terminal deoxyuridine end labeling, was recognized as the most optimal method for determining apoptosis in cells [33], since numerous strand breaks occur in DNA during apoptosis under the action of

endonucleases, which results in formation of many 3'ends [34]. The TUNEL method is based on binding of biotin-labeled deoxyuridine triphosphate to the 3'-end of a DNA strand. The binding is implemented by deoxynucleotide transferase. There are other optimal biochemical, molecular, and genetic methods for determining apoptosis [35]. In the context of existing information about the abnormalities of lymphocyte apoptosis in systemic, autoimmune, and allergic diseases, the study of apoptosis in BA has become relevant and important.

Persistence of allergic inflammation in the airways is associated with excessive activation of immunocompetent cells (ICC), which, in turn, leads to accumulation of autoreactive clones with a simultaneous decrease in the activity of apoptosis (positive activation and absence of apoptosis) [36–38]. It is possible that lymphocyte apoptosis is closely related to their migration to the focus of inflammation as a result of allergen exposure. In this case, apoptosis acts as a mechanism of antigen-mediated selection of lymphocytes [39].

LYMPHOCYTE APOPTOSIS AND BA

In BA, several pathways of death are triggered in the ICC, and they are able to switch in the course of the disease [40]. In BA, marked inhibition of the mechanisms of lymphocyte apoptosis takes place, associated with an increase in the expression of IL-5 mRNA and the main anti-apoptotic factors (bcl-2, bcl-XL) inhibiting apoptosis via the receptor and mitochondrial pathways, which directly correlates with the severity of BA [41].

The molecular mechanisms playing an important role in the regulation of PCD include transcription factors, such as Janus kinases and signal transducer and activator of transcription (JAK-STAT), PAX-5, NF-KB, p53, etc. Transcription factors regulate expression of proteins (IL-4, -15, -13, IgE, Fas receptor molecules, etc.) and proliferation of cells, such as lymphocytes and eosinophils [42, 43].

It is proved that decreased gene expression, reduced activity of transcription factor p53, and stimulation of NF-kB activity have anti-apoptotic effects. High levels of STAT6 expression at reduced PAX-5 and elevated levels of NF-KB lead to development of BA and aggravation of its course [44].

The Bcl-2 / Bax ratio in patients with severe BA is much higher. This fact was confirmed by many studies demonstrating that NF-kB stimulates the expression and activity of Bcl-2, which itself acts as a powerful anti-apoptotic molecule and inhibits pro-apoptotic Bax molecules [45, 46].

Recent studies have found that the levels of Bcl-2 or the Bcl-2 / Bax ratio are higher in BA patients than in healthy people. The level of NF-kB expression positively correlates with the bcl2 / Bax ratio in patients diagnosed with BA [47].

It is remarkable that the Fas / FasL system that triggers the receptor-mediated pathway of apoptosis is less active in Th2 lymphocytes typical of BA, which suggests their evolutionary predisposition to a reduced apoptosis rate [48, 49]. According to the studies, steroids are able to enhance apoptosis but reduce the expression of Fas (CD95) and CD25 regulators, switching apoptosis to a different pathway [50].

A number of studies reported the association between the activity of apoptosis and the severity of BA [51]. Specifically, an inverse correlation was demonstrated between the number of apoptotic cells and BA severity [52]. It was noted in another study, that the number of lymphocytes in apoptosis was significantly lower in severe BA (compared to mild BA) against the background of 6-day incubation in the solution with the addition of an apoptosis inducer (dexamethasone). According to the authors, this may indicate inhibition of type I PCB in patients with severe BA [53].

Necrosis

Currently, an important role is assigned to the study of the consequences of not only apoptosis, but also of type III PCD (necrosis) for surrounding cells and the body as a whole. Necrotic cell death is accompanied by destruction of the cell membrane and entry of intracellular molecules into the extracellular space with alterations of surrounding cells and inflammation. Phagocytosis of dead cells is accompanied by development of a full-fledged immune response [54]. Necrotic death of lymphocytes is always associated with excretion of inflammatory mediators having cytotoxic and histochemical effects. It is also accompanied by active proliferation and migration of new effector cells into the airways, which aggravates airway inflammation [55].

According to A.P. Parakhonsky, the development of BA is closely associated not only with abnormalities in the implementation of apoptosis in blood ICC, but also with an increase in lymphocyte necrosis [56]. Few existing studies showed that necrotic death of lymphocytes prevails in patients with severe persistent BA [57].

PLASMA MEMBRANE BLEBBING (PMB) AND BA

During oxidative stress, apoptosis, and necrosis of lymphocytes, membrane microvesicles (MV) are formed as a result of intensive PMB [58, 59].

Development of PMB occurs following disruption of membrane-cytoskeleton interactions at the end of enzymatic reaction activation, electrolyte imbalance in the cell, and oxidative damage to the cytoskeleton. PMB is preceded by a disruption of actin-myosin interactions and externalization of phosphatidylserine, which is characterized by a bubble-like protrusion of the membrane. It is accompanied by migration of organelles and antigens into the resulting blebs, which acquire inflammatory and autoantigenic potential. For example, extracellular particles originating from T-cells carry CD4, CD3, or CD8 antigens [60, 61].

Microvesicles

Evidence for the role of ICC plasma membrane vesicles is still significantly limited. Their role in regulating homeostasis by transporting signals between cells, up to presentation of an antigen to immune cells, is still under discussion [62].

Microvesicles (MV) can affect cells in two main ways: interaction with receptors and microRNA transfer [63]. MV can regulate gene expression and cell differentiation, which involves them in the pathogenesis of many processes [64]. Many studies are devoted to MV microRNAs, which are highly conserved non-coding RNAs with a length of 18–24 nucleotides, as well as to their proinflammatory role [65].

Another factor affecting the inflammatory process can be the ability of MV to induce apoptosis of ICC. Apoptogenic activity of MV can be explained by the influence of caspases and other biologically active substances contained in them that induce apoptosis [66].

However, very few studies have been devoted to the pathogenic effect of lymphocyte microvesicles (LMVs) on the course of pathological processes, in particular, on the pathogenesis of BA. LMVs are known to induce activation of NADPH-oxidase, leading to activation of oxidative stress [67].

High content of circulating MV in patients with BA can serve as a useful biomarker of the activity of apoptosis and airway inflammation and a potential predictor of BA severity. It reflects the effect of an important element in the pathogenesis of BA and not just the appearance of inert “cell dust”, as it has been generally accepted for a long time.

APOPTOSIS AND OBESITY

There are very few studies and publications devoted to apoptosis of ICC (lymphocytes, in particular) in obesity. Metabolic changes in the white adipose tissue of obese people are known to lead to the persistence of ICC in the adipose tissue. Locally, elevated levels of proinflammatory cytokines and free radicals are observed that contribute to oxidative stress and progression of systemic inflammation [68].

In obesity, an increase in the intensity of lymphocyte apoptosis is noted, in which the interaction of the Fas receptor with FasL leads to activation of caspase-8, -10, and -3. In obese patients, the concentration of apoptosis inducer (p53 protein) is elevated, and its level directly correlates with the body mass index, waist circumference, and Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) index [69, 70].

CONCLUSION

In terms of physiopathology, BA is a chronic inflammatory airway disease. The study of PCD, in particular, apoptosis, is determined by the essential role of lymphocytes in the pathogenesis of BA. Despite a large body of data on BA, the concept of apoptosis in the pathogenesis of BA is still contradictory. The findings of immunological studies provide evidence for changes in the functional state of lymphocytes in BA patients. Studies of apoptosis related to BA with comorbid obesity are especially relevant. It is worth noting that there is growing evidence that obesity has a causal association with BA. Better understanding of this association at the pathogenetic level may result in development of new treatment methods for the therapeutically resistant cohort of patients.

Therefore, further study of all aspects of apoptosis and lymphocyte PMB in BA and establishment of their cytological, molecular, and biochemical markers can contribute to more complete understanding of the mechanisms of BA pathogenesis, improve diagnosis, and create a new landmark for differential diagnosis. It can also serve as the basis for developing highly effective, modern methods for treatment and prognosis of treatment effectiveness in obstinate and treatment-resistant forms of BA.

REFERENCES

1. Global Strategy for asthma management and prevention. Global Initiative for Asthma. GINA. URL: http://www.slideshare.net/cristobalbunuel/gina-report-2016; 2016. URL: http://www.ginasthma.org/local / uploads/files/GINA.Report_ 2016.pdf.

2. Federal clinical guidelines on bronchial asthma. 2016: 64. URL: http://spulmo.ru/obshchestvo news/news-812/ (in Russ.).
3. Reddel H.K., Hurd S.S., FitzGerald J.M. World Asthma Day. GINA 2014: A global asthma strategy for a global problem. *Int. J. Tuberc. Lung Dis*. 2014; 18 (5): 505–506. DOI: 10.5588/ijtld.14.0246.
4. Beltyukov E.K., Bratukhin K.P. Epidemiology of allergic rhinitis and bronchial asthma in the Sverdlovsk Region. *Doctor.ru*. 2015; 108 (7): 11–14 (in Russ.).
5. Ushakova D.B., Nikonov E.L. Epidemiology of bronchial asthma. *Therapy.* 2018; 20 (2): 90–95 (in Russ.).
6. Fedoseev G.B., Emelyanov A.V., Sergeeva G.R., Ivanova N.I., Zibrina T.M., Maksimenko I.N., Tsukanova I.V. Prevalence of bronchial asthma and allergic rhinitis among adult population of Saint-Petersburg. *Archives of Therapy*. 2003; 75 (1): 523–526 (in Russ.).
7. Guzzardi M.A, Iozzo P., Salonen M.K., Kajantie E., Eriksson J.G. Maternal adiposity and infancy growth predict later telomere length: a longitudinal cohort study. *Int. J. Obes*. 2016; 40 (7): 1063–1069. DOI: 10.1038/ijo.2016.58.
8. Mineev V.N., Lalayeva T.M., Vasilyeva T.S., Trofimov V.I. The asthma – obesity phenotype. *Pulmonology.* 2012; (2): 102–107 (in Russ.). DOI: 10.18093/0869-0189-2012-0-2-102-107.
9. Baffi C.W., Winnica D.E., Holguin F. Asthma and obesity: mechanisms and clinical implications. *Asthma Res. Pract.* 2015; 1: 1. DOI: 10.1186/s40733-015-0001-7.
10 Glushkova E.F., Shartanova N.V., Luss L.V. Obesity and bronchial asthma: clinical and allergological characteristics. *Russian Medical Journal.* 2018; 8 (1): 4–8 (in Russ.).
11. Vodounon C.A., Chabi C.B., Skibo Yu.V., Ezin V., Aikou N., Kotchoni S.O., Akpona S.A., Baba-Moussa L., Abramova Z.I. Influence of the programmed cell death of lymphocytes on the immunity of patients with atopic bronchial asthma. *Allergy Asthma Clin. Immunol.* 2014; 10 (1): 14. DOI: 10.1186/1710-1492-10-14.
12. Shore S.A. Obesity and asthma: possible mechanisms. *J. Allergy Clin. Immunol*. 2008; 121 (5): 1087–1093. DOI: 10.1016/j.jaci.2008.03.004.
13. Miranda C., Busacker A., Balzar S., Trudeau J., Wenzel S.E. Distinguishing severe asthma phenotype: role of age at onset and eosinophilic inflammation. *J. Allergy Clin. Immunol.* 2004; 113 (1): 101–108. DOI: 10.1016/j.jaci.2003.10.041.
14. Wiik A.S., Gordon T.P., Kavanaugh A.F., Lahita R.G., Reeves W., van Venrooij W.J., Wilson M.R., Fritzler M. Cutting edge diagnostics in rheumatology: the role of patients, clinicians, and laboratory scientists in optimizing the use of autoimmune serology. *Arthritis Rheum.* 2004; 51 (2): 291–298. DOI: 10.1002/art.20229.
15. Jameson S.C. Maintaining the norm: T-cell homeostasis. *Nat. Rev. Immunol.* 2002; 8 (2): 547–556. DOI: 10.1038/nri853.
16. Plas D.R., Rathmell J.C., Thompson C.B. Homeostatic control of lymphocyte survival: potential origins and implications. *Nat. Immunol.* 2002; 3 (6): 515–521. DOI: 10.1038/ni0602-515.
17. Badovinac V.P., Harty J.T. Programming, demarcating, and manipulating CD8+ T-cell memory. *Immunol. Rev.* 2006; 211 (1): 67–80. DOI: 10.1111/j.0105-2896.2006.00384.x.
18. Skibo Yu.V., Ponomareva A.A., Reshetnikova I.D., Abramova Z.I. Induction of autophagy in peripheral blood T lymphocytes in patients with atopic bronchial asthma. *Cellular Transplantation and Tissue Engineering.* 2012; 7 (3): 146–150 (in Russ.).
19. Green D.R. The end and after: how dying cells impact the living organism. *Immunity.* 2011; 35 (4): 441–445. DOI: 10.1016/j.immuni.2011.10.003.
20. Chernikov V.P., Belousova T.A., Kakturskiy L.V. Morphological and biochemical criteria of cell death. *Archives of Pathology.* 2010; 72 (3): 48–54 (in Russ.).
21. Romao S., Gannage M., Munz C. Checking the garbage bin for problems in the house, or how autophagy assists in antigen presentation to the immune system. *Semin. Cancer Biol.* 2013; 23 (5): 391–396. DOI: 10.1016/j.semcancer.2013.03.001.
22. Walsh C.M., Edinger A.L. The complex interplay between autophagy, apoptosis and necrotic signals promotes T-cell homeostasis. *Immunol. Rev.* 2010; 236 (1): 95–109. DOI: 10.1111/j.1600-065X.2010.00919.x.
23. Varga O.Yu., Ryabkov V.A. Apoptosis: concept, mechanisms of implementation, and value. *Human Ecology.* 2006; (7): 28–32 (in Russ.).
24. Zhao Y.X., Zhang H.R., Yang X.N., Zhang Y.H., Feng S., Yu F.X., Yan X.X. Fine particulate matter-induced exacerbation of allergic asthma via activation of T-cell immunoglobulin and mucin domain 1. *Chinese Medical Journal* 2018; 131 (20): 2461–2473. DOI: 10.4103/0366-6999.243551.
25. Chechina O.E., Biktasova A.K., Sazonova E.V. The role of cytokines in redox regulation of apoptosis. *Bulletin of Siberian Medicine.* 2009; 8 (2): 67–71 (in Russ.). DOI: 10.20538/1682-0363-2009-2-67-71.
26. Tian B.P., Xia L.X., Bao Z.Q., Zhang H., Xu Z.W., Mao Y.Y. Bcl-2 inhibitors reduce steroid-insensitive airway inflammation. *J. Allergy Clin Immunol.* 2017; 140 (2): 418–430. DOI: 10.1016/j.jaci.2016.11.027.
27. Lunev D.A., Zaklyakova L.V., Ovsyannikova E.G., Sarsengalieva A.K. The role of apoptosis in maintaining homeostasis in living systems. *Astrakhan Medical Journal.* 2010; 5 (1): 11–20 (in Russ.).
28. Brison D.R. Apoptosis in mammalian preimplantation embryos: regulation by survival factors. *Pathol. Int.* 2001; 51 (12): 948–953.
29. Hengartner M.O. The biochemistry of apoptosis. *Nature.* 2000; 407 (6805): 770–776. DOI: 10.1038/35037710.
30. Marsden V.S., O'connor L., O'reilly R.A.. Apoptosis initiated by Bcl-2-regulated caspase activation independently of the cytochrome c/Apaf-1/caspase-9 apoptosome. *Nature.* 2002; 419 (6907): 614–617. DOI: 10.1038/nature01101.
31. Kroemer G., Martin S.J. Caspase-independent cell death. *Nat. Med.* 2005; 11 (7): 725–730. DOI: 10.1038/nm1263.
32. Milner T.A., Waters E.M., Robinson D.C., Pierce J.P. Degenerating processes identified by electron microscopic immunocytochemial methods. *Meth. Mol. Biol*. 2011; 793: 23–59. DOI: 10.1007/978-1-61779-328-8_3.
33. Whelan R.S., Kaplinskiy V., Kitsis R.N. Cell death in the pathogenesis of heart disease: mechanisms and signifi cance.

Annu. Rev. Physiol. 2010; 72 (1): 19–44. DOI: 10.1146/annurev.physiol.010908.163111.
34. Yang F., Tu J., Pan J.Q., Luo H.L., Liu Y.H., Wan J., Zhang J., Wei P.F., Jiang T., Chen Y.H., Wang L.P. Light-controlled inhibition of malignant glioma by opsin gene transfer. *Cell Death Dis.* 2013; 4(10): 893–907. DOI: 10.1038/cddis.2013.425.
35. Zeng W., Wang X., Xu P., Liu G., Eden H.S., Chen X. Molecular imaging of apoptosis: from micro to macro. *Theranostics*. 2015; 5 (6): 559–582. DOI: 10.7150/thno.11548.
36. Sergeeva T.F., Shirmanova M.V., Zagaynova E.V., Lukyanov K.A. Modern methods of studying apoptosis in cells. *Modern Technology in Medicine.* 2015; 7 (3): 172–182 (in Russ.). DOI: 10.17691/stm2015.7.3.21.
37. Todo-Bom A., Mota P.A., Alves V., Vale P.S., Santos R.M. Apoptosis and asthma in the elderly. *J. Investig Allergol Clin. Immunol.* 2007; 17 (2): 107–112.
38. Deponte M. Programmed cell death in protists. *Biochem Biophys Acta*. 2008; 1783 (7): 1396–1405. DOI: 10.1016/j.bbamcr.2008.01.018.
39. Potapinska O., Demkow U. T-lymphocyte apoptosis in asthma. *Eur. J. Med. Res.* 2009: 14 (4): 192–195. DOI: 10.1186/2047-783X-14-S4-192.
40. Rottem M., Shoenfeld Y. Asthma as a paradigm for autoimmune desease. *Int. Arch. Allergy. Immunol*. 2003; 132 (3): 210–214. DOI: 10.1159/000074301.
41. Yarovaya G.A., Neshkova E.A., Martynova E.A., Blokhina T.B. The role of proteolytic enxymes in controlling different stages of apoptosis. *Laboratory Medicine.* 2011; (11): 39–53 (in Russ.).
42. Ogorodova L.M., Deev I.A., Ivanchuk I.I. Clinical and pathogenetic effectiveness of different pharmacotherapy regimens in severe bronchial asthma in children. *Pediatric Pharmacology*. 2006; 3 (3): 26–31 (in Russ.).
43. Murray P.J. The JAK-STAT signaling pathway: input and output integration. *J. Immunol*. 2007; 178 (5): 2623–2629. DOI: 10.4049/jimmunol.178.5.2623.
44. Mineev V.N., Sorokina L.N., Nyoma M.A., Ivanov V.A., Lipkin G.I. The role of transcription factor PAX-5 in the pathogenesis of bronchial asthma. *Medical Immunology.* 2012; 14 (4–5): 347–352 (in Russ.).
45. Bittner S. Ehrenschwender M. Multifaceted death receptor 3 signaling-promoting survival and triggering death. *FEBS Letters.* 2017; 591 (17): 2543–2555. DOI: 10.1002/1873-3468.12747.
46. Chen G.G., Liang N.C., Lee J.F., Chan U.P., Wang S.H., Leung B.C., Leung K.L. Over-expression of Bcl-2 against Pteris semipinnata L-induced apoptosis of human colon cancer cells via a NF-kappa B-related pathway. *Apoptosis.* 2004; 9 (5): 619–627. DOI: 10.1023/b:appt.0000038041.57782.84.
47. Viatour P., Bentires-Alj M., Chariot A., Deregowski V., de Leval L., Merville M.P., Bours V. NF-kappa B2/p100 induces Bcl-2 expression. *Leukemia.* 2003; 17 (7): 1349–1358. DOI: 10.1038/sj.leu.2402982.
48. Abdulamir A.S., Kadhim H.S., Hafi R.R., Ali M.A., Faik I., Abubakar F., Abbas K.A. Severity of asthma: the role of CD25+, CD30+, NF-kappaB, and apoptotic markers. *J. Investig. Allergol. Clin. Immunol.* 2009; 19 (3): 218–224.
49. Ying S., Khan L.N., Meng Q., Barnes N.C., Kay A.B. Cyclosporin A, apoptosis of BAL T-cells and expression of Bcl-2 in asthmatics. *Eur. Respir. J.* 2003; 22 (2): 207–212. DOI: 10.1183/09031936.03.00098902.
50. Akha A.S., Miller R.A. Signal transduction in the aging immune system. *Curr. Opin. Immunol.* 2005; 17 (5): 486-491. DOI: 10.1016/j.coi.2005.07.004
51. Melis M., Siena L., Pace E., Gjomarkaj M., Profi ta M., Pirazzoli A., Todaro M., Stassi G., Bonsignore G., Vignola A.M. Fluticasone induces apoptosis in peripheral T-lymphocytes: a comparison between asthmatic and normal subjects. *Eur. Respir. J.* 2002; 19 (2): 257–266. DOI: 10.1183/09031936.02.00239202.
52. Buc M., Dzurilla M., Bucova M. Immunophathogenesis of bronchial asthma. *Arch. Immunol. Ther. Ex.* 2009; 57 (5): 331–434. DOI: 10.1007/s00005-009-0039-4.
53. Chubarova S.V., Chernova I.A., Kraposhina A.Yu., Solovieva I.A., Demko I.V., Salmina A.B., Malinovskaya N.A. Peripheral blood lymphocyte apoptosis in bronchial asthma of varying severity. *Bulletin of Physiology and Pathology of Respiration.* 2013; (48): 28–33 (in Russ.).
54. Vodounon C.A., Chabi C.B., Skibo Y.V., Ezin V., Aikou N., Kotchoni S.O., Akpona S.A., Baba-Moussa L., Abramova Z.I. Influence of the programmed cell death of lymphocytes on the immunity of patients with atopic bronchial asthma. *Allergy, Asthma and Clinical Immunology.* 2014; 10 (1): 1–11. DOI: 10.1186/1710-1492-10-14.
55. Peter C., Wesselborg S., Herrman M., Lauber K. Dangerous attraction: phagocyte recruitment and danger signals of apoptotic and necrotic cells. *Apoptosis.* 2010; 15 (9): 1007–1028. DOI: 10.1007/s10495-010-0472-1.
56. Potapnev M.P. Autophagy, apoptosis, cell necrosis, and immune recognition. *Immunology.* 2014; 35(2): 95–102 (in Russ.).
57. Parakhonskiy A.P., Egorova S.V., Tsyganok S.S. Mechanisms of programmed cell death in peripheral blood of patients with bronchial asthma. *Advances of Modern Natural Science.* 2008; (8): 107–108 (in Russ.).
58. Nsangu M.M.D., Vodunon A.S., Abramova Z.I., Luntsov A.V., Tsibulkina V.N. Characteristics of morphological parameters and quantitative assessment of peripheral blood lymphocytes in patients with atopic bronchial asthma. *Kazan Medical Journal.* 2007; 88 (2): 168–171 (in Russ.).
59. Carmen M.M., Angela T., Fatina Z. Shed membrane microparticles from circulating and vascular cells in regulating vascular function. *Am. J. Physiol. Heart Circ. Physiol.* 2005; 288 (3): 1004–1009. DOI: 10.1152/ajpheart.00842.2004.
60. Inzhutova A.I., Salmina A.B., Petrova M.M. Detection of peripheral blood lymphocyte plasma membrane blebbing as a rapid method for assessing the severity of patient's condition in complicated forms of hypertension. *Bulletin of the Siberian Branch of RAS.* 2007; (1): 6–10 (in Russ.).
61. Akers J.C., Gonda D., Kim R., Carter B.S., Chen C.C. Biogenesis of extracellular vesicles (EV): exosomes, microvesicles, retrovirus-like vesicles, and apoptotic bodies. *J. Neurooncol.* 2013; 113 (1): 1–11. DOI: 10.1007/s11060-013-1084-8.
62. Buzas E.I., György B., Nagy G. Emerging role of extracellular vesicles in inflammatory diseases. *Nat. Rev. Rheumatol.* 2014; 10 (6): 356–364. DOI: 10.1038/nrrheum.2014.19.

63. Hwang I. Cell-cell communication via extracellular membrane vesicles and its role in the immune response. *Molecules and Cells*. 2013; 36 (2): 105–111. DOI: 10.1007/s10059-013-0154-2.
64. Panteleev M.A., Abaeva A.A., Balandina A.N., Belyaev A.V., Nechipurenkova D.Yu., Obydenniy S.I., Sveshnikova A.N., Shibeko A.M., Ataullakhanov F.I. Extracellular vesicles in blood plasma: composition, origin, and properties. *Biological Membranes.* 2017; 34 (3): 155–161 (in Russ.). DOI: 10.7868/S0233475517030069.
65. Hargett L.A., Bauer N.N. On the origin of microparticles: From "platelet dust" to mediators of intercellular communication. *Pulm. Circ.* 2013; 3 (2): 329–340. DOI: 10.4103/2045-8932.114760.
66. Redzic J.S., Balaj L., van der Vos K.E., Breakefield X.O. Extracellular RNA mediates and marks cancer progression. *Semin Cancer Biol.* 2014; 28: 14–23. DOI: 10.1016/j.semcancer.2014.04.010.
67. Mostefai H.A., Agouni A., Carusio N., Mastronardi M.L., Heymes C., Henrion D., Andriantsitohaina R., Martinez M.C. Phosphatidylinositol 3-kinase and xanthine oxidase regulate nitric oxide and reactive oxygen species productions by apoptotic lymphocyte microparticles in endothelial cells. *J. Immunol*. 2008; 180 (7): 5028–5035. DOI: 10.4049/jimmunol.180.7.5028.
68. Franceschi C., Campisi J. Chronic inflammation (inflammaging) and its potential contribution to age-associated diseases. *J. Gerontol. A Biol. Sci. Med. Sci.* 2014; 69 (1): 4–9. DOI: 10.1093/gerona/glu057.
69. Locksley R.M., Killeen N., Lenardo M.J. The TNF and TNF receptor superfamilies: integrating mammalian biology. *Cell.* 2001; 104 (4): 487–501. DOI: 10.1016/S0092-8674(01)00237-9.
70. Chernysheva E.N., Panova T.N. Association between apoptosis and premature aging in patients with metabolic syndrome. *Saratov Scientific and Medical Journal.* 2012; 8 (2): 251–255 (in Russ.).

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Modern methods for studying atherosclerosis and coronary artery disease: flow cytometry

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ABSTRACT

The problem of atherosclerosis, which forms the pathological basis of coronary artery disease (CAD), is one of the most discussed ones in development of cardiovascular diseases. This chronic inflammatory disease involves interactions between different cells, and an atherosclerotic plaque is a complex immunological environment. Modern quantitative methods increase the understanding of the pathophysiological processes responsible for progression of atherosclerotic plaques. Flow cytometry is a powerful modern method that allows for a complex and simultaneous cell analysis. This review is devoted to studies on atherosclerosis and CAD performed using flow cytometry.

Key words: flow cytometry, atherosclerosis, inflammation, T-lymphocytes, monocytes.

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Современные методы исследования атеросклероза и ишемической болезни сердца: проточная цитометрия

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РЕЗЮМЕ

Проблема атеросклероза, формирующего патологическую основу ишемической болезни сердца, является одной из наиболее обсуждаемых в развитии сердечно-сосудистых заболеваний. Это хроническое воспалительное заболевание включает комплекс сложных взаимодействий между различными клетками, а атеросклеротическая бляшка представляет собой сложную иммунологическую среду. Современные количественные методы повышают понимание патофизиологических процессов, ответственных за прогресси-

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рование атеросклеротической бляшки. Проточная цитометрия представляет собой мощный современный метод, позволяющий проводить комплексный анализ клеток одновременно. Данный обзор посвящен научным исследованиям атеросклероза и ишемической болезни сердца, выполненным с помощью метода проточной цитометрии.

Ключевые слова: проточная цитометрия, атеросклероз, воспаление, Т-лимфоциты, моноциты.

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INTRODUCTION

More than 50 years ago, such a new diagnostic method as pulse cytophotometry was developed. Since 1978, it has been called “flow cytometry”. Today, flow cytometry is a technique for studying dispersive substances using single particle analysis in the dispersed phase via signals received during fluorescence and light scattering. Flow cytometry is based on a combination of modern cytochemical fluorescent methods for analyzing the structural components of cells and their antigens. It is a modern and highly functional method that allows for a comprehensive analysis of various cell populations [1].

PRINCIPLES OF FLOW CYTOMETRY

The physical principles of flow cytometry are simple: a cell suspension, previously incubated with fluorochromes, is placed in a flow of liquid passed through a flow cell. This generates the effect of hydrodynamic focusing: the cells under investigation line up in a chain and, in this order, are guided through laser beams. This is how an individual cell is analyzed.

The light emitted from fluorochromes is focused using an optical system consisting of several mirrors and lenses, and then decomposed into certain components. The received light signals are converted into electrical impulses and analyzed using special software. In just a few seconds, thousands of cells pass through the flow cell, allowing a researcher to identify the composition and characteristics of the cell suspension.

Flow cytometry is a powerful method with many advantages: rapid analysis of a large number of cells (up to 10^7 cells per second), objective measurement of fluorescence intensity, obtainment of data for a single cell population, simultaneous analysis of different processes, an ability to characterize rare events [1].

FLOW CYTOMETRY IN STUDIES ON CAD AND ATHEROSCLEROSIS

The problem of atherosclerosis and the complications it causes is one of the most discussed ones in the development of cardiovascular diseases (CVDs). Atherosclerosis develops for a long time and forms the pathological basis for CAD. This complex chronic inflammatory disease involves interactions between different cells. New technologies, in particular, flow cytometry, allow to perform a complex analysis of different cells simultaneously.

One of the main pathophysiological mechanisms in the development of atherosclerosis is a disruption of the structural integrity and functional activity of the vascular endothelium. Circulating endothelial cells detached from the endothelial wall in the course of its damage can act as a direct cellular marker of endothelial dysfunction [2, 3].

Using flow cytometry, it was revealed that the presence of more than 3 circulating endothelial cells per 3×10^5 leukocytes in the peripheral blood increased the relative risk of CAD development in young and middle-aged women by 4 times. In women with CAD, it increased the risk of developing acute myocardial infarction by 8 times [2].

Endothelial progenitor cells, which are capable of self-renewal and differentiation, are involved in repair of the endothelium and formation of new blood vessels. Recruitment and migration of endothelial progenitor cells in the body are controlled by cells located directly in the damaged area [4]. Most progenitor cells mature from hematopoietic stem cells, mainly found

in the bone marrow, peripheral blood, and umbilical cord, but they are also present in the spleen, intestines, liver, adipose tissue, and adventitia. Regardless of their source, all hematopoietic stem cells have markers CD34+ and CD133+ [4].

Endothelial progenitor cells are characterized by expression of surface markers, such as vascular endothelial growth factor receptor-2 (VEGFR-2), CD31, endothelial nitric oxide synthase (eNOS), and vascular endothelial cadherin (VE-cadherin). Therefore, to identify endothelial progenitor cells, the following surface markers of hematopoietic and endothelial cell lines are used: CD34, CD133, VEGFR-2, or kinase domain receptor (KDR) [4, 5]. In patients with arterial hypertension with a high level of low -density lipoproteins (LDL), the number and migration capacity of circulating endothelial progenitor cells are reduced [6, 7]. The number and functional activity of circulating endothelial progenitor cells in the blood are independent predictors of morbidity and mortality from CVDs [8].

The involvement of endothelial progenitor cells in atherogenesis is beyond doubt. In the study by S. Ai et al., when evaluating the expression of the vitamin D receptor (VDR), the authors found that the VDR expression on circulating endothelial progenitor cells was significantly reduced in CAD patients and negatively correlated with the glycated hemoglobin levels. Consistently high serum glucose decreased the VDR expression on endothelial progenitor cells, potentially accelerating the pathological process of atherosclerosis. Thus, low VDR expression on circulating endothelial progenitor cells may serve as a potential risk factor for CAD development [9].

Monocytes are cells of the immune system that are involved in the formation of innate and adaptive immunity. Monocytes play a key role in the atherogenesis, since, after being recruited into the lipid and lipoprotein-rich areas of the arterial intima, they differentiate into macrophages under the influence of the macrophage colony-stimulating factor (M-CSF) produced by the activated endothelium [10, 11]. In the peripheral blood of patients with atherosclerosis, monocytes are preactivated and have some features of macrophages [10]. Their adhesion to the endothelium is 1.5 times higher than that of monocytes in healthy individuals, and they express a number of receptors (type I and type II Fcγ-receptor, intercellular adhesion molecule-1 (ICAM-1)) [12]. Monocytes have heterogeneous composition. In atherosclerosis, an increase in the relative count of monocytes of the intermediate (CD14++/CD16+) and nonclassical (CD14+/CD16++) subpopulations is detected – by 2.3 and 1.8 times, respectively [10, 13].

K.A. Arnold et al. investigated correlations of monocyte subtypes and macrophages cultured from them with CAD progression. Groups of study participants were formed after a coronary angiogram in accordance with CAD severity: a group without CAD (minor disturbances in the vascular lumen); a group with CAD with a single-vessel lesion; a group with CAD with a multi-vessel lesion. In CAD patients (with both single-vessel and multi-vessel lesions), the blood levels of both intermediate and non-classical monocytes were elevated compared to patients without CAD ($p < 0.05$). The count of regulatory macrophages (CD206+) was reduced in patients with both single-vessel and multi-vessel lesions ($p < 0.001$) [14].

A relationship between the atherogenic lipoprotein phenotype and innate immunity in atherosclerotic patients with CAD was shown. The atherogenic fraction of LDLP was associated with an increase in the content of non-classical monocytes (CD14+CD16++) and a decrease in the content of the classical subpopulation (CD14++CD16-) [15].

Lymphocytes play a key role in the development of inflammatory responses in CVDs. The content and phenotype of lymphocytes in the peripheral blood, subcutaneous adipose tissue, and epicardial adipose tissue in patients with and without CAD, who had undergone an elective heart surgery, were studied. It was found that the epicardial adipose tissue in CAD patients was characterized by an increased number of T-lymphocytes, B-lymphocytes, a higher total lymphocyte count, and a reduced number of natural killer (NK) cells in comparison with patients without CAD [16].

The complement system is involved in the CVD pathogenesis by inducing inflammation and interacting with the coagulation system [17]. The balance between activation and inhibition of the complement system is critical for controlling the degree of inflammation. Molecules involved in the regulation of the complement system activation include complement receptor type 1 (CR1, CD35), membrane cofactor protein (MCP, CD46), decay-accelerating factor (DAF, CD55), and membrane inhibitor of reactive lysis (MIRL, CD59). Each protein is different in the mechanism of action for the complement system regulation [18]. Patients with CAD showed lower expression of CD46 and CD55 on the surface of lymphocytes,

monocytes, and granulocytes and higher surface expression of CD35 and CD59 on granulocytes ($p < 0.0001$), compared to healthy donors. High CD59 expression on granulocytes positively correlated with the severity of the disease and may serve as a potential marker of disease progression [18].

FLOW CYTOMETRY IN THE STUDIES ON ATHEROSCLEROTIC PLAQUES

The structure of atherosclerotic plaques is one of the key directions in the study of the pathogenesis of atherosclerosis. There is a large number of experimental studies devoted to formation, maturation, and rupture of atherosclerotic plaques, but the mechanisms of these phenomena remain largely unclear. Traditionally, blood is used as a material for scientific research on atherosclerosis, since collection of tissues for research is associated with certain difficulties. However, for the study of the pathophysiological mechanisms of atherosclerosis, the atherosclerotic lesion itself is of the greatest interest.

Modern quantitative methods can increase the understanding of the pathological processes responsible for plaque progression. It was flow cytometry and development of original protocols for enzymatic isolation of cells from atherosclerotic plaques that allowed to perform the most complete analysis of lymphocytes, their role in the immunological mechanisms of maturation and rupture, and their distribution in atherosclerotic plaques [19, 20].

Earlier there were some attempts to study the role of lymphocytes in an atherosclerotic lesion. However, at the same time, scientists isolated lymphocytes from the tissue into a culture medium with various activators of the lymphocyte migration, i.e. studied individual lymphocytes migrating from the atherosclerotic plaque tissue into the culture medium. This made it possible not to practice the technique of isolating living cells but to study the cellular composition of the atherosclerotic plaque tissue itself [21].

At the same time, attempts were made to accurately determine the cellular composition of the tissue of human atherosclerotic plaques using flow cytometry, where the material obtained during carotid endarterectomy was subjected to enzymatic treatment with collagenase I (250 U / ml) at 37° C. This study showed that about 50% of the cells in the atherosclerotic plaques were mononuclear inflammatory cells (T-lymphocytes and monocytes (macrophages)) [22].

In further research, scientists developed original protocols for isolating cells from the tissue of atherosclerotic plaques and studied the whole complexes of antibodies labeled with fluorochromes. In addition, the use of several fluorochromes simultaneously made it possible to study various processes in the cell. The study by L. Sh. Grievel et al. showed that the phenotypic composition of T-lymphocytes in the plaque differed from that in the blood. When comparing the expression levels of cellular markers CD3, CD4, CD16, CD45, CD45RA, CCR7 CD28, CD27, HLA-DR, and CD38, high levels of CD4 and CD8 T-cells in the plaques were revealed [19].

The dominant inflammatory cell in atherosclerosis is a macrophage. However, interactions with other inflammatory cells may also play a role in the atherogenesis. Using immunohistochemistry, the presence of mast cells in the atherosclerotic lesion, constituting a heterogeneous population, was established. In a 3-year study of 270 patients with carotid stenosis, plasma mast cell levels were shown to be associated with future acute cardiovascular events. Despite the fact that mast cells are present in a plaque in small numbers, they can contribute to destabilization of an atherosclerotic plaque [23].

Mast cells are involved in inflammatory responses in various tissues, including the arterial intima. Mast cells are activated for degranulation, releasing large amounts of inflammatory mediators (histamine, heparin, proteases, and cytokines) stored in their cytoplasm. Activated mast cells in the atherosclerotic lesion can promote leukocyte chemotaxis, adhesion to the activated endothelium, and subsequent transendothelial migration [24].

In order to characterize the population of mast cells in human atherosclerotic lesions in more detail and to determine its activity, E. Kritikou et al. used several markers simultaneously (CD45, CD117, CD63, and IgE, Tryptase/TPSAB1) [25]. The authors confirmed that the main but not the only pathway of mast cell activation inside a plaque is IgE-mediated one, but there is a group of mast cells that are activated without IgE binding [25].

Flow cytometry is a powerful tool for detecting the diversity of leukocyte subsets in the atherosclerotic plaque [26]. The atherosclerotic plaque is a complex immunological environment. Since the discovery of T-cells in atherosclerotic plaques, they have been found to play an important role in the development of atherosclerosis and CVDs. T-helpers can differentiate into several phenotypes (Th1, Th2, Th9, Th17, Treg, etc.), produce various interleukins, and have both pro- and anti-inflammatory mechanisms. The study by

Grönberg et al. suggested that therapeutic inhibition of T-cell differentiation into Th1-cells is a promising strategy for reducing the progression of atherosclerosis [27].

T-lymphocytes play an essential role in atherogenesis, but the atherogenic or atheroprotective role of CD8+ T-cells in late stages of atherosclerosis development remains controversial. The study by J. van Duijn et al. demonstrated the local, protective role of CD8+ T-cells in progressive atherosclerosis by comparing the phenotypes of CD8+ T-cells obtained from plaques from the aorta, spleen, and blood of mice. In progressive atherosclerosis in a mouse model, aortic CD8+ T-cells produced lower amounts of IFNγ and TNFα compared to their systemic counterparts, with a simultaneous increase in the expression of CD39 ectonucleotidase. At the same time, pharmacological inhibition of CD39 in apoE-/- mice partially restored the production of cytokines by CD8+ T-cells. The studies on the samples of atherosclerotic plaques in the human carotid and femoral arteries confirmed these results [28, 29].

Tissue cell death is a characteristic feature of progressive atherosclerotic plaques, including unstable lesions, which are largely responsible for complications of CVDs. The data on the accumulation of cytotoxic lymphocytes in human lesions strongly suggest that these lymphocytes promote cell death in atherosclerotic foci and lead to potential rupture of plaques [30].

NK cells can induce cell death in various ways, using killer activation receptors (KARs) or the cytolytic components perforin and granzyme B to form an immunological synapse, through which the release of cytolytic granules ultimately leads to lysis of target cells [31, 32]. T-cells (CD8, CD4, γδ-cells) and NK cells are involved in the atherogenesis. These cells are present in large numbers in unstable plaques, which indicates that their killer function is important for the progression of atherosclerotic process [30].

Inflammatory cells in the atherosclerotic plaque are derived from hematopoietic stem cells (HSPCs). When analyzing mononuclear blood cells by flow cytometry using antibodies CD38+CD45RA+CD34+HSPCs, the authors found that in CAD patients (coronary stenosis ≥ 50%), the level of circulating HSPCs in the peripheral blood was 1.8 times higher than in individuals without CAD. The level of HSPCs in the blood was associated with the degree of coronary stenosis. In addition, according to the multivariable logistic regression analysis, the level of circulating HSPCs was the only marker that was associated with the odds ratio (OR) of mild to severe (≥ 70%) coronary stenosis (OR 2.08 (95% confidence interval (CI) 1.35–3.21), p = 0.0009). That allowed the authors to propose HSPCs as an important marker for the assessment of atherosclerotic coronary stenosis [33].

CONCLUSION

Flow cytometry has been rapidly developing as a modern method for diagnosing and researching disorders of the immune system. Atherosclerosis is a multi-factorial chronic inflammatory disease that includes complex interactions between the vascular wall and blood cells, and an atherosclerotic plaque is a complex immunological environment.

Scientific knowledge about the atherosclerotic plaque composition and the role of cells found in the lesion focus is constantly growing due to fundamental research. However, a simple and accessible diagnosis of early atherosclerosis remains a problem, since measurement and determination of changes in cell populations in the atherosclerotic focus are impracticable in clinical practice.

It is possible that flow cytometry will become a priority method for determining new prognostic peripheral blood markers for the severity of atherosclerosis.

REFERENCES

1. Khaidukov S.V., Zurochka A.V., Chereshnev V.A. Cytometric analysis in clinical immunology. Yekaterinburg: UB RAS, 2011: 220 (in Russ.).
2. Feoktistova V.S., Vavilova T.V., Sirotkina O.V., Boldueva S.A., Gaykovaya L.B., Leonova I.A., Laskovets A.B., Ermakov A.I. The new approach to evaluation of endothelium dysfunction: detection of number of circulating endothelium cells using flow cytometry technique. *Russian Clinical Laboratory Diagnostics*. 2015; 60 (4): 23–39 (in Russ.).
3. Boos C.J., Balakrishnan B., Blann A.D., Lip G.Y.H. The relationship of circulating endothelial cells to plasma indices of endothelial damage/dysfunction and apoptosis in acute coronary syndromes: implications for prognosis. *J. Thromb. Haemost*. 2008; 6 (11): 1841–1850. DOI: 10.1111/j.1538-7836.2008.03148.x.
4. Semenova A.E., Sergienko I.V., Dombrovskiy A.L., Rvacheva A.V. Endothelial progenitor cells and atherosclerotic process. *The Journal of Atherosclerosis and Dyslipidemias*. 2012, 3 (8):14–24 (in Russ.).
5. Fadini G.P., de Kreutzenberg S.V., Coracina A., Baesso I., Agostini C., Tiengo A., Avogaro A. Circulating CD34+ cells, metabolic syndrome, and cardiovascular risk. *Eur. Heart J.* 2006; 27 (18): 2247–2255. DOI: 10.1093/eurheartj/ehl198.
6. Zhou B., Ma F.X., Liu P.X., Fang Z.H., Wang S.L., Han Z.B., Poon M.C., Han Z.C. Impaired therapeutic vasculogenesis by transplantation of OxLDL-treated endothelial progenitor

cells. *J. Lipid. Res*. 2007; 48 (3): 518–527. DOI: 10.1194/jlr.M600251-JLR200.
6. Vasa M., Fichtlscherer S., Aicher A., Adler K., Urbich C., Martin H., Zeiher A.M., Dimmeler S. Number and migratory activity of circulating endothelial progenitor cells inversely correlate with risk factors for coronary artery disease. *Circ. Res*. 2001; 89 (1): E1–7. DOI: 10.1161/hh1301.093953.
7. Sen S., McDonald S.P., Coates P.T., Bonder C.S. Endothelial progenitor cells: novel biomarker and promising cell therapy for cardiovascular disease. *Clin. Sci. (Lond.).* 2011; 120 (7): 263–283. DOI: 10.1042/CS20100429.
8. Ai S., He Z., Ding R., Wu F., Huang Z., Wang J., Huang S., Dai X., Zhang J., Chen J., Liu L., Wu Z., Liang C. Reduced vitamin D receptor on circulating endothelial progenitor cells: a new risk factor of coronary artery diseases. *J. Atheroscler. Thromb*. 2018; 25 (5): 410–421. DOI: 10.5551/jat.40808.
9. Chelombitko M.A., Shishkina V.S., Ilyinskaya O.P., Kaminnyi A.I., Pavlunina T.O., Samovilova N.N., Gracheva E.V., Tararak E.M., Prokazova N.V. A cytofluorometric study of membrane rafts in human monocyte subsets in atherosclerosis. *Acta Naturae*. 2014, 6 (4): 86–94 (in Russ.).
10. Thomas G.D., Hamers A.A.J., Nakao C., Marcovecchio P., Taylor A.M., McSkimming C., Nguyen A.T., McNamara C.A., Hedrick C.C. Human blood monocyte subsets: a new gating strategy defined using cell surface markers identified by mass cytometry. *Arterioscler. Thromb. Vasc. Biol.* 2017; 37 (8): 1548–1558. DOI: 10.1161/ATVBAHA.117.309145.
11. Luu N.T., Madden J., Calder P.C., Grimble R.F., Shearman C.P., Chan T., Tull S.P., Dastur N., Rainger G.E., Nash G.B. Comparison of the pro-inflammatory potential of monocytes from healthy adults and those with peripheral arterial disease using an in vitro culture model. *Atherosclerosis*. 2007. 193 (2): 259–268. DOI: 10.1016/j.atherosclerosis.2006.08.050.
12. Kapellos T.S., Bonaguro L., Gemünd I., Reusch N., Saglam A., Hinkley E.R., Schultze J.L. Human monocyte subsets and phenotypes in major chronic inflammatory diseases. *Front. Immunol.* 2019; 10: 2035. DOI: 10.3389/fimmu.2019.02035.
13. Arnold K.A., Blair J.E., Paul J.D., Shah A.P., Nathan S., Alenghat F.J. Monocyte and macrophage subtypes as paired cell biomarkers for coronary artery disease. *Exp. Physiol.* 2019; 104 (9): 1343–1352. DOI: 10.1113/EP087827.
14. Krychtiuk K.A., Kastl S.P., Pfaffenberger S., Lenz M., Hofbauer S.L., Wonnerth A., Koller L., Katsaros K.M., Pongratz T., Goliasch G., Niessner A., Gaspar L., Huber K., Maurer G., Dostal E., Wojta J., Oravec S., Speidl W.S. Association of small dense LDL serum levels and circulating monocyte subsets in stable coronary artery disease. *PLoS One*. 2015; 10 (4): e0123367. DOI: 10.1371/journal.pone.0123367.
15. Mráz M., Cinkajzlová A., Kloučková J., Lacinová Z., Kratochvílová H., Lipš M., Pořízka M., Kopecký P., Pierzynová A., Kučera T., Melenovský V., Stříž I., Lindner J., Haluzík M. Coronary artery disease is associated with an increased amount of T lymphocytes in human epicardial adipose tissue. *Mediators Inflamm*. 2019; 2019: 4075086. DOI: 10.1155/2019/4075086.
16. Oikonomopoulou K., Ricklin D., Ward P.A., Lambris J.D. Interactions between coagulation and complement–their role in inflammation. *Semin. Immunopathol*. 2012; 34 (1): 151–165. DOI: 10.1007/s00281-011-0280-x.
17. Mishra N., Mohata M., Narang R., Lakshmy R., Hazarika A., Pandey R.M., Das N., Luthra K. Altered expression of complement regulatory proteins CD35, CD46, CD55, and CD59 on leukocyte subsets in individuals suffering from coronary artery disease. *Front. Immunol.* 2019; 10: 2072. DOI: 10.3389/fimmu.2019.02072.
18. Grivel J.-Sh., Ivanova O.I., Pinegina N.V., Blank P.S., Shpektor A.V., Margolis L.B., Vasilieva E.Yu. A new method for analyzing the cellular composition of atherosclerotic plaques. *Creative Cardiology*. 2012; 1: 26–40 (in Russ.).
19. Lebedeva A., Vorobyeva D., Vagida M., Ivanova O., Felker E., Fitzgerald W., Danilova N., Gontarenko V., Shpektor A., Vasilieva E., Margolis L. *Ex vivo* culture of human atherosclerotic plaques: A model to study immune cells in atherogenesis. *Atherosclerosis*. 2017; 267: 90–98. DOI: 10.1016/j.atherosclerosis.2017.10.003.
20. Curry M.P., Norris S., Golden-Mason L., Doherty D.G., Deignan T., Collins C., Traynor O., McEntee G.P., Hegarty J.E., O'Farrelly C. Isolation of lymphocytes from normal adult human liver suitable for phenotypic and functional characterization. *J. Immunol. Methods*. 2000; 242 (1-2): 21–31. DOI: 10.1016/s0022-1759(00)00204-0.
21. Bonanno E., Mauriello A., Partenzi A., Anemona L., Spagnoli L.G. Flow cytometry analysis of atherosclerotic plaque cells from human carotids: a validation study. *Cytometry*. 2000; 39 (2): 158–165. DOI: 10.1002/(sici)1097-0320(20000201)39:2<158::aid-cyto9>3.0.co;2-8.
22. Willems S., Vink A., Bot I., Quax P.H., de Borst G.J., de Vries J.P., van de Weg S.M., Moll F.L., Kuiper J., Kovanen P.T., de Kleijn D.P., Hoefer I.E., Pasterkamp G. Mast cells in human carotid atherosclerotic plaques are associated with intraplaque microvessel density and the occurrence of future cardiovascular events. *Eur. Heart J.* 2013; 34 (48): 3699–3706. DOI: 10.1093/eurheartj/eht186.
23. Kovanen P.T., Bot I. Mast cells in atherosclerotic cardiovascular disease – Activators and actions. *Eur. J. Pharmacol.* 2017; 816: 37–46. DOI: 10.1016/j.ejphar.2017.10.013.
24. Kritikou E., Depuydt M.., de Vries M., Mulder K., Govaert A., Smit M., van Duijn J., Foks A., Wezel A., Smeets H., Slütter B., Quax P., Kuiper J., Bot I. Flow cytometry-based characterization of mast cells in human atherosclerosis. *Cells.* 2019; 8 (4): 334. DOI: 10.3390/cells8040334.
25. Winkels H., Ehinger E., Ghosheh Y., Wolf D., Ley K. Atherosclerosis in the single-cell era. *Curr. Opin. Lipidol*. 2018; 29 (5): 389–396. DOI: 10.1097/MOL.0000000000000537.
26. Grönberg C., Nilsson J., Wigren M. Recent advances on CD4+ T cells in atherosclerosis and its implications for therapy. *Eur. J. Pharmacol.* 2017; 816: 58–66. DOI: 10.1016/j.ejphar.2017.04.029.
27. Van Duijn J., van Elsas M., Benne N., Depuydt M., Wezel A., Smeets H., Bot I., Jiskoot W., Kuiper J., Slütter B. CD39 identifies a microenvironment-specific anti-inflammatory CD8+ T-cell population in atherosclerotic lesions. *Atherosclerosis*. 2019; 285: 71–78. DOI: 10.1016/j.atherosclerosis.2019.04.217.
28. Van Duijn J., Kritikou E., Benne N., van der Heijden T., van Puijvelde G.H., Kröner M.J., Schaftenaar F.H., Foks A.C.,

Wezel A., Smeets H., Yagita H., Bot I., Jiskoot W., Kuiper J, Slütter B. CD8+ T-cells contribute to lesion stabilization in advanced atherosclerosis by limiting macrophage content and CD4+ T-cell responses. *Cardiovasc. Res*. 2019; 115 (4): 729–738. DOI: 10.1093/cvr/cvy261.

29. Kyaw T., Tipping P., Toh B.-H., Bobik A. Killer cells in atherosclerosis. *Eur. J. Pharmacol.* 2017; 816: 67–75. DOI: 10.1016/j.ejphar.2017.05.009.
30. Pegram H.J., Andrews D.M., Smyth M.J., Darcy P.K., Kershaw M.H. Activating and inhibitory receptors of natural killer cells. *Immunol. Cell Biol*. 2011; 89 (2): 216–224. DOI: 10.1038/icb.2010.78.
31. Griffiths G.M., Tsun A., Stinchcombe J.C. The immunological synapse: A focal point for endocytosis and exocytosis. *J. Cell Biol*. 2010; 189 (3): 399–406. DOI: 10.1083/jcb.201002027.
32. Zhu F.L., Zhang N., Ma X.J., Yang J., Sun W.P., Shen Y.Q., Wen Y.M., Yuan S.S., Zhao D., Zhang H.B., Feng Y.M. Circulating hematopoietic stem/progenitor cells are associated with coronary stenoses in patients with coronary heart disease. *Sci. Rep*. 2019; 9 (1): 1680. DOI: 10.1038/s41598-018-38298-5.

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Diffusion magnetic resonance imaging data: development of methods and tools for diagnosis and treatment of brain diseases

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ABSRACT

The use of quantitative mapping of diffusion characteristics carries great potential for diagnosis and therapy of brain diseases, since it potentially allows to classify tumors, determine the degree of their malignancy, differentiate various morphological structures of tumor and non-tumor pathologies (such as tumor stroma, necrotic zones, cysts, various types of edema, etc.), and predict the course and outcome of diseases, in particular, a clinical response to treatment. Based on diffusion weighted magnetic resonance imaging (MRI), it is possible to perform 3D modeling of the white matter pathways of the brain, which is called tractography. In addition to a unique ability to visualize the location of tracts in relation to intracranial pathologies, this technology allows to build and analyze complex maps of communication networks in the brain (connectomics).

The review is devoted to the discussion of the physical and technical concept of diffusion weighted MRI, the key ways of its application in tumor and non-tumor processes, and problems that complicate correct interpretation of results. Since the problem of developing software for diffusion MRI data remains relevant, this review presents our own experience in developing an application as part of the project on creating effective methods for processing diffusion MRI data and modeling white matter tracts.

Key words: magnetic resonance imaging, diffusion weighted magnetic resonance imaging, tractography, brain, neuroscience.

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Диффузионные данные магнитно-резонансной томографии: разработка методологии и инструментов использования в диагностике и лечении заболеваний головного мозга

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РЕЗЮМЕ

Использование различных карт количественных характеристик диффузии несет в себе большой потенциал для медицинской диагностики и терапии патологии головного мозга, так как позволяет классифицировать опухоли, определять степень их злокачественности, дифференцировать различные морфологические структуры опухолевых и неопухолевых патологических процессов (таких как строма опухоли, зоны некроза, кисты, различные виды отека и т.д.), прогнозировать течение и исход заболеваний, в частности клинический ответ на проведенное лечение. На основе диффузионно-взвешенной томографии возможна реализация трехмерной реконструкции волокон белого вещества головного мозга, называемая трактографией. Помимо уникальной возможности визуализировать расположение трактов относительно интракраниальных патологических изменений, данная технология позволяет строить и анализировать комплексные карты сложных сетей связей в головном мозге (коннектомика).

Обзор посвящен обсуждению физико-технической концепции диффузионно-взвешенной томографии, ключевых направлений применения в случае опухолевых и неопухолевых процессов, а также проблем, затрудняющих процесс корректной интерпретации результатов. Так как в настоящий момент остается актуальной задача применения программного обеспечения для работы с диффузионными показателями магнитно-резонансной томографии, то в представленном обзоре показан собственный опыт разработки приложения в рамках проекта по созданию эффективных методик интерпретации диффузионных данных и построению трактов белого вещества головного мозга.

Ключевые слова: магнитно-резонансная томография, диффузионно-взвешенная томография, трактография, головной мозг, нейронауки.

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

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INTRODUCTION

Introscopy is a key discipline in medical diagnostics. Technical advances in computed tomography machine design and software made it possible to solve existing problems and open up new scientific directions that previously could not be invasively studied on people. In this context, diffusion weighted imaging (DWI) has become a widely used technique for studying the structure and functions of the brain, since it allows to measure the orientation of white matter fibers *in vivo*. This is especially relevant in the fields of stereotactic radiosurgery and neurosurgery, where it is important to measure the degree of involvement of functionally significant areas in the pathological process, while in certain cases, the

standard set of images (T1-weighted (T1w), T1w contrast-enhanced, T2-weighted (T2w), fluid-attenuated inversion recovery (FLAIR) images, etc.) may not be enough to differentiate various morphological structures in tumor and non-tumor pathological processes.

PHYSICAL AND TECHNICAL FEATURES OF DIFFUSION MRI DATA

DWI is a technique of magnetic resonance imaging (MRI) that provides information about microscopic displacements of water molecules that occurs in biological tissues due to physical diffusion.

Vector $\mathbf{r}_0$ is the initial position of the particle at $t = 0$, and vector $\mathbf{r}$ is its subsequent position at $t = \tau$. Generalizing the Einstein's equation [1] to the case of an anisotropic medium, we obtain:

$$D = \frac{1}{6\tau}\langle \mathbf{R}^T \cdot \mathbf{R} \rangle = \begin{bmatrix} Dxx & Dxy & Dxz \\ Dyx & Dyy & Dyz \\ Dzx & Dzy & Dzz \end{bmatrix}, \quad (1)$$

where D is the diffusion tensor, and the vector $\mathbf{R} = \mathbf{r}\text{-}\mathbf{r}_0$ shows particle displacement. It can be shown that this second-rank tensor is symmetric and positive definite, and that its eigenvalues are real.

It is necessary to obtain several DWI in different noncollinear directions of the gradient g_k ($k = 1, ..., N$) to calculate the diffusion tensor D($\mathbf{r}$) [2]. In general, the diffusion tensor can be calculated by solving the following system of equations:

$$S_k(\mathbf{r}) = S_0(\mathbf{r}) e^{-b\hat{g}_k^T \cdot D(\mathbf{r}) \cdot \hat{g}_k}, \text{ где } \hat{g}_k = \frac{g_k}{\|g_k\|}, \quad (2)$$

where $S_0(\mathbf{r})$ is the signal in the absence of diffusion gradients (i.e., $\|g\| = 0$).

Coefficient b is the so-called b-factor, introduced in [3] and defined as:

$$b_k = \gamma^2 \delta^2 \left(\Delta - \frac{\delta}{3}\right) \|g\|^2, \quad (3)$$

where γ is the gyromagnetic ratio of hydrogen (42.58 MHz / T), δ is the signal duration, and Δ is the time between the pulses.

The probability density function $p(\mathbf{r}|\mathbf{r}_0, \tau)$ for an anisotropic medium:

$$p(\mathbf{r} \,|\, \mathbf{r_0}, \tau) = \frac{1}{\sqrt{(4\pi\tau)^3 |D|}} e^{-\frac{(\mathbf{r}-\mathbf{r_0})^T \cdot D^{-1} \cdot (\mathbf{r}-\mathbf{r_0})}{4\tau}}, \quad (4)$$

Also, since the $\|g_k\|$ are different, the b-factor can be expressed as follows:

$$b_k = \gamma^2 \delta^2 (\Delta - \frac{\delta}{3}) \|g_k\|^2, \quad (5)$$

It is more convenient to rewrite the system of equations (2) in the form:

$$\begin{bmatrix} (\hat{g}_{1x})^2 & 2\hat{g}_{1x}\hat{g}_{1y} & 2\hat{g}_{1x}\hat{g}_{1z} & (\hat{g}_{1y})^2 & 2\hat{g}_{1y}\hat{g}_{1z} & (\hat{g}_{1z})^2 \\ (\hat{g}_{2x})^2 & 2\hat{g}_{2x}\hat{g}_{2y} & 2\hat{g}_{2x}\hat{g}_{2z} & (\hat{g}_{2y})^2 & 2\hat{g}_{2y}\hat{g}_{2z} & (\hat{g}_{2z})^2 \\ \vdots & \vdots & \vdots & \vdots & \vdots & \vdots \\ (\hat{g}_{Nx})^2 & 2\hat{g}_{Nx}\hat{g}_{Nz} & 2\hat{g}_{Nx}\hat{g}_{2N} & (\hat{g}_{Ny})^2 & 2\hat{g}_{Ny}\hat{g}_{Nz} & (\hat{g}_{Nz})^2 \end{bmatrix} \begin{bmatrix} D_{xx}(\mathbf{r}) \\ D_{xy}(\mathbf{r}) \\ D_{xz}(\mathbf{r}) \\ D_{yy}(\mathbf{r}) \\ D_{yz}(\mathbf{r}) \\ D_{zz}(\mathbf{r}) \end{bmatrix} = \begin{bmatrix} \frac{1}{b}\ln\frac{S_0(\mathbf{r})}{S_1(\mathbf{r})} \\ \frac{1}{b}\ln\frac{S_0(\mathbf{r})}{S_2(\mathbf{r})} \\ \vdots \\ \frac{1}{b}\ln\frac{S_0(\mathbf{r})}{S_N(\mathbf{r})} \end{bmatrix} \quad (6)$$

$$\mathrm{G} \qquad \widetilde{D(\mathbf{r})} \qquad \mathrm{B}(\mathbf{r})$$

If more than 6 directions are obtained, then the system of equations (6) can be solved using the least squares approximation. For example, if you use a linear unweighted least squares approximation, then:

$$\widetilde{D(\mathbf{r})} = (G^T \cdot G)^{-1} \cdot G^T \cdot B(\mathbf{r}). \quad (7)$$

In diffusion tensor imaging (DTI), it is assumed [4] that distribution of molecules undergoing diffusion is characterized by the conditional probability density function $p(\mathbf{r}|\mathbf{r}_0, \tau)$ given by the expression (4). Thus, D can be associated with an ellipsoid, which is a probability density isosurface of molecule diffusion. Since D is a symmetric positive definite second-rank tensor, it can be decomposed into eigenvectors (which form an orthogonal basis) and real eigenvalues [5]:

$$D = E \cdot \Lambda \cdot E^{-1}, \quad (8)$$

$$E = [e_1\ e_2\ e_3] \text{ и } \Lambda = \begin{bmatrix} \lambda_1 & 0 & 0 \\ 0 & \lambda_2 & 0 \\ 0 & 0 & \lambda_3 \end{bmatrix}. \quad (9)$$

WORKING WITH DIFFUSION MRI DATA

The development of methods and tools for working with diffusion MRI data involves a comprehensive consideration of several aspects.

In this section, the key questions are: "Why do we use diffusion weighted images? What capabilities do they have? What information can be obtained with their use along with other MRI modalities?"

DWI IN THE STUDY OF TUMOR PROCESSES

Due to processing of diffusion MRI data and analysis of their parameters (apparent diffusion coefficient (ADC), fractional anisotropy (FA), average diffusion coefficient, etc.) together with other MRI modalities available for a particular patient, it is possible to non-invasively:

1. Classify a process.

Central nervous system (CNS) lymphoma often exhibits a lower ADC [6–9] than other potentially similar formations on structural MRI, such as glial tumors, metastases, or infectious lesions.

Some of the most common brain tumors in children significantly differ in their diffusion properties, which can be useful in differential diagnosis. In particular, medulloblastomas tend to have a significantly lower ADC [10] than ependymomas or pilocytic astrocytomas.

In most cases, DWI makes it possible to distinguish an abscess from tumor necrosis (on structural MRI, the signals from them often mimic each other), since the ADC for purulent exudate is usually much lower than for necrosis [11].

2. Determine the tumor grade.

In the study [12], a statistically significant correlation was demonstrated between tumor cellularity and the minimum ADC, which was not detected in relation to T2w images. It was noted that the ADC for high-grade glial tumors ($(0.82–2.46) \cdot 10^{-3}$ mm^2/ s, the average ADC value = $(1.26 \pm 0.40) \cdot 10^{-3}$ mm^2/ s)) significantly exceeded the value for low-grade tumors ($(1.94 –3.31) \cdot 10^{-3}$ mm^2/ s, the average ADC value = $(2.7 \pm 0.7) \cdot 10^{-3}$ mm^2/ s).

3. Predict the course of the disease, its outcome, and a clinical response to treatment.

Since not all brain tumors respond to a particular therapy in the same way, early detection of treatment failures will allow for faster implementation of alternative treatment. It was shown that DWI is sensitive to tissue cellularity and, therefore, can potentially be used as a therapeutic biomarker [13]. For example, a method of functional diffusion mapping was proposed in [14]. It was calculated as an ADC change between the maps constructed upon admission of the patient for treatment (baseline) and at the current moment. Each voxel is mapped with one of 3 colors: blue voxels – a decrease in ADC, indicating an increase in cell density in the tumor; red voxels – an increase in ADC, indicating a decrease in cell density; green voxels – no significant change in the ADC. Thus, this method makes it possible to quantitatively assess the tumor response to treatment at runtime, provided that structural MRI cannot always reliably demonstrate this response.

4. Perform tractography.

In its simplest form, tractography can be interpreted as follows [15–17]: each voxel is characterized by one predominant fiber orientation and combines these local orientations to derive global trajectories. Mathematically, a set of local fiber orientations can be considered as a three-dimensional (3D) vector field, and global trajectories – as its streamlines [18–20]. A streamline is any curve that is tangential to the vector field along a path and can be represented as a three-dimensional spatial curve $\boldsymbol{r}(s)$, parameterized by arc length s. To align the streamline with the vector field, the tangent over the arc length s must be equal to the vector in the appropriate ratio:

$$\frac{d\boldsymbol{r}(s)}{ds} = \boldsymbol{v}[\boldsymbol{r}(s)], \quad (10)$$

where $\boldsymbol{r}(s)$ denotes the position along the streamline and **v** is the three-dimensional vector field.

The above-described equation is differential and can be solved as follows:

$$r(s) = \int_{s_0} \boldsymbol{v}[\boldsymbol{r}(s)]ds, \quad (11)$$

where $\boldsymbol{r}(\boldsymbol{s_0}) = \boldsymbol{r_0}$ represents the starting point of the streamline.

The above described streamline orientation is commonly called streamline reconstruction or tractography, and the resulting trajectories are called tracts or paths. A tractogram is a set of tracts reconstructed using tractography.

There are two different approaches in tractography: deterministic and probabilistic (there is also a global one [21–23], but its detailed consideration remains outside this article). With their help, it is possible to visualize various information [24, 25], such as brain tracts and connection maps, respectively. A lack of information about the error in the fiber tracking procedure is a significant limitation of deterministic tractography methods. To assess this uncertainty, probabilistic tractography algorithms create a large set of tracts (or a distribution of possible trajectories from each starting point). The results of probabilistic tractography are often presented in the form of quan-

titative maps showing the number of tracts that pass through a voxel, since it is assumed that the areas of the brain that are displayed with higher densities of the resulting trajectories have a higher probability of a connection with the starting point [26, 27].

DWI IN THE STIDY OF NON-TUMOR PROCESSES

The parameters of diffusion MRI can be considered as predictors of pathology long before it appears morphologically. For example, DTI allows to detect specific changes in the quantitative diffusion parameters of patients with functional pathologies, such as epilepsy, multiple sclerosis (MS), Parkinson's and Alzheimer's diseases, obsessive-compulsive disorder (OCD), etc.

According to the data of post-mortem studies, there is widespread involvement of the gray matter in the demyelinating process in MS. At the same time, some cortical tumors remain invisible on structural MRI (even using 3.0 T MRI scanners [28]). In a study [29], it was shown that the FA values for intracortical MS lesions are higher than for lesions in the gray matter. This self-contradictory discovery may actually reflect loss of dendrites within the lesion and activation of microglial cells. In another work [30], a relationship between T2 relaxation time and quantitative DTI data was investigated. MS lesions with longer times showed the most pronounced diffusion anomalies, which strongly correlated with a reduced content of myelin water. Taken together, these results support the idea that DTI is capable of detecting and assessing the severity of functionally significant tissue damage in MS at early stages.

It was shown that in the potentially epileptogenic areas [31], a decrease in FA and an increase in the average diffusion coefficient were observed. At the same time, tractography detected changes in the brain fibers responsible for memory and speech in patients with temporal lobe epilepsy.

The cingulum has long been known to be involved in the pathogenesis of OCD, but the results of its research are ambiguous. Studies on the left cingulum revealed both higher [32] and lower FA values compared to the control [33]. Other works [33; 34] demonstrated lower FA values in the right cingulum in patients with OCD. In addition to the data on the cingulum, high FA values were found in the inner capsule, corpus callosum [34; 35], and centrum semiovale [36]; low FA level was observed in the parietal lobe, supramarginal gyri, and left lingual gyrus in the occipital lobe [33]. The data described above are successfully used for functional stereotactic radiosurgery [37] (Fig. 1).

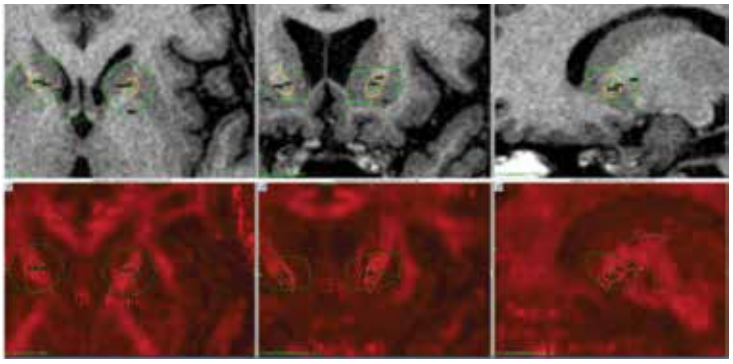

Fig. 1. Gamma Knife radiosurgery plan for a patient with obsessive-compulsive disorder: as a target, the inner capsule is chosen ((orange outline), prescribed radiation dose of 80 Gy along the 50% isodose line); inner green line – 140 Gy, yellow line – 80 Gy, outer green line – 15 Gy

RADIOSENSITIVITY OF THE BRAIN WHITE MATTER TRACTS

Another way of using diffusion weighted imaging relates to visualization of critical structures and optimization of the dose they receive during stereotactic radiosurgery. In modern clinical practice, we have limited recommendations on the tolerance doses for critical structures. If we discuss the effect of the dose on the brain, there is no differentiation of dose restrictions for the white matter, cerebral cortex, and various structures of the gray matter. The need for such differentiation and the need for visualizing and isolating tracts adjacent to or involved in a tumor and reducing the radiation dose applied to them are being discussed.

The study [38] demonstrated that with an increase in the radiation dose applied to the area of interest and time after the course of radiation therapy, the values of the coefficients of mean, axial (λ‖), and radial (λ⊥) diffusion significantly increased with a corresponding decrease in FA, which indicated changes in fibers and their radiosensitivity to therapeutic radiation doses. The changes were significant after 4–6 months or more (p <0.001). Hence, it can be concluded that when planning the irradiation of targets located in the vicinity of functional zones, negligence of adjacent white matter tracts is likely to result in neurocognitive impairments.

INSTRUMENTS FOR WORKING WITH DIFFUSION MRI DATA

The key questions discussed in this section are: "How to develop instruments for working with diffusion MRI data? What problems and limitations can be faced and how to solve them? What software is available today?"

Problems of working with diffusion MRI data:

1. Processing of ambiguous local geometries.

When using the tensor model for working with diffusion MRI data, it is impossible to distinguish at the voxel level such fiber configurations as crossing, kissing, bending, and fanning [39]. In this case, it may be necessary to use higher order diffusion models [40–47].

2. Reconstructions near the cortex.

Tracking fibers near the cortex and gyri is a challenging task [48, 49], since it is determined by large modeling errors in the local assessment of fiber orientation when approaching the gray matter. Higher order algorithms provide high quality assessment of fiber orientation in white matter voxels. However, along with this, they can give unreliable results in voxels partially containing gray matter. To solve this problem, it is necessary to use local models that take into account the presence of other types of tissues or microstructural compartments.

3. Spatial resolution.

Currently, acquisition of whole-brain DWI for routine use within a reasonable scan time is limited to 2 mm^3 spatial resolution. At the same time, it is obvious that in order to characterize the fine structures of the white matter and, in particular, the intricate structures of the cerebral cortex or small structures near the gray matter (nuclei, brain stem), higher spatial resolution is required. Increasing it is not a trivial task, since it is usually accompanied by either a significant decrease in the signal-to-noise ratio or a significant increase in the acquisition time.

4. Angular resolution.

Local orientation models fail to distinguish between two fiber populations, when the angle between them falls below a certain threshold. Such effects could potentially generate entirely artificial fibers. Despite the existence of such a term as high angular resolution diffusion imaging, angular resolution of DWI is rather limited. As a consequence, even with the most advanced high angular resolution diffusion imaging and advanced fiber orientation techniques, angles below 30° between fibers are rarely resolved.

5. False positives.

As shown in [50–52], due to the fact that different fiber configurations can lead to the same MRI signal in the voxel, a large number of false positive tracts and connections are generated. For 96 tractograms shown at the ISMRM 2015 Tractography Challenge by the 21st International Group, there were on average four false positives per a valid fiber.

6. Artifacts.

DWI, like any echo planar imaging, is characterized by a number of artifacts: physical hardware (magnetic susceptibility, eddy currents, gradient artifact, etc.) and artifacts associated with the object of the study (artifacts of movement, blood pulsations, chemical shift, fat suppression, etc.).

IMAGE DISTORTION CORRECTION

Before working with DWI, it is necessary to process the image, namely, to correct the distortions caused by the above-described artifacts. At the same time, new methods (both hardware and software) for dealing with them already exist and are constantly developing.

The most interesting is the correction method implemented in FSL toolbox (FMRIB, England). It is based on the choice of a covariance function that predicts the signal from voxels even with complex fiber configurations.

Diffusion MRI data (in each voxel) are obtained by measuring the signal after applying diffusion weighting (characterized by a b-factor and a unit-length vector **g** defining the direction). A complete diffusion protocol consists of multiple measurements in different gradient directions. Hence, the data can be viewed as a response variable (signal) obtained at the surface of the sphere.

Two important aspects should be noted: 1) the signal changes smoothly when the angle of the diffusion

weighting direction changes; 2) the signal is axially symmetric, that is, the signal along $\boldsymbol{g}$ is identical to the signal along -$\boldsymbol{g}$.

Since the diffusion signal is distributed over the sphere, the methods used in geostatistics can be used to work with it. One such method is kriging, it is a special case of the Gaussian process observed on the sphere. For these methods, covariance is often defined as a function of the angle θ between two vectors from the center of the sphere to x (observed points) and x' (points predicted in the absence of distortion). These vectors are easily represented as the $\boldsymbol{g}$-vectors described above. Two popular covariance functions that determine the relationship between observed points and predicted (sought) points in geostatistics are:

1) exponential model:

$$C(\theta) = e^{-\theta/a} \text{ for } 0 \le \theta \le \pi, \quad (12)$$

where a is the positive parameter of the scale;

2) spherical model

$$C(\theta) = \begin{cases} 1 - \frac{3\theta}{2a} + \frac{\theta^3}{2a^3} & \text{for } \theta \le a \\ 0 & \text{for } \theta \ge a \end{cases}, \quad (13)$$

where a is a positive parameter of the scale, which here defines the distance at which the covariance θ tends to zero.

SOFTWARE FOR WORKING WITH DWI

The process of working with diffusion images is divided into the following stages: pre-processing, including correction of artifacts and extraction of the brain mask, direct work with the image and visualization, namely, estimation of diffusion tensors, construction of maps for diffusion parameters and tracts, clustering, and then quantitative analysis. If we consider the whole variety of software that works with DWI, relying on the stages of working with it, we can distinguish several programs: AFNI (NIMH, USA), FSL (FMRIB, England), MRTrix (collaboration of a large number of institutions from different countries of the world), 3D Slicer (NIH, USA), Camino (University College London, England), TrackVis (MGH GCRC and NIMH, USA), etc. However, none of them covers the entire range of tasks. In addition, many software products developed for processing diffusion images involve writing and / or understanding of scripts, which implies a lot of manual work that is not transparent to non-programmers. At the moment, when planning radiation therapy, the function of diffusion MRI data processing is not available in commercial specialized software.

Therefore, the task of developing software that most fully covers the tasks of working with diffusion MRI data remains relevant.

DEVELOPMENT OF SELF-DESIGNED SOFTWARE

We are developing the MRDiffusion application in the standard C ++ language. The subject part has been moved to separate class libraries and can be used on various platforms. The current user interface is Windows 10. Forms are written in XAML. Graphics is generated using the DirecX environment. Open source math libraries are used for some computational tasks.

Using the interface, you can upload (Fig. 2) files obtained during MRI in DICOM with series of DTI, which will be displayed with the ability to select a specific series. It is convenient in terms of navigating through images and is effective in the work setting.

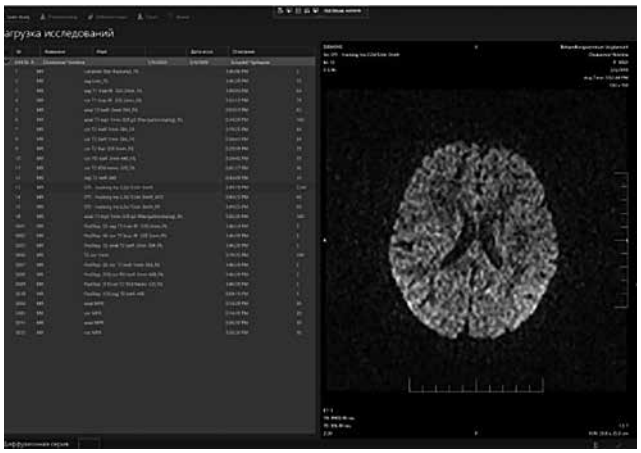

Fig. 2. Loading images into MRDiffusion

For further image processing, it is necessary to extract the brain mask, which is performed on images with a zero gradient, that is, on T2w (Fig. 3, *a*).

Using the application, it is possible to calculate such quantitative parameters as average diffusion and FA, presented in two versions: conventional and with color coding of directions (Fig. 3. *b*).

At the moment, tractography is implemented by a simple deterministic method – fiber assignment by continuous tracking (FACT). Its essence is as follows.

Path selection begins at the center of each voxel with an FA value above the predetermined threshold and continues parallel to the main diffusion direction. Black arrows (Fig. 4, *a*) indicate the diffusion tensor eigenvectors with the largest eigenvalue. At the point where the path crosses the voxel, the direction changes according to the new main direction. Iteratively

continuing such actions, restoration of the path will be interrupted when the conditions for stopping the algorithm occur: if the FA value in the voxel is less than the threshold or if the angle between the new and the old main directions of diffusion turns out to be higher than the predetermined angle threshold. The path is restored in both directions from the starting point: in the direction of the main vector and against it. Moreover, each piece of the tract has a color obtained by mixing red, green, and blue, which, in turn, indicate the main direction of diffusion in one direction or another (Fig. 4, *b*).

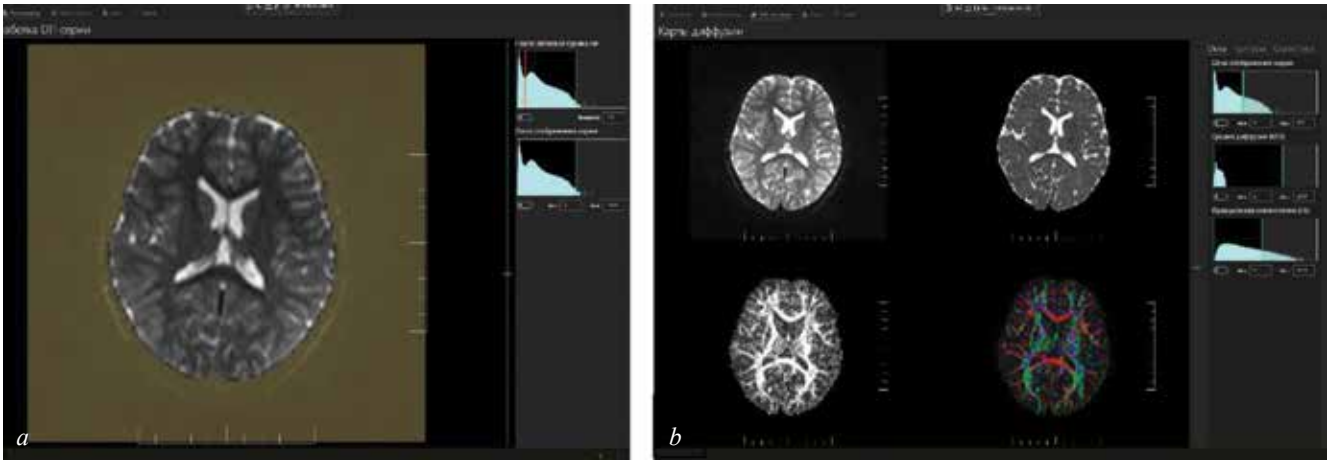

Fig. 3. Extraction of the brain mask (*a*); quantitative diffusion maps (*b*)

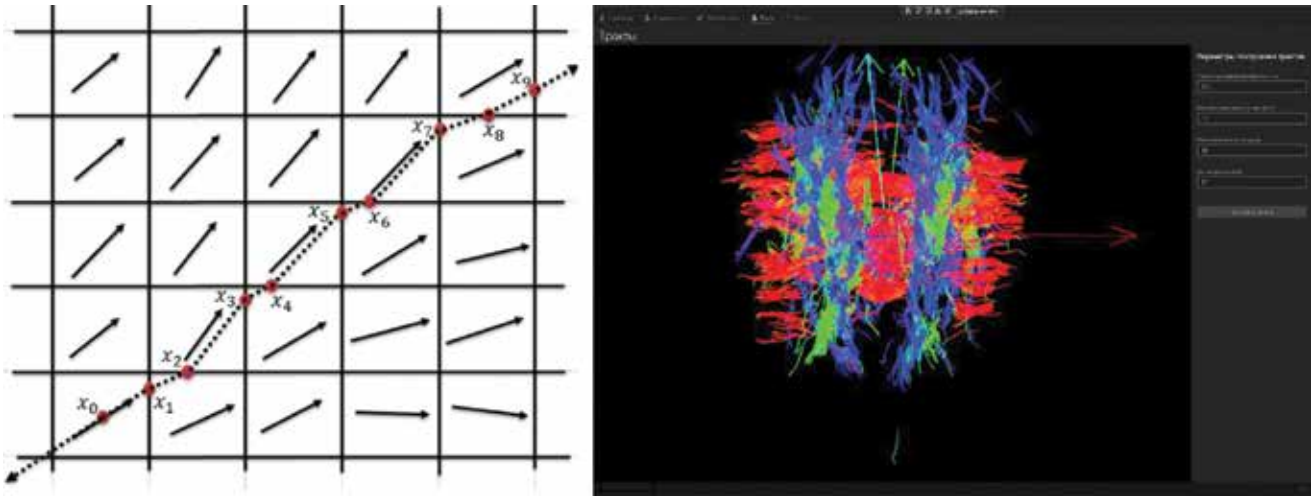

Fig. 4. Scheme of FACT algorithm (*a*), an example of FACT tractography (*b*)

CONCLUSION

Since its emergence in the mid-1990s, diffusion weighted MRI has been widely used in medical imaging for data processing and analysis, and especially in brain research. This is due to its unique capabilities (compared to other modalities), namely, the ability to quantitatively and qualitatively investigate tissue structure and function at the cellular level without the use of contrast agents or ionizing radiation. The most promising application of this technique, in addition to routine medical research, is its use in planning radiation therapy and neurosurgical operations and studying epilepsy, Alzheimer's and Parkinson's diseases, multiple sclerosis, and other disorders. The use of this technology in other areas is strongly limited by the ability to correctly interpret the results.

Diffusion weighted MRI of the brain is undoubtedly a promising non-invasive technology, but working with it is associated with a number of problems: 1) a

high probability of appearance of false positive fibers; 2) the impossibility of finding a difference and, as a consequence, a reliable reconstruction of intersecting, kissing, and merging fibers; 3) a lack of reproducibility of the result, dependence on the user; 4) the impossibility of displaying short tracts; 5) the instability of algorithms when working with pathology.

REFERENCES

1. Einstein A. Über die von der molecularkinetischen Theorie der Wärme geforderte Bewegung von in ruhenden Flüssigkeiten suspendierten Teilchen. An-nalen der Physik. 1905; 322 (8): 549–560.
2. Leemans A. Modeling and processing of diffusion tensor magnetic resonance images for improved analysis of brain connectivity. PhD thesis. University of Antwerp, Antwerpen, 2006.
3. Hashemi R., Bradley W., Lisanti C. MRI. The Basics. Lippincott Williams & Wilkins. Ph., 2004: 353.
4. Laun F., Fritzsche K., Kuder T., Stieltjes B. Introduction to the basic principles and techniques of diffusion-weighted imaging. *Radiology*. 2011; 51 (3):170–179. DOI: 10.1007/s00117-010-2057-y.
5. Basser P., Mattiello J., Le Bihan D. MR diffusion tensor spectroscopy and imaging. *Biophysical Journal*. 1994; 66 (1): 259–267. DOI: 10.1016/S0006-3495(94)80775-1.
6. Kitis O., Altay H., Calli C., Yunten N., Akalin T., Yurtseven T. Minimum apparent diffusion coefficients in the evaluation of brain tumors. *Eur. J. Radiol*. 2005; 55 (3): 393-400. DOI: 10.1016/j.ejrad.2005.02.004.
7. Yamasaki F., Kurisu K., Satoh K., Arita K., Sugiyama K., Ohtaki M. et al. Apparent diffusion coefficient of human brain tumors at MR imaging. *Radiology*. 2005; 235 (3): 985–991. DOI: 10.1148/RADIOL.2353031338.
8. Al-Okaili R., Krejza J., Woo J.H., Wolf R., O'Rourke D., Judy K. et al. Intraaxial brain masses: MR imaging-based diagnostic strategy – initial experience. *Radiology*. 2007; 243 (2): 539–550. DOI: 10.1148/radiol.2432060493.
9. Toh C., Castillo M., Wong A., Wei K., Wong H., Ng S. et al. Primary cerebral lymphoma and glioblastoma multiforme: differences in diffusion characteristics evaluated with diffusion tensor imaging. *AJNR Am. J. Neuroradiol*. 2008; 29 (3): 471–475. DOI: https://doi.org/10.3174/ajnr.A0872.
10. Wilke M., Eidenschink A., Muller-Weihrich S., Auer D. MR diffusion imaging and 1H spectroscopy in a child with medulloblastoma. A case report. *Acta Radiol.* 2001; 42 (1): 39–42.
11. Guzman R., Barth A., Lovblad K., El-Koussy M., Weis J., Schroth G. Use of diffusion-weighted magnetic resonance imaging in Diffusion Imaging in Brain Tumors differentiating purulent brain processes from cystic brain tumors. *J. Neurosurg*. 2002; 97 (5): 1101–1107. DOI: 10.3171/jns.2002.97.5.1101.
12. Sugahara T., Korogi Y., Kochi M., Ikushima I., Shigematu Y., Hirai T., Okuda T., Liang L., Ge Y., Komohara Y. et. al. Usefulness of diffusion-weighted MRI with echo-planar technique in the evaluation of cellularity in gliomas. *J. Magn. Reson. Imaging.* 1999; 9 (1): 53–60. DOI: 10.1002/(SICI)1522-2586(199901)9:1<53::AID-JMRI7>3.0.CO;2-2.
13. Schmainda K. Diffusion-weighted MRI as a biomarker for treatment response in glioma. *CNS Oncology*. 2012; 1 (2):169–180. DOI: 10.2217/cns.12.25.
14. Ellingson B., Malkin M., Rand S. et al. Validation of functional diffusion maps (fDMs) as a biomarker for human glioma cellularity. *J. Magn. Reson. Imaging*. 2010; 31 (3): 538–548. DOI: 10.1002/jmri.22068.
15. Mori S., Zijl P. Fiber tracking: Principles and strategies – A technical review. *NMR in Biomedicine.* 2002; 15 (7-8): 468–480. DOI: 10.1002/nbm.781.
16. Lazar M. Mapping brain anatomical connectivity using white matter tractography. *NMR Biomed.* 2010; 23 (7): 821–835. DOI: 10.1002/nbm.1579.
17. Wasserthal J., Neher P., Maier-Hein K. Tract orientation mapping for bundle-specific tractography. Springer, 2018. DOI: 10.1007/978-3-030-00931-1_5.
18. Conturo T., Lori N., Cull T. et al. Tracking neuronal fiber pathways in the living human brain. *Proc. Natl. Acad. Sci.* 1999; 96 (18): 10422–10427. DOI: 10.1073/pnas.96.18.10422.
19. Mori S., Crain B.J., Chacko V.P., van Zijl P.C. Three-dimensional tracking of axonal projections in the brain by magnetic resonance imaging. *Ann. Neurol.* 1999; 45 (2): 265–269. DOI: 10.1002/1531-8249(199902)45:2<265::aid-ana21>3.0.co;2-3.
20. Basser P., Pajevic S., Pierpaoli C. et al. In vivo fiber tractography using DT-MRI data. *Magn. Reson. Med.* 2000; 44 (4): 625–632. DOI: 10.1002/1522-2594(200010)44:4<625::aid-mrm17>3.0.co;2-o.
21. Reisert M., Mader I., Anastasopoulos C. et al. Global fiber reconstruction becomes practical. *Neuroimage*. 2011; 54 (2): 955–962. DOI: 10.1016/j.neuroimage.2010.09.016.
22. Mangin J-F., Fillard P., Cointepas Y. et al. Toward global tractography. *Neuroimage*. 2013; 80: 290–296. DOI: 10.1016/j.neuroimage.2013.04.009.
23. Christiaens D., Reisert M., Dhollander T. et al. Global tractography of multi-shell diffusion-weighted imaging data using a multi-issue model. *Neuroimage*. 2015; 123: 89–101. DOI: 10.1016/j.neuroimage.2015.08.008.
24. Behrens T., Woolrich M., Jenkinson M. et al. Characterization and propagation of uncertainty in diffusion-weighted MR imaging. *Magn. Reson. Med.* 2003; 50 (5): 1077–1088. DOI: 10.1002/MRM.10609.
25. Behrens T., Berg H., Jbabdi S., Rushworth M., Woolrich M. Probabilistic diffusion tractography with multiple fibre orientations: what can we gain? *Neuroimage.* 2007; 34 (1): 144–155. DOI: 10.1016/j.neuroimage.2006.09.018.
26. Parker G., Alexander D. Probabilistic anatomical connectivity derived from the microscopic persistent angular structure of cerebral tissue. *Philos. Trans. R. Soc. Lond. B Biol. Sci*. 2005; 360 (1457): 893–902. DOI: 10.1098/rstb.2005.1639.
27. Parker G., Haroon H., Wheeler-Kingshott C. A Framework for a streamline-based probabilistic index of connectivity (PICo) using a structural interpretation of MRI diffusion measurements. *J. Magn. Reson. Imaging*. 2003; 18 (2): 242–254. DOI: 10.1002/jmri.10350.
28. Polman C., Reingold S., Banwell B. et al. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. *Annals of Neurology*. 2011; 69 (2): 292–302. DOI: 10.1002/ana.22366.

29. Poonawalla A., Hasan K., Gupta R., Ahn C., Nelson F., Wolinsky J., Narayana P. Diffusion-tensor MR imaging of cortical lesions in multiple sclerosis: initial findings. *Radiology.* 2008; 246(3): 880–886. DOI: 10.1148/radiol.2463070486.
30. Kolind S., Laule C., Vavasour I., Li D.K., Traboulsee A., Mädler B., Moore G., Mackay A. Complementary information from multi-exponential T2 relaxation and diffusion tensor imaging reveals differences between multiple sclerosis lesions. *Neuroimage*. 2008; 40(1): 77–85. DOI: 10.1016/j.neuroimage.2007.11.033.
31. Hugg J., Butterworth E., Kuzniecky R. Diffusion mapping applied to mesial temporal lobe epilepsy: preliminary observations. *Neurology*. 1999; 53(1): 173 – 176. DOI: 10.1212/wnl.53.1.173.
32. Cannistraro P., Makris N., Howard J., Wedig M., Hodge S., Wilhelm S., Kennedy D., Rauch S. A diffusion tensor imaging study of white matter in obsessive-compulsive disorder. *Depress Anxiety*. 2007; 24(6): 440–446. DOI: 10.1002/da.20246.
33. Szeszko P., Ardekani B., Ashtari M., Malhotra A., Robinson D., Bilder R., Lim K. White matter abnormalities in obsessive-compulsive disorder: a diffusion tensor imaging study. *Arch. Gen Psychiatry*. 2005; 62(7): 782–790. DOI: 10.1001/archpsyc.62.7.782.
34. Cannistraro P., Makris N., Howard J., Wedig M., Hodge S., Wilhelm S. et al. A diffusion tensor imaging study of white matter in obsessive-compulsive disorder. *Depress Anxiety.* 2006; 24: 440–446. DOI: 10.1007/978-1-59745-495-7_3.
35. Yoo S., Jang J., Shin Y., Kim D., Park H., Moon W., Chung E., Lee J., Kim I., Kim S., Kwon J. White matter abnormalities in drug-naive patients with obsessive-compulsive disorder: a diffusion tensor study before and after citalopram treatment. *Acta Psychiatr. Scand*. 2007; 116 (3): 211–219. DOI: 10.1111/j.1600-0447.2007.01046.x.
36. Nakamae T., Narumoto J., Shibata K., Matsumoto R., Kitabayashi Y., Yoshida T., Yamada K., Nishimura T., Fukui K. Alteration of fractional anisotropy and apparent diffusion coefficient in obsessive-compulsive disorder: a diffusion tensor imaging study. *Prog. Neuropsychopharmacol. Biol. Psychiatry.* 2008; 32(5): 1221–1226. DOI: 10.1016/j.pnpbp.2008.03.010.
37. Gavin C., Ian Sabin H. Stereotactic diffusion tensor imaging tractography for Gamma Knife radiosurgery. *J. Neurosurgery*. 2016; 125 (1):139–146. DOI: 10.3171/2016.8.GKS161032.
38. Connor M., Karunamuni R., McDonald C., White N., Pettersson N., Moiseenko V., Seibert T., Marshall D., Cervino L., Bartsch H., Kuperman J., Murzin V., Krishnan A. et al. Dose-dependent white matter damage after brain radiotherapy. *Radiothion. Oncol*. 2016; 121 (2): 209–216. DOI: 10.1016/j.radonc.2016.10.003.
39. Tournier J.-D., Calamante F., Connelly A. MRtrix: diffusion tractography in crossing fiber regions. *Int. J. Imaging Syst. Technol.* 2012; 22: 53–66. DOI: 10.1002/IMA.22005.
40. Wedeen V., Hagmann P., Tseng W. Mapping Complex Tissue Architecture with Diffusion Spectrum Magnetic Resonance Imaging. *Magnetic Resonance in Medicine*. 2005; 54 (6): 1377–1386. DOI: 10.1002/MRM.20642.
41. Campbell J., Siddiqi K., Rymar V. Flow-based fiber tracking with diffusion tensor and q-ball data: validation and comparison to principal diffusion direction techniques. *Neuroimag*e. 2005; 27 (4): 725–736. DOI: 10.1016/j.neuroimage.2005.05.014.
42. Tuch, D. Q-ball imaging. *Magn. Reson. Med*. 2004; 52 (6): 1358–1372. DOI: 10.1002/mrm.20279.
43. Assaf Y., Basser P. Composite hindered and restricted model of diffusion (CHARMED) MR imaging of the human brain. *Neuroimage*. 2005; 27 (1): 48–58. DOI: 10.1016/j.neuroimage.2005.03.042.
44. Özarslan E., Shepherd T., Vemuri B. et al. Resolution of complex tissue microarchitecture using the diffusion orientation transform (DOT). *Neuroimage* 2006; 31 (3): 1086–1103. DOI: 10.1016/j.neuroimage.2006.01.024.
45. Liu C., Bammer R., Acar B. et al. Characterizing non-Gaussian diffusion by using generalized diffusion tensors. *Magn. Reson. Med*. 2004; 51 (5): 924–937. DOI: 10.1002/mrm.20071
46. Tournier J.-D., Calamante F., Gadian D. et al. Direct estimation of the fiber orientation density function from diffusion-weighted MRI data using spherical deconvolution. *Neuroimage*. 2004; 23 (3): 1176–1185. DOI: 10.1016/j.neuroimage.2004.07.037.
47. Tournier J.-D., Calamante F., Connelly A. Robust determination of the fibre orientation distribution in diffusion MRI: non-negativity constrained super resolved spherical deconvolution. *Neuroimage.* 2007; 35 (4): 1459–1472. DOI: 10.1016/j.neuroimage.2007.02.016.
48. Jbabdi S., Johansen-Berg H. Tractography: where do we go from here? *Brain Connect*. 2011; 1 (3): 169–183. DOI: 10.1089/brain.2011.0033.
49. Thomas C., Ye F., Irfanoglu M. et al. Anatomical accuracy of brain connections derived from diffusion MRI tractography is inherently limited. *Proc. Natl. Acad. Sci. USA*. 2014; 111 (46): 16574–16579. DOI: 10.1073/pnas.1405672111.
50. Maier-Hein K., Neher P., Houde J.-C. et al. Tractography-based connectomes are dominated by false-positive connections. *bioRxiv*. 2016; 1: 84137. DOI: 10.1101/084137.
51. Fillard P., Descoteaux M., Goh A., Gouttard S., Jeurissen B., Malcolm J., Ramirez-Manzanares A., Reisert M., Sakaie K., Tensaouti F. et al. Quantitative evaluation of 10 tractography algorithms on a realistic diffusion MR phantom. *Neuroimage*. 2011; 56 (1): 220–234. DOI: 10.1016/j.neuroimage.2011.01.032.
52. Maier-Hein K., Neher P., Houde J.., Côté M., Garyfallidis E., Zhong J., Chamberland M., Yeh F., Lin Y., Descoteaux M. et al. The challenge of mapping the human connectome based on diffusion tractography. *Nature Communications*. 2017; 8 (1): 1349. DOI: 10.1038/s41467-017-01285-x.

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Methods for diagnosing endothelial dysfunction

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ABSTRACT

Endothelial dysfunction as a crucial factor in the pathogenesis of cardiovascular diseases requires precise and effective diagnostic methods. The review highlights the currently used methods which can be divided into morphological, instrumental, and laboratory ones. Special attention is paid to the so called jar test, which was introduced by Professor V.A. Valdman in 1936. The jar test may serve as a prototype of modern methods for endothelial function assessment.

These diagnostic methods can help to identify functional endothelial disorders at the earliest stage. It will significantly expand the possibilities of primary prevention of cardiovascular and a number of other diseases through non-pharmacological and pharmacological correction of endothelial dysfunction.

Key words: endothelium, endothelial dysfunction, methods of endothelial function assessment.

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Методы диагностики эндотелиальной дисфункции

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РЕЗЮМЕ

Проблема эндотелиальной дисфункции как важного звена в патогенезе заболеваний сердечно-сосудистой системы предполагает внедрение эффективных и точных диагностических методов. В обзоре освещаются наиболее актуальные из применяемых в научной и клинической практике методов, которые условно подразделены на морфологические, инструментальные и лабораторные. Отдельно упомянута предложенная еще в 1936 г. профессором В.А. Вальдманом эндотелиальная баночная проба, которая может служить прообразом современных методов исследования эндотелия.

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Данные диагностические методики могут способствовать выявлению функциональных нарушений эндотелия на самом раннем донозологическом уровне, что позволит существенно расширить возможности первичной профилактики сердечно-сосудистых и ряда других заболеваний путем немедикаментозной и фармакологической коррекции эндотелиальной дисфункции.

Ключевые слова: эндотелий, эндотелиальная дисфункция, методы оценки эндотелиальной функции.

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 endothelium is a thin layer of cells that line the interior surface of blood vessels, lymphatic vessels, and the endocardium, which implements coordination and an optimal course of diverse metabolic and physiological processes [1]. As the most important regulatory and endocrine organ, the endothelium performs such crucial functions as control over leukocyte adhesion, vascular tone, and angiogenesis, regulation of platelet adhesion and aggregation, and involvement in fibrinolysis and inflammatory processes [2].

Endothelial dysfunction is one of the most important links in the pathogenesis of cardiovascular diseases. The relevance of the problem implies introduction of efficient and accurate diagnostic methods that can help identify functional disruptions of the endothelium at the earliest stage. It will significantly expand the possibilities of primary prevention of cardiovascular and a number of other diseases through non-pharmacological and pharmacological correction of endothelial dysfunction.

The currently used methods for the endothelial function can be conditionally divided into morphological, instrumental, and laboratory ones.

MORPHOLOGICAL METHODS

The so called jar test, which was proposed back in the 1930s, can serve as a prototype of morphological methods for endothelial function assessment. Professor V.A. Valdman, the founder and the first head of the Department of Intermediate Level Therapy of the Leningrad Pediatric Medical Institute, was one of the first to call the vascular endothelium "the arena where the first collision of a macroorganism with microbes occurs". Studying vascular pathology, in 1936, he proposed a test as a method for detecting hyperergic swelling of the vascular endothelium (endotheliosis) [3]. In fact, the jar test can be rightfully considered as one of the first attempts to assess the functional state of the vascular endothelium.

In 1978, J. Hladovec introduced a method for assessing endothelial dysfunction by examining the level of circulating desquamated endothelial cells (DEC), which are cells that separate from the vessel wall during its damage [4]. Currently, a computer-assisted analysis of a DEC image is carried out (the cytometry method). On average, the amount of DEC in adults normally varies from 2 to 4 cells / 100 μl of plasma [5]. In patients with cardiovascular diseases, it was proposed to distinguish the degree of endothelial dysfunction by the DEC concentration. A moderate degree of endothelial dysfunction is defined when the number of DEC is from 6 to 10, an average degree is from 11 to 25, and a pronounced degree – 26 or more [6].

Recently, increased attention has been paid to apoptotic endothelial microparticles (EMPs) as new markers of endothelial dysfunction. EMPs are membrane vesicles released into the extracellular space during activation or apoptosis of endothelial cells [7]. EMPs are known to express markers of cell damage, such as intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), E-selectin, and von Willebrand factor (vWF) [8]. The EMP concentration is determined by flow cytometry, and their morphology and size are determined by cryotransmission electron microscopy using receptor-specific labeling [9].

INSTRUMENTAL METHODS

One of the first instrumental methods to estimate the vasoregulatory function of the endothelium was coronary angiography with the administration of

acetylcholine for evaluating endothelium-dependent vasodilation (EDVD) and sodium nitroprusside for evaluating endothelium-independent vasodilation (EIVD) (S.W. Werns et al., 1989) [10]. Usually, acetylcholine is used for intracoronary administration during coronary angiography; interacting with the intact endothelium, it has a stimulating effect on the production of nitric oxide (NO) and other endothelium-derived relaxing factors.

In endothelial dysfunction, or damaged endothelium, acetylcholine causes either weak vasodilation or even vasoconstriction [11]. To assess EIVD, exogenous sources of NO (nitroglycerin, sodium nitroprusside), which directly affect vascular smooth muscles, are administered via the intracoronary route. Changes in the diameter of the arteries are recorded using digital quantitative image analysis systems and intravascular ultrasound catheters [12]. The method is the most accurate for assessing the vasoregulatory function of the endothelium due to direct assessment of vascular reactivity in the coronary arteries. However, it has limitations due to the invasiveness of the procedure and the high cost.

D.S. Celermajer et al. (1992) developed a non-invasive method for diagnosing the functional state of the endothelium by evaluating postocclusive changes in the diameter of the brachial artery using high-resolution ultrasound [13]. This method for evaluating endothelial function is called flow-mediated dilation (FMD). During the test, endothelium-mediated NO release occurs, which leads to vasodilation. The degree of vasodilation can be quantified as an indicator of vasomotor function [14]. The method consists in an increase in shear stress as a result of elevated blood flow through the peripheral artery. This is achieved by creating reactive hyperemia, which implies a five-minute occlusion of the brachial artery by inflating the cuff to a pressure 50 mmHg higher than systolic blood pressure. Then, after five minutes, the cuff is decompressed, which leads to increased blood flow and increased shear stress. Using a high-resolution ultrasound sensor, blood flow velocity parameters are measured within 15–20 seconds, and the artery diameter is estimated 45–60 seconds after the decompression, when its maximum increase is noted [13]. The EDVD index of the brachial artery is calculated by the formula:

$$EDVD = (D_2 - D_1) / D_1 \times 100\%,$$

where D_1 is the initial diameter of the brachial artery, and D_2 is the diameter of the brachial artery after decompression [15]. The advantage of the method is its non-invasiveness. It allows to repeat measurements to evaluate the effectiveness of various interventions that may affect the state of the vascular endothelium [16].

T. Anderson et al. (1995) demonstrated a close correlation between the EDVD parameters obtained during coronary angiography with acetylcholine and parameters obtained during ultrasound examination of the brachial artery. It allowed to use the brachial artery as a universal model for further evaluation of the effects of various factors on endothelial function [17].

T. Gori et al. (2008) described a new non-invasive method – low flow-mediated constriction (L-FMC), which allows to assess arterial tone at rest. L-FMC consists in narrowing the brachial artery due to a decrease in blood flow after arterial occlusion using a distal cuff. L-FMC is a simple and fast method that complements FMD and extends diagnostic capabilities [18].

The simplest and fastest method for instrumental diagnosis of endothelial dysfunction is analysis of the volume pulse wave shape, which is recorded using a photoplethysmographic sensor located on the nail phalanx of the patient's finger, with subsequent processing of the received signal on the computer. During the study, the contour of a volume pulse wave is recorded by merging two waves: systolic (direct) and reflected. The reflection index (RI) is calculated as the ratio of direct and reflected wave amplitudes to the reflection time (T), by which the reflected wave lags behind the systolic one. The stiffness index (SI) is calculated as the ratio of the patient's height L (in meters) to the reflection time (in seconds). In various pathologies of the cardiovascular system, a decrease in vascular elasticity is observed, which is indicated by an increase in the RI and SI [19].

LABORATORY METHODS

Laboratory methods for evaluating endothelial function consist in determining the concentration of certain factors synthesized by the endothelium and performing various functions.

Mainly, the levels of NO and its metabolites are primarily used to assess the endothelial vasomotor function [20]. It is known that NO is synthesized as a result of oxidation of L-arginine by the oxygen atom with the participation of NO-synthase:

$$2\text{L-arginine} + 3\text{NADPH} + 4O_2 + 3\text{H+} \rightarrow 2\text{L-citrulline} + 2\text{NO} + 3\text{NADPH} + 4H_2O$$

According to their structure, NO-synthase (NOS) isoforms are divided into endothelial (eNOS), neuro-

nal (nNOS), and inducible (iNOS). According to the mechanism of action, they are divided into constitutional (cNOS) and inducible (iNOS) [21, 22]. cNOS are involved in NO synthesis in hypoxic conditions with vasoconstriction of blood vessels, iNOS – after their induction by bacterial endotoxins, some inflammatory mediators, and reactive oxygen species (ROS) [21]. NO-synthases can exert their effect only in the presence of cofactors, such as flavins, NADPH oxidase (NOX), and tetrahydrobiopterin [23].

Since NO has a half-life of only 0.1 seconds [24], the levels of end products of NO oxidation – nitrites and nitrates (NO_x) – are usually determined. The study material can be plasma, blood serum, or culture fluids [25]. The most common method for determining the NO_x levels is the spectrophotometric method with Griess reagent.

At the first stage of this study, the nitrite ion is determined by the reaction of nitrites with Griess reagent, which contains an aqueous solution of 0.05% N-(1-naphthyl)ethylenediamine and a 1% solution of sulfonamide in acetic acid [26]. The reaction produces a stained diazo compound with an absorption maximum at the wavelength of 548 nm. Since Griess reagent detects only nitrites, reactions of reducing nitrates to nitrites are performed using either nitrate reductase or Vanadium (III) [27, 28].

In various cardiovascular diseases, a decrease in NO_x concentration is observed, which may be caused by a decrease in the expression and activity of eNOS, a decrease in the level of L-arginine and NO-synthase cofactors, or an increase in endogenous NO inhibitors. In some patients, there is an increase in the level of NO_x with functional signs of reduced EDVD, which indicates severe endothelial dysfunction. An increase in the concentration of the end products of NO oxidation may be associated with activation of iNOS by inflammatory mediators (tumor necrosis factor-α, interleukin-6 (IL-6), IL-1β), lipopolysaccharides (LPS), and ROS. With excess NO, toxic peroxynitrite is formed, which has a cytostatic and cytotoxic effects [25].

To assess endothelial dysfunction, the concentration of asymmetric dimethylarginine (ADMA), an endogenous inhibitor of NO synthase, is also determined. ADMA is an amino acid synthesized from arginine by protein arginine methyltransferase (PRMT). There are two isoforms of PRMT, one of which, PRMT-1, activates ADMA formation, and the second one, PRMT-2, activates symmetric dimethylarginine (SDMA) formation. It is ADMA that has an inhibitory effect on three NOS isoforms [29]. The ADMA concentration is determined using high-performance liquid chromatography with highly sensitive laser-induced fluorescence detection [30].

To assess vasoconstrictor functions of the endothelium, the level of endothelin-1 (ET-1) is determined. High concentrations of ET-1 contribute to the development of endothelial dysfunction. ET-1 increases production of endothelial superoxide, which leads to EDVD disruption and triggering of proinflammatory processes [31]. High concentrations of ET-1 in the blood serum are found in patients with coronary artery disease, arterial hypertension, kidney disease, as well as obstetric and gynecologic pathology [32].

Evaluation of endothelial function also involves determining the concentration and functional activity of von Willebrand factor (vWF), which mediates platelet aggregation and adhesion to the vascular endothelium. vWF is a multimeric plasma glycoprotein that is excessively synthesized by endotheliocytes and megakaryocytes in the form of a propeptide (vWFpp), which then undergoes post-translational modifications. The vWFpp, together with vWF, is stored inside endothelial cells in Weibel-Palade bodies and in α-granules of megakaryocytes [33–35]. Inflammatory cytokines are able to stimulate production of vWF and vWFpp in high concentrations from Weibel-Palade bodies and α-granules (IL-8 and TNF-α) and inhibit the cleavage of vWF (IL-6). As a result, the hyperreactive factor accumulates in the blood and on the surface of endothelial cells, which leads to increased activation of platelet aggregation and adhesion to vascular endothelium [36]. Studies showed the importance of increasing the vWF concentration as a predictor of a risk of recurrent myocardial infarction [37].

There are several laboratory tests for assessing the level and activity of vWF. Quantitative determination of the vWF antigen is performed by the enzyme immunoassay (vWF:Ag) [34]. It is also proposed to evaluate the level of vWFpp in the blood plasma as a marker of endothelial dysfunction, since vWFpp is not used in platelet aggregation, in contrast to the vWF antigen (vWF:Ag) [38]. Analysis of the vWFpp / vWF:Ag ratio is used to assess the degree of vWF clearance. In normal human plasma, the vWFpp / vWF:Ag ratio is 1.0. In patients with type 1C von Willebrand disease, a decrease in the vWF concentration is observed due to its rapid elimination, which leads to an increase in this ratio [33, 39].

Activity evaluation (ristocetin-cofactor (RCoF) activity) is based on the ability of vWF to activate platelet aggregation and adhesion through interaction of the factor A1 domain with the platelet membrane receptor – glycoprotein Ib (GPIb). The process is imitated by adding ristocetin to the suspension of washed red blood cells in the presence of the patient's plasma, which promotes binding of vWF to GPIb [40]. The aggregation rate is measured by an aggregator. Normal values of the method range from 50 to 200 IU / dl [41]. The test does not exclude false results due to possible defects in the ability of vWF to bind ristocetin [42]. A new analysis was developed that does not involve the use of ristocetin. It is based on the use of recombinant GPIb with enhanced function, which spontaneously binds vWF *in vitro* [43].

vWF is able to form bonds with collagen through the A3 domain when the endothelial lining is damaged. This fact is the basis for another diagnostic test – quantitative determination of the collagen-binding activity of vWF by enzyme immunoassay (vWF:CB) [44].

Through the domains D and D3, vWF binds to coagulation factor VIII (FVIII) and transports it through the bloodstream in an inactive form. If the endothelial lining is damaged, vWF delivers FVIII to the site of injury, where FVIII forms a complex with coagulation factor IX [34, 40, 41]. The vWF / FVIII binding test (vWF:FVIIIB), which is mainly used for the diagnosis of type 2N von Willebrand disease, allows to evaluate the affinity of vWF to FVIII [45].

To analyze the anticoagulant function of the endothelium, the level of thrombomodulin (TM), which is a transmembrane glycoprotein expressed on the surface of endothelial cells of blood vessels, is examined. At the site of endothelial damage, thrombin binds to TM, forming a thrombin – TM complex that promotes activation of protein C, which splits factors Va and VIIIa, thereby preventing excessive fibrin clotting. The concentration of TM in the plasma of healthy people is relatively low. In case of damage to the endothelium, accompanied by proteolysis, the concentration of TM in the blood plasma increases by 1.5–2 times. The level of TM in the blood is measured by the enzyme immunoassay [46].

To assess fibrinolytic function, the level of tissue plasminogen activator (tPA) produced in endothelial cells is examined. The tPA provides an external pathway for plasminogen activation by forming a triple complex (fibrin + plasminogen + tPA), due to which the resulting plasmin provides proteolytic degradation of fibrin [47]. The tPA concentration is also determined by the enzyme immunoassay [32].

The endothelium is known to play an important role in adhesion and migration of leukocytes through secretion of adhesive molecules, such as ICAM-1, VCAM-1, P-selectin, E-selectin, and VE-cadherin. Changes in the concentration of these substances indicate endothelial damage and the severity of inflammation, and can be used to determine a prognosis of the disease [48].

The endothelium is directly involved in activation of vascular growth in hypoxia and tissue damage. One of the most important regulators of angiogenesis is vascular endothelial growth factor (VEGF), which is used as a marker of endothelial dysfunction. Determination of VEGF concentration is most important for the diagnosis of cancer, since the growth of blood vessels in a tumor contributes to its activation, rapid growth, and metastasis. VEGF, which interacts with vascular endothelial receptors, is the most powerful mediator of this process [49].

CONCLUSION

Currently, a clinical researcher has a wide range of methods for endothelial function assessment: laboratory, instrumental and morphological techniques. Further research of the endothelial function in normal and pathological conditions could clarify components of the pathogenesis of cardiovascular diseases that are still unclear and suggest measures of primary and secondary prevention. Parameters that characterize the functions of the endothelium, of course, are only surrogate intermediate points for evaluating the effectiveness of certain effects. However, on the other hand, the assessment of endothelial function can have a great prognostic value, since endothelium dysfunction is one of the earliest preclinical markers of vascular damage. And, conversely, restoration of impaired parameters is probably one of the first markers of the effectiveness of a preventive or therapeutic intervention. Therefore, the study of the properties and functions of the endothelium, as well as development and optimization of diagnostic methods for assessing its condition are necessary and relevant.

REFERENCES

1. Kupriyanov V.V. Endothelium. Big medical encyclopedia 3rd ed. Moscow : Sov. encycl., 1986. Vol. 28: 192–193 (in Russ.).

2. Petrishchev N.N., Vlasov T.D. Physiology and pathophysiology of the endothelium. In: Petrishchev N.N., Vlasov T.D. Endothelial dysfunction. Causes, mechanisms, pharmacological correction. SPb.: Publishing house of SPSMU, 2003: 4–38 (in Russ.).
3. Valdman V.A. Endotheliitis and a "jar test". *Clinical Medicine*. 1938; 16 (3): 313–318 (in Russ.).
4. Hladovec J., Prerovský I., Staněk V., Fabián J. Circulating endothelial cells in acute myocardial infarction and angina pectoris. *Klin. Wochenschr.* 1978; 56 (20): 1033–1036. DOI: 10.1007/bf01476669.
5. Mironov A.V., Torchinov A.M., *Umakhanova* M.M., Galachiev O.V., Osmanova S.R., Filippov A.V., Lebedev V.A. Assessment of the endothelial dysfunction severity in pregnancy complications. *Difficult Patient.* 2018; 16 (8–9): 30–36 (in Russ.). DOI: 10.24411/2074-1995-2018-10006.
6. Astashev P.E., Gudymovich V.G. Markers of endothelial dysfunction as a prognostic sign of the course of the intracardiac infectious process. *Bulletin of Pirogov National Medical & Surgical Center*. 2011; 6 (3): 3–8 (in Russ.).
7. Chironi G.N., Boulanger C.M., Simon A., Dignat-George F., Freyssinet J.M., Tedgui A. Endothelial microparticles in diseases. *Cell Tissue Res*. 2009; 335 (1): 143–151. DOI: 10.1007/s00441-008-0710-9.
8. Leroyer A.S., Tedgui A., Boulanger C.M. Role of microparticles in atherothrombosis. *J. Intern. Med*. 2008; 263 (5): 528–537. DOI: 10.1111/j.1365-2796.2008.01957.x.
9. Lacroix R., Judicone C., Mooberry M., Boucekine M., Key N.S., Dignat-George F. Standardization of pre-analytical variables in plasma microparticle determination: results of the International Society on Thrombosis and Haemostasis SSC Collaborative workshop. *J. Thromb. Haemost.* 2013: 10.12207. DOI: 10.1111/jth.12207.
10. Werns S.W., Walton J.A., Hsia H.H., Nabel E.G., Sanz M.L., Pitt B. Evidence of endothelial dysfunction in angiographically normal coronary arteries of patients with coronary artery disease. *Circulation.* 1989; 79 (2): 287–291. DOI: 10.1161/01.cir.79.2.287.
11. Ludmer P.L., Selwyn A.P., Shook T.L., Wayne R.R., Mudge G. H., Alexander R.W., Ganz P. Paradoxical vasoconstriction induced by acetylcholine in atherosclerotic coronary arteries. *N. Engl. J. Med.* 1986; 315 (17): 1046–1051. DOI: 10.1056/NEJM198610233151702.
12. Polonetsky O. L., Polonetsky L. Z Endothelial dysfunction and atherosclerosis. *Medical News.* 2012; (6): 6–11 (in Russ.).
13. Celermajer D.S., Sorensen K.E., Gooch V.M., Spiegelhalter D.J., Miller O.I., Sullivan I.D., Lloyd J.K., Deanfield J.E. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. *Lancet*. 1992; 340 (8828): 1111–1115. DOI: 10.1016/0140-6736(92)93147-f.
14. Joannides R., Haefeli W.E., Linder L., Richard V., Thuillez C., Lüscher T.F. Role of nitric oxide in flow-dependent vasodilation of human peripheral arteries in vivo. *Arch. Mal. Coeur. Vaiss*. 1994; 87 (8): 983–985.
15. Kovalyaeva I.B., Myasoedova S.E., Bogatova I.K., Bukina E.A. Endothelial function in postmenopausal women with arterial hypertension. *Cardiovascular Therapy and Prevention.* 2004; 3 (6): 4–8 (in Russ.).
16. Corretti M.C., Anderson T.J., Benjamin E.J., Celermajer D., Charbonneau F., Creager M.A., Deanfield J., Drexler H., Gerhard-Herman M., Herrington D., Vallance P., Vita J., Vogel R. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. *J. Am. Coll. Cardiol.* 2002; 39 (2): 257–265. DOI: 10.1016/s0735-1097(01)01746-6.
17. Anderson T.J., Uehata A., Gerhard M.D., Meredith I.T., Knab S., Delagrange D., Lieberman E.H., Ganz P., Creager M.A., Yeung A.C. Close relation of endothelial function in the human coronary and peripheral circulations. *J. Am. Coll. Cardiol*. 1995; 26 (5): 1235–1241. DOI: 10.1016/0735-1097(95)00327-4.
18. Gori T., Dragoni S., Lisi M., , Di Stolfo G., Sonnati S., Fineschi M., Parker J.D. Conduit artery constriction mediated by low flow a novel noninvasive method for the assessment of vascular function. *J. Am. Coll. Cardiol*. 2008; 51 (20): 1953–1958. DOI: 10.1016/j.jacc.2008.01.049.
19. Kalakutskiy L.I., Lebedev P.A., Komarova M.V. The methodology for analyzing the pulse wave circuit in the diagnosis of vascular endothelial function. *Izvestiya SFedU. Engineering Sciences.* 2008; 82 (5): 43–47 (in Russ.).
20. Tsikas D. Methods of quantitative analysis of the nitric oxide metabolites nitrite and nitrate in human biological fluids. *Free Radic. Res*. 2005; 39 (8): 797–815. DOI: 10.1080/10715760500053651.
21. Kuznetsova V.L., Solovyova A.G. Nitric oxide: properties, biological role, mechanisms of action. *Modern Problems of Science and Education.* 2015; (4): 462 (in Russ.).
22. Melnikova Yu.S., Makarova T.P. Endothelial dysfunction as a central link in the pathogenesis of chronic diseases. *Kazan Medical Journal*. 2015; 96 (4): 659–665 (in Russ.).
23. Averkin N.S., Latynova I.V., Fedorova M.G., Chudaeva D.G., Vishnyakova Zh.S., Pivovarov E.V., Samartsev A.D. The value of oxidative stress in the aging mechanisms of the arterial wall. *Modern Problems of Science and Education.* 2018; (6): 42 (in Russ.).
24. Kelm M., Schrader J. Control of coronary vascular tone by nitric oxide. *Circ. Res.* 1990; 66 (6): 1561–1575. DOI: 10.1161/01.res.66.6.1561.
25. Lapshina L.A., Kravchun P.G., Titova A.Ju., Glebova O.V. The importance of determining nitrite-nitrates as markers of endothelial dysfunction in cardiovascular disease. *Ukrainian Medical Journal.* 2009; (6): 49–53 (in Russ.).

26. Mazhitova M.V. Spectrophotometric determination of the level of nitrogen monoxide metabolites in blood plasma and brain tissue of white rats. *Modern Problems of Science and Education.* 2011; (3) (in Russ.). URL: http://science-education.ru/ru/article/view?id=4655
27. Ramesh S.S., Prasanthi A., Bhat D.I., Devi B.I., Cristopher R., Philip M. Correlation between plasma total nitric oxide levels and cerebral vasospasm and clinical outcome in patients with aneurysmal subarachnoid hemorrhage in Indian population. *J. Neurosci. Rural. Pract.* 2014; 5 (1): 22–27. DOI: 10.4103/0976-3147.145196.
28. Miranda K.M., Espey M.G., Wink D.A. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. *Nitric. Oxide.* 2001; 5 (1): 62–71. DOI: 10.1006/niox.2000.0319.
29. Landim M.B., Casella Filho A., Chagas A.C. Asymmetric dimethylarginine (ADMA) and endothelial dysfunction: implications for atherogenesis. *Clinics (Sao Paulo)*. 2009; 64 (5): 471–478. DOI: 10.1590/s1807-59322009000500015.
30. Bassareo P.P., Puddu M., Flore G., Deidda M., Manconi E., Melis A., Fanos V., Mercuro G. Could ADMA levels in young adults born preterm predict an early endothelial dysfunction? *Int. J. Cardiol*. 2012; 159 (3): 217–219. DOI: 10.1016/j.ijcard.2011.02.069.
31. Cerrato R., Cunnington C., Crabtree M.J., Antoniades C., Pernow J., Channon K.M., Böhm F. Endothelin-1 increases superoxide production in human coronary artery bypass grafts. *Life Sci*. 2012; 91 (13-14): 723–728. DOI: 10.1016/j.lfs.2012.03.024.
32. Ivanov A.N., Grechikhin A.A., Norkin I.A., Puchinyan D.M. Diagnostic methods for endothelial dysfunction. *Regional Blood Circulation and Microcirculation*. 2014; 13 (4): 4–11 (in Russ.).
33. Stufano F., Boscarino M., Bucciarelli P., Baronciani L., Maino A., Cozzi G., Peyvandi F. Evaluation of the utility of von Willebrand factor propeptide in the differential diagnosis of von Willebrand disease and acquired von Willebrand Syndrome. *Semin. Thromb. Hemost*. 2019; 45 (1): 36–42. DOI:10.1055/s-0038-1660481.
34. Likhacheva E.A., Polyanskaya T.Yu., Zorenko V.Yu. International experience in the diagnosis and treatment of von Willebrand disease. *Russian Journal of Hematology and Transfusiology*. 2013; 58 (4): 45–49 (in Russ.).
35. Isaev D.P., Kasumova R.D. Determination of the von Willebrand factor concentration in the blood serum of patients with acute hepatitis B complicated by polyarteritis nodosa. *Biomedicine.* 2019; (2): 18–21 (in Russ.).
36. Bernardo A., Ball C., Nolasco L., Moake J.F., Dong J.F. Effects of inflammatory cytokines on the release and cleavage of the endothelial cell-derived ultralarge von Willebrand factor multimers under flow. *Blood*. 2004; 104 (1): 100–106. DOI: 10.1182/blood-2004-01-0107.
37. Springer T.A. Von Willebrand factor, Jedi knight of the bloodstream. *Blood*. 2014; 124 (9): 1412–1425. DOI: 10.1182/blood-2014-05-378638.
38. Marianor M., Zaidah A.W., Maraina Ch.C. von Willebrand Factor Propeptide: A potential disease biomarker not affected by ABO blood groups. *Biomark. Insights.* 2015; 10: 75–79. DOI: 10.4137/BMI.S24353.
39. Jacobi P.M., Kanaji S., Jakab D., Gehrand A.L, Johnsen J.M., Haberichter S.L. Von Willebrand factor propeptide to antigen ratio identifies platelet activation and reduced von Willebrand factor survival phenotype in mice. *J. Thromb. Haemost*. 2018; 16 (3): 546–554. DOI: 10.1111/jth.13934.
40. Chernova E.V. Von Willebrand factor. *Newsletter of North-Western State Medical University named after I.I. Mechnikov.* 2018; 10 (4): 73–80 (in Russ.). DOI: 10.17816/mechnikov201810473-80.
41. Kosterina A.V. Diagnosis and treatment of von Willebrand disease. *Practical Medicine.* 2018; 16 (7-2): 51–54 (in Russ.).
42. Sharma R., Flood V.H. Advances in the diagnosis and treatment of Von Willebrand disease. *Blood*. 2017; 130 (22): 2386–2391. DOI: 10.1182/blood-2017-05-782029.
43. Patzke J., Budde U., Huber A., Méndez A., Muth H., Obser T., Peerschke E., Wilkens M., Schneppenheim R. Performance evaluation and multicentre study of a von Willebrand factor activity assay based on GPIb binding in the absence of ristocetin. *Blood Coagul. Fibrinolysis*. 2014; 25 (8): 860–870. DOI: 10.1097/MBC.0000000000000169.
44. Ni Y., Nesrallah J., Agnew M., Geske F.J., Favaloro E.J. Establishment and characterization of a new and stable collagen-binding assay for the assessment of von Willebrand factor activity. *Int. J. Lab. Hematol*. 2013; 35 (2): 170–176. DOI: 10.1111/ijlh.12019.
45. Federici A.B. Current and emerging approaches for assessing von Willebrand disease in 2016. *Int. J. Lab. Hematol*. 2016; 38 (Suppl 1): 41–49. DOI: 10.1111/ijlh.12540.
46. Demeulenaere M., Devreese K., Vanbelleghem H., De Zaeytijd J., Walle J.V., Biesen W.V., Laecke S.V. Thrombomodulin and endothelial dysfunction: A disease-modifier shared between malignant hypertension and atypical hemolytic uremic syndrome. *Nephron*. 2018; 140 (1): 63–73. DOI: 10.1159/000490201.
47. Tultseva S.N. Use of recombinant tissue plasminogen activator in the treatment of retinal vein occlusion. *Ophthalmic Statements.* 2010; 3 (1): 42–50 (in Russ.).
48. Reinhart K., Bayer O., Brunkhorst F., Meisner M. Markers of endothelial damage in organ dysfunction and sepsis. *Crit. Care Med*. 2002; 30 (5): 302–312. DOI: 10.1097/00003246-200205001-00021.
49. Glybochko P.V., Zaharova N.B., Ponukalin A.N., Durnov D.A., Shakhpazyan N.K. Diagnostic value of angiogenesis indicators in kidney cancer. *Oncourology.* 2011; (3): 25–31 (in Russ.).

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The problem of overdiagnosis of vertebral artery compression syndrome

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ABSTRACT

Vertebral artery compression is a syndrome that occurs as a result of hemodynamically significant partial or complete obstruction of vertebral arteries by extravascular structures. In clinical practice, this condition is most often called vertebral artery syndrome. Any vertebral segments can be compressed, but most often the lesion is determined at the level of C1–C2. Russian authors consider vertebral artery compression to be a common cause of a wide range of patient complaints, including dizziness, headaches, and subjective tinnitus. In some studies, it is reported that vertebral artery syndrome develops in 50% of patients with degenerative changes in the cervical spine.

In the world literature, vertebral artery compression syndrome which is often referred to as “bow hunter’s syndrome” is called a rare pathology. Such a pronounced difference in the frequency of detection of vertebral artery compression in Russian and world literature may be associated with a lack of common diagnostic criteria, low awareness of alternative diagnoses, and incorrect interpretation of patient complaints. It is obvious that these factors need to be corrected in order to reduce the likelihood of overdiagnosis of vertebral artery compression syndrome and improve the quality of medical care.

Key words: vertebral artery syndrome, compression, ultrasound imaging, criteria, overdiagnosis.

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Проблема гипердиагностики синдрома экстравазальной компрессии позвоночных артерий

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РЕЗЮМЕ

Синдромом экстравазальной компрессии позвоночных артерий называют симптомокомплекс, возникающий в результате гемодинамически значимой частичной или полной обструкции позвоночных артерий экстравазальными структурами. В клинической практике данное состояние чаще всего называют синдромом позвоночной артерии. Компрессии может быть подвержен любой сегмент, но наиболее часто поражение определяют на уровне С1–С2. Отечественные авторы считают компрессию позвоночных артерий распространенной причиной широкого спектра жалоб пациента, включая головокружение, головные боли и субъективный шум в голове. В отдельных работах сообщают, что синдром позвоночной артерии развивается у 50% пациентов с дегенеративными изменениями шейного отдела позвоночника.

В мировой литературе синдром компрессии позвоночных артерий, который чаще именуется «синдром лучника» (bow hunter's syndrome), называют редкой патологией. Чем обусловлена столь выраженная разница частоты выявления компрессии позвоночных артерий в отечественной и мировой литературе? К возможным причинам указанного феномена могут быть отнесены: отсутствие единых диагностических критериев, низкая осведомленность об альтернативных диагнозах и неверная интерпретация жалоб пациента. Очевидным является необходимость коррекции указанных факторов с целью снижения вероятности гипердиагностики синдрома позвоночной артерии и повышения качества оказания медицинской помощи.

Ключевые слова: синдром позвоночной артерии, компрессия, ультразвуковая диагностика, критерии, гипердиагностика.

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

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INTRODUCTION

Extravascular compression of the vertebral arteries (VA) is a syndrome arising as a result of hemodynamically significant partial or complete obstruction of the VA by extravascular structures. In clinical practice, this condition is most often referred to as vertebral artery compression syndrome (VACS). Any vertebral segments can be compressed (Fig. 1), but most often the lesion is determined at the level of C1–C2 [1].

Russian authors consider VA compression to be a common cause of a wide range of patient complaints, including dizziness, headaches, and subjective tinnitus [2]. There are publications in which VA compression was detected in 45% of patients with dizziness [3]. Also, some studies reported that VACS developed in 50% of patients with degenerative changes in the cervical spine [4].

At the same time, in the world literature, VACS, which is often referred to as “bow hunter’s syndrome”, is called a rare pathology [5, 6]. This is perfectly demonstrated by V. Rastogi et al. (2015) who had founded only 153 cases of VACS from 1966 and 2013 [7]. Such a pronounced difference in the frequency of VACS detection in Russian and world literature may be associated with a lack of common diagnostic criteria, low awareness of alternative diagnoses, and incorrect interpretation of patient complaints.

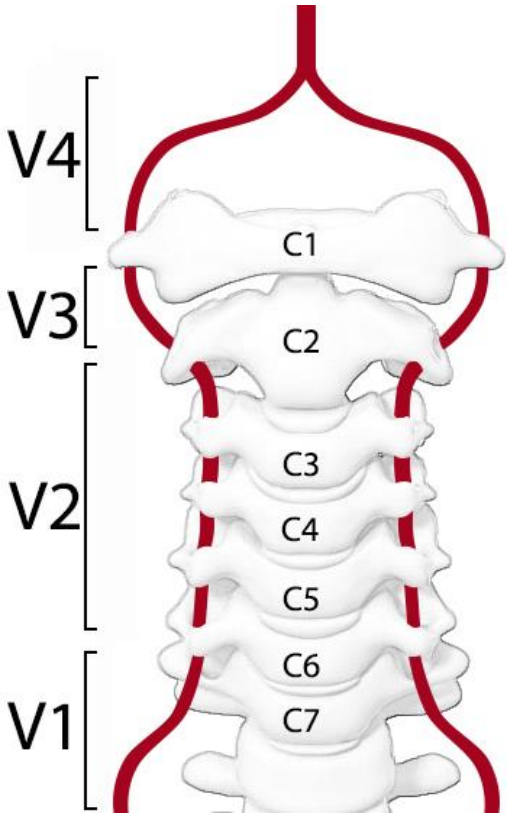

Fig. 1. Vertebral artery segments: V1 – origin to the transverse foramen of C6; V2 – VA ascends through the transverse foramina of the cervical vertebrae; V3 – from the C2 transverse foramen until it enters the skull; V4 – intracranial segment

LACK OF COMMON DIAGNOSTIC CRITERIA FOR VACS

Nowadays, there are no clinical guidelines that propose recommendations for the diagnosis of VACS [8, 9], as a result, different authors use different criteria and methods of patient examination. Computed tomography (CT) and magnetic resonance imaging (MRI) contribute to the diagnosis of VACS, however, obligatory head rotation (maintaining this position for 2 minutes) to demonstrate compression carries a potential risk of neurological complications [10], therefore, researchers use conventional angiography for diagnosis [5, 11]. Most often, the diagnostic criteria for VACS are considered to be intraluminal filling defects in the VA during head rotation with the onset of clinical manifestations. Due to a high cost and potential complications of the procedure, angiography is commonly performed after ultrasound examination [12].

It should be noted that the ultrasound criteria for VACS vary from author to author. For example, in a duplex study, T.V. Zakhmatova et al. (2014) evaluated the effect of extravascular structures on VA by calculating the ratios of blood flow indicators (V3 / V1 maximum blood flow rate; V3 / V1 time-averaged maximum blood flow rate). With the V3 / V1 ratio of more than 1.0, the blood flow was regarded as compensated, and with the ratio being less than 0.7 – as decompensated [13]. M.L. Dicheskul and V.P. Kulikov (2011) demonstrated that the peak systolic velocity (PSV) in the V4 segment in patients with cervical spine pathology during head rotation was significantly decreased compared to healthy volunteers.

The authors suggested a decrease in PSV ≥ 30% as a diagnostic threshold [14]. The same group of authors studied blood flow parameters in the VA during head rotation in patients with vertebrobasilar insufficiency (VBI). Duplex scanning of the suboccipital and intracranial segments of the VA was performed in 28 healthy volunteers and 70 patients with stage I–II VBI according to the classification by A.B. Sitel [15]. The authors concluded that a reduction of PSV ≥ 30% in the V4 segment of the VA during head rotation was a highly specific indicator of extravascular compression – 98% (CI 94.8−99.8%) with a predictive value of 94.6% (CI 81.8−99.2%); the PSV was significantly lower in patients with VBI ($p < 0.05$). It was also noted that the absence of changes in the PSV during head rotation did not guarantee the absence of extravascular compression of VA [16]. The main methodological defect of the study was the use of VBI classification proposed by A.B. Sitel (Table 1).

Table 1

VBI classification proposed by A.B. Sitel (2008)

Stage	Clinical features
I	It is characterized by the predominance of subjective symptoms over objectively detectable disturbances of movement and sensitivity, there is an autonomic dysfunction, mild short-term dizziness, episodes of blurred vision. Functional blockages are detected in the craniocervical junction, the region of the cervicothoracic junction, and the lower back. This stage is also characterized by the inferior oblique muscle syndrome, contracture of the muscles of the neck, anterior chest wall syndrome, and interscapular pain syndrome
II	It is characterized by more intense and longer episodes of dizziness and headache attacks usually starting with pain in the neck or behind the ear region. There are periods of hearing impairment in the form of hearing loss, the appearance of tinnitus and imbalance; visual disturbances in the form of floaters and cloudy vision; pain in the region of the heart, not related to stress or premature ventricular contractions. Functional blockages, in contrast to the first stage, are detected in both the middle and upper cervical regions, the lumbar lordosis is straightened
III	It is characterized by more pronounced clinical presentation: severe headache attacks, analgesics and non-steroidal anti-inflammatory drugs do not bring relief, dizziness with nausea and vomiting, and drop attacks. Attacks are triggered by throwing the head back or turning to the side and using the rolling stairs or transport. Functional blockages are detected in the motion segments of the cervical and lumbar spine
IV	Stage IV clinically coincides with stage III discirculatory encephalopathy, since patients have signs of prior strokes in the carotid arteries and brain stem and / or persistent neurologic deficit

Clinical signs of stages I and II of VBI classification by A.B. Sitel are non-specific and can be manifestations of a wide range of pathological or physiological conditions. Unfortunately, this fact challenges the conclusions of M.L. Dicheskul and V.P. Kulikov, since the study was conducted on a heterogeneous group of patients whose complaints were most likely caused by various diseases (primary headaches [17], benign paroxysmal positional vertigo (BPPV) [18] and others [19]).

The absence of common diagnostic ultrasound criteria for VACS is observed not only in the Russian Federation, but in other countries as well. For example, in Japan. M. Kamouchi et al. (2003) evaluated the qualitative parameters of blood flow in the VA (reduction of the PSV in the V2 segment and disappearance

of the diastolic component during rotation) in patients with suspected VACS [20]. Y. Iguchi et al. (2006) assessed the qualitative characteristics of the blood flow in the posterior cerebral artery (PCA) and basilar artery during transcranial doppler ultrasound [11].

It should be noted that the disappearance of the diastolic component in the VA during head rotation as a sign of VA compression was also described by J. Yeh et al. (2005) [21]. An additional criterion was reduction of PSV by more than 50% in the extracranial segments of the VA during head rotation. The assessment of the blood flow parameters in the PCA with transcranial doppler ultrasound is used in other countries as well. In particular, reduction of PSV in the PCA ≥ 50% (compared to the baseline value) during head rotation is a criterion for VACS in some ultrasound laboratories in the USA and Europe [5, 12, 22].

The criteria are presented in Table 2. In our practice, in patients with suspected VACS, we evaluate blood flow in the V2 segment during head rotation. The patient is recommended to perform angiography to verify the diagnosis, if PSV decreased by more than 50%, the diastolic component is absent, and typical clinical signs of VACS are observed, which is quite rare.

Table 2

Ultrasound criteria of extravascular compression of the vertebral arteries

Author	Method	Criteria
M.L.Dicheskul, 2012 [16]	Duplex study	Reduction of PSV ≥ 30% in the V4 segment of the VA during rotation
T.V. Zakhmatova, 2014 [13]	Duplex study	The V3 / V1 maximum blood flow rate or V3 / V1 time-averaged maximum blood flow rate of more than 1.0 – compensated blood flow; less than 0.7 – decompensated blood flow
M. Kamouchi, 2003 [20]	Duplex study	Qualitative changes in the blood flow in the VA (reduction of the PSV in the V2 segment and disappearance of the diastolic component during head rotation)
J. Yeh, 2005 [21]	Duplex study	Reduction of PSV ≥ 50% in extracranial VA segments and disappearance of the diastolic component during head rotation
Y. Iguchi, 2006 [11]	Transcranial Doppler	Qualitative changes in the blood flow in the PCA and basilar artery (gradual reduction of the blood flow and blood flow cessation with maximum head rotation)
M.D. Vilela, 2005 [12], M. Sturzenegger, 1994 [22], G.F. Jost, 2015 [5]	Transcranial Doppler	Reduction of PSV in PCA ≥ 50% with head rotation followed by reactive hyperemia when returning the head to the neutral position with an increase in PSV > 10% compared to the baseline value

From the data demonstrated above, the absence of common ultrasound criteria for VACS is clearly observed, which affects the difference in the rate of VACS diagnosis in individual diagnostic centers. However, ultrasound examination is preceded by the analysis of complaints and history, which misinterpretation can also lead to overdiagnosis of VACS.

EVALUATION OF PATIENT COMPLAINTS AND HISTORY IN SUSPECTED VACS

Interpretation of patient complaints and history is of great importance, since this is the starting point for formulation of a diagnostic hypothesis [23] and differential diagnosis [24]. The common complaints in VACS include dizziness, headache, nausea, vomiting, and visual disturbances during head rotation relieved by returning to a neutral position [10]. In the literature, these complaints are considered as a part of VBI, which includes only transient ischemic attack and ischemic stroke in the vertebrobasilar arteries. So, VBI is defined solely as stroke (acute cerebrovascular disease).

In Russian literature, VBI is understood as a variant of chronic cerebral ischemia [25], a diagnosis often masked by other diseases (tension-type headache, depressive disorders, etc.) [26]. Perhaps, the difference in the perception of VBI (as an acute condition in the world literature and as a chronic process in the Russian one) contributes to the overdiagnosis of VACS in Russia. At the same time, clinical manifestations of VA compression are non-specific and can occur in various pathologies. For example, BPPV is the most common cause of vertigo in adults [27], and migraine is a common cause of headaches [28]. Both conditions are often accompanied by nausea, vomiting, and visual disturbances. Considering that in a patient with BPPV, a vertigo attack can be triggered by turning the head [29], it is BPPV that should be suspected upon examination, and not rare VACS [7]. However, low awareness of doctors in medical and diagnostic centers about BPPV [30] and chronic migraine [31] can also lead to misinterpretation of patient complaints, followed by overdiagnosis of VACS.

Of course, the reasons for VACS overdiagnosis are not limited to the absence of uniform criteria and misinterpretation of patient complaints. Cognitive biases should also be taken into consideration. They include overconfidence bias, confirmation bias, and anchoring bias. Table 3 provides descriptions and examples of the listed psychological phenomena. More detailed information about them can be found in the study by E.D. O'Sullivan and S.J. Schofield [32].

Table 3

A short list of possible cognitive biases

Cognitive biases	Definition	Example
Overconfidence bias	Overestimation of the likelihood of a diagnosis based on the ease with which it comes to mind	Establishing a diagnosis based on a previous patient with similar clinical presentation
Confirmation bias	Selective preference for evidence supporting the diagnosis	Use of scientific articles that describe criteria for confirming the diagnosis, ignoring alternative publications
Anchoring bias	Striving to adhere to a specific diagnosis despite evidence of refutation	Doctor's refusal to change the diagnosis of VACS despite a peer-confirmed diagnosis of BPPV

CONCLUSION

Different approaches to the interpretation of diagnostic criteria for VACS are reflected in different frequency of diagnosis of VA compression. It is obvious that unified criteria for diagnosis of VACS and raising awareness of doctors about alternative diagnoses (BPPV, migraine, and other diseases) are required in order to reduce the likelihood of VACS overdiagnosis and improve the quality of medical care.

REFERENCES

1. Jadeja N., Nalleballe K. Pearls & Oy-sters: Bow hunter syndrome: A rare cause of posterior circulation stroke: Do not look the other way. *Neurology*. 2018; 91 (7): 329–331. DOI: 10.1212/WNL.0000000000006009.
2. Molokov D.D., Yagunov P.V., Molokov V.D. Rehabilitation therapy for vertebral artery compression syndrome. *Siberian Medical Journal.* 2010; 97 (6): 266–267 (in Russ.).
3. Alekseeva N.S. Vertigo mediated by cervical spine pathology. *Treating Doctor.* 2009; (7): 60–63 (in Russ.).
4. Zinovyeva G.A., Babanina L.P. Vertebral artery compression syndrome in vertebrogenic pathology of the cervical spine. *Bulletin of BolgSMU.* 2006; 1 (17): 9–13 (in Russ.).
5. Jost G.F., Dailey A.T. Bow hunter's syndrome revisited: 2 new cases and literature review of 124 cases. *Neurosurgical Focus*. 2015; 38 (4): 1–15. DOI: 10.3171/2015.1.FOCUS14791.
6. Simpkin C.T., Davis K.E., Davis B.S., Vosko A.M., Jorgensen M.E. Bow hunter's syndrome in a patient with vertebral artery atresia, an arcuate foramen, and unilateral deafness: a case report. *Radiol. Case Rep*. 2017; 12 (3): 597–601. DOI: 10.1016/J.RADCR.2017.04.001.
7. Rastogi V., Rawls A., Moore O. et al. Rare Etiology of Bow Hunter's Syndrome and Systematic Review of Literature. *J. Vasc. Interv. Neurol.* 2015; 8 (3): 7–16.
8. Go G., Hwang S.H., Park I.S., Park H. Rotational vertebral artery compression: Bow Hunter's Syndrome. *J. Korean Neurosurg Soc*. 2013; 54 (3): 243–245. DOI: 10.3340/JKNS.2013.54.3.243.
9. Mileva N.B., Vassilev D.I., Serbezova I., Rigatelli G., Gill R.J. Vertebral artery stenting in a patient with Bow Hunter's Syndrome. *JACC: Case Reports*. 2019; 1 (1): 73–74. DOI: 10.1016/J.JACCAS.2019.05.010.
10. Duan G., Xu J., Shi J., Cao Y. Advances in the Pathogenesis, Diagnosis and Treatment of Bow Hunter's Syndrome: A Comprehensive Review of the Literature. *Interv. Neurol.* 2016; 5(1-2): 29–38. DOI:10.1159/000444306.
11. Iguchi Y., Kimura K., Shibazaki K., Iwanaga T., Ueno Y., Inoue T. Transcranial doppler and carotid duplex ultrasonography findings in Bow hunter's syndrome. *J. Neuroimaging*. 2006; 16 (3): 278–280. DOI: 10.1111/J.1552-6569.2006.00040.X.
12. Vilela M.D., Goodkin R., Lundin D.A., Newell D.W. Rotational vertebrobasilar ischemia: hemodynamic assessment and surgical treatment. *Neurosurgery*. 2005; 56 (1): 36–45. DOI: 10.1227/01.NEU.0000146441.93026.CE.
13. Zakhmatova T.V., Shchedrenok V.V., Moguchaya O.V., Sebelev K.I., Krasnoshlyk P.V. Comparative analysis of vertebral artery hemodynamics in injuries and degenerative diseases of the cervical spine using Duplex color scanning. *Russian Biomedical Journal.* 2014; 15 (2): 352–365 (in Russ.).
14. Dicheskul M.L., Kulikov V.P. The effect of maximum head rotation on the blood flow parameters in the intracranial segment of the vertebral arteries. *Manual Therapy.* 2011; 41 (1): 27–32 (in Russ.).
15 Sitel A.B., Nefedov A.Yu. Treating vertebrobasilar insufficiency with manual therapy – prevention of cerebral stroke. *Manual Therapy.* 2008; 29 (1): 22–31 (in Russ.).
16. Dicheskul M.L., Kulikov V.P. Ultrasound-guided assessment of blood flow dynamics in the vertebral arteries during head rotations. *Fundamental Research*. 2012; (5-1): 22–25 (in Russ.).
17. Yeh W.Z., Blizzard L., Taylor B.V. What is the actual prevalence of migraine? *Brain Behav.* 2018; 8 (6): 1–6. DOI: 10.1002/BRB3.950.
18. Ciorba A., Cogliandolo C., Bianchini C. et al. Clinical features of benign paroxysmal positional vertigo of the posterior semicircular canal. *SAGE Open Med.* 2019; 7: 2050312118822922. DOI: 10.1177/2050312118822922.

19. Nicolas-Puel C., Faulconbridge R.L., Guitton M., Puel J.L., Mondain M., Uziel A. Characteristics of tinnitus and etiology of associated hearing loss: a study of 123 patients. *Int. Tinnitus J.* 2002; 8 (1): 37–44.
20. Kamouchi M., Kishikawa K., Matsuo R. et al. Ultrasonographic detection of extracranial vertebral artery compression in bow hunter's brain ischemia caused by neck rotation. *Cerebrovasc. Dis*. 2003; 16 (3): 303–305. DOI: 10.1159/000071134.
21. Yeh J.F., Lin Y.J., Po H.L. et al. A case of bow hunter's stroke caused by non-dominant vertebral artery. *Acta Neurol. Taiwan.* 2005; 14 (2): 69–73.
22. Sturzenegger M., Newell D.W., Douville C., Byrd S., Schoonover K. Dynamic transcranial Doppler assessment of positional vertebrobasilar ischemia. *Stroke*. 1994; 25 (9): 1776–1783. DOI: 10.1161/01.STR.25.9.1776.
23. Nierenberg R. The chief complaint driven medical history: implications for medical education. *Int. J. Med. Educ*. 2017; 8: 205–206. DOI: 10.5116/IJME.5907.74D8.
24. Heneghan C., Glasziou P., Thompson M. et al. Diagnostic strategies used in primary care. *BMJ*. 2009; 338: b946. DOI: 10.1136/BMJ.B946.
25. Barulin A.E., Kurushina O.V., Puchkov A.E. Vertebrobasilar insufficiency. *Bulletin of VolfSMU.* 2014; 3 (51): 3–8 (in Russ.).
26. Parfenov V.A. Modern aspects of diagnosis and treatment of chronic cerebral ischemia: the use of naftidrofuryl. *Medical Council.* 2015; 18: 11–17 (in Russ.) DOI: 10.21518/2079-701X-2015-18-11-17.
27. Hornibrook J. Benign Paroxysmal Positional Vertigo (BPPV): History, pathophysiology, office treatment and future directions. *Int. J. Otolaryngol.* 2011; 2011: 835671. DOI: 10.1155/2011/835671.
28. Ahmed F. Headache disorders: differentiating and managing the common subtypes. *British Journal of Pain.* 2012; 6 (3): 124–132. DOI: 10.1177/2049463712459691.
29. Jung H.K., Soo B.I., Je H.J., Dong S.S., Sang M.Y., Jong H.M. Bow Hunter Syndrome treated via an anterior approach and selective decompression only. *Biomed. J. Sci. Tech. Res*. 2018; 9 (5): 1–4. DOI: 10.26717/ BJSTR.2018.09.001870.
30. Lebedeva N.V., Zamergrad M.V., Parfenov V.A., Antonenko L.M. Diagnosis and treatment of aptients with benign paroxysmal positional vertigo in routine clinical practice. *Archives of Therapy*. 2017; 89 (1): 57–61 (in Russ.). DOI: 10.17116/terarkh201789157-61.
31. Amelin A.V., Bogdanova Yu.N., Koreshkina M.I., Protsenko E.V., Skoromets A.A., Tarasova S.V. Diagnosis of primary and symptomatic forms of daily chronic headache. *Journal of Neurology and Psychiatry named after S.S. Korsakov.* 2011; 111 (4): 82–84 (in Russ.).
32. O'Sullivan E.D., Schofield S.J. Cognitive bias in clinical medicine. *J. R. Coll. Physicians Edinb.* 2018; 48 (3): 225–232. DOI: 10.4997/JRCPE.2018.306.

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Тромбоциты и регенерация

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РЕЗЮМЕ

Представлен анализ данных, доказывающих участие тромбоцитов в механизмах регуляции репаративной регенерации тканей. Показано их влияние на повреждение, апоптоз, пролиферацию клеток, ремоделирование экстрацеллюлярного матрикса, ангиогенез и нейрогенез. Дана оценка их взаимодействию с макрофагами в процессе восстановления структуры поврежденных тканей. Охарактеризованы некоторые тромбоцитарные регенеративные факторы.

Ключевые слова: тромбоциты, регенерация, ангиогенез, нейрогенез, макрофаги.

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Platelets and regeneration

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ABSTRACT

The review presents the analysis of data proving the role of platelets in the mechanisms of regulation of reparative tissue regeneration. The influence of platelets on damage, apoptosis, and proliferation of cells, as well as on extracellular matrix remodeling, angiogenesis, and neurogenesis is shown. Their interaction with macrophages in restoring the structure of damaged tissues is assessed. Several regenerative properties of platelets are characterized.

Key words: platelets, regeneration, angiogenesis, neurogenesis, macrophages.

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INTRODUCTION

It has long been thought that platelets are involved only in the processes of hemostasis. However, studies over the past two decades have shown the polyfunctionality of these formed blood elements, which makes them an important link in body adaptation to various extreme factors, such as hypoxia, ionizing radiation, infection, stress, etc. [1].

Platelets play a particular role in reparative processes, being the first to react to damage, along with neutrophils and macrophages. However, experimental studies showed that depletion of the neutrophil storage pool does not affect the regeneration process [2]. Platelets accumulate at the ends of damaged blood vessels, turn fibrinogen into fibrin, and form a blood clot consisting of cross-linked fibrin, fibronectin, vitronectin, thrombospondin, red blood cells, and platelets [3, 4]. In this clot, platelets are responsible for activating and releasing important biomolecules from their α-granules, including platelet-specific proteins, growth factors, clotting factors, adhesion molecules, cytokines, angiogenic factors, proteoglycans, and cytokines / chemokines [5]. Secretion of cytokines, chemokines, and growth factors, in turn, induces proliferation and activation of cells involved in wound healing, such as fibroblasts, neutrophils, monocytes, smooth muscle cells, and mesenchymal stem cells (MSCs) [6].

Antiplatelet drugs inhibit regeneration by slowing down the secretion of growth factors, such as vascular endothelial growth factor (VEGF), by platelets. So, in rats with gastric ulcer, the adenosine diphosphate (ADP) receptor inhibitor – ticlopidine – not only significantly suppresses platelet aggregation, but also disrupts gastric ulcer healing and angiogenesis. Moreover, platelet transfusions can reverse this effect [7]. In this case, platelets are present in the bloodstream but do not release their granules.

It can be assumed that the regenerative effect of platelets is due to one of three components: growth factors, chemokines, or proteins localized on the granule membrane and embedded in the platelet membrane after activation.

In trauma, the injury site is filled with a 3-dimensional polymerized fibrin clot containing plasma rich in wound healing factors, platelets, MSCs, and fibroblasts. Within several days, the cells inside the wound form a complex cocktail of wound healing, neurotrophic, and other factors. These observations served as the basis for the use of platelet-rich plasma ((PRP), platelet concentrate, platelet gel) for stimulating regeneration.

Blood plasma with a high platelet content is a complex of physiologically active substances that are defined as platelet-derived wound healing factors (PDWHF) [8–10]. They include several isotypes of platelet-derived growth factors (PDGF): ADP, adenosine triphosphate (ATP), calcium, serotonin, platelet factor 4, fibronectin, β-thromboglobulin, von Willebrand factor (vWF), fibrinogen, blood clotting factors V and XIII, transforming growth factor-β (TGFβ), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), basic fibroblast growth factor (β-FGF), fibroblast growth factor-2 (FGF-2), platelet factor 4 (PF4), ciliary neurotrophic factor (CNTF), insulin-like growth factor-1 (IGF-1), and platelet-derived angiogenesis factor (PDAF) [9, 11]. Some of these factors are involved in recruiting progenitor cells involved in tissue healing to the site of damage, while others are targeted by cells of the damaged tissue.

Although PRP is increasingly used for wound closure and activation of wound healing [12], specific mechanisms of platelet regulation of reparative processes have only recently attracted the attention of researchers. The accumulated clinical and experimental data allow to look at the physiology of blood plates from a new perspective.

Platelets are non-nuclear elements of blood, the cytoplasm of which can contain up to 100 membrane granules. The granules act as storage tanks for active substances. Several types of platelet granules were described, of which α-granules, dense granules, and lysosomes are of the greatest interest [13, 14].

α-granules are the most numerous (40–80%) and the largest (200–400 nm). They store more than 300 different proteins, such as blood coagulation proteins (factor V, factor IX, factor XIII, antithrombin, plasminogen, plasminogen activator inhibitor-1 (PAI-1)), adhesion molecules (fibrinogen, vWF, thrombospondin), chemokines, and growth factors (vascular

endothelial growth factor (VEGF), PDGF, FGF, EGF, hepatocyte growth factor (HGF), TGFβ). The release of these biologically active substances is not random but proceeds under the influence of an external stimulus. The contents of the granules are released after adhesion to collagen, other matrix components, or in response to soluble agonists, such as ADP or thrombin [5]. α-Granules are considered to be the key organelles with regard to platelet function.

Dense granules are smaller in size and quantity, store high concentrations of calcium, magnesium, nucleotides (ATP, ADP, cyclic adenosine monophosphate (cAMP), uridine triphosphate (UTP)), and pyrophosphates, serotonin (5-HT) and histamine. Secretion of dense platelet granules plays a major role in enhancing platelet response and thrombosis.

Platelet lysosomes, like in other cells, contain enzymes, such as cathepsin, elastase, collagenase, carboxypeptidase, glucosidase, glucuronidase, and acid phosphatase, which are associated with degradation of proteins, carbohydrates, and lipids [15–17].

In addition to protein synthesis, platelets also act as a source of active metabolites, such as eicosanoids synthesized from arachidonic acid, which are released from membrane phospholipids. Thromboxane A2 (TXA2) is a potent vasoconstrictor and is also associated with a proliferative response of damaged vessels [18]. Sphingosine-1-phosphate (S1P), which has the mitogenic action, is secreted by activated platelets at the time of clot formation and stimulates assembly of the fibronectin matrix and expression of TF (tissue factor) in endothelial cells [19]. Platelet-activating factor (PAF) is an active platelet-derived lipid that suppresses leukocyte migration and activates endothelial cells [20].

The platelet membrane contains many functional cell adhesion molecules, such as P-selectin, GPIIb/IIIa, GPIb, and integrins, which are not only involved in clot formation, but also allow platelets to interact with endothelial cells, white blood cells, including macrophages, and progenitor cells [21, 22]. P-selectin is present on the activated platelet membrane after the release of α-granules. This ensures that only activated platelets interact with immune and endothelial cells. The main leukocyte ligand for P-selectin is PSGL-1. Parallel release of cytokines and chemokines affects the interaction of platelets with leukocytes. This leads to increased regulation of leukocyte transcription factors and production of more cytokines and chemokines [5].

After damage, substances that are usually found inside cells enter the extracellular space. These endogenous molecules, which include proteins and nucleic acids, are called dampers and are a key signal for initiating immune responses and regeneration. Dampers can activate various types of receptors, including Toll-like receptors, nucleotide-binding oligomerization domain (NOD)-like receptors, retinoic acid-inducible gene 1 (RIG-1)-like receptors, C-type lectin receptors (CLR), receptors for advanced glycation end products (RAGE), G-protein-coupled receptors (GPCR), and ion channels [23], located on epithelial cells, endothelial cells, fibroblasts, neutrophils, macrophages, platelets, dendritic cells, etc.

The platelet response begins with the interaction of the P2Y1 and P2Y12 receptors located on the platelet membrane with ADP molecules emerging from damaged cells, and the G2 and Gi proteins, respectively, are secondary messengers in transmitting this signal. These receptors are known for their central role in platelet activation and aggregation [5, 23].

Platelet activation causes release of substances from dense granules through exocytosis involving Rab proteins. The released components include ADP (activating neighboring platelets in the above-described way), ATP, inorganic polyphosphate, pyrophosphate, serotonin, and calcium.

Activated platelets also release their α-granules containing biologically active substances, such as chemokine ligand 5 ((CCL5) or regulated upon activation, normal T cell expressed, and secreted (RANTES)), thrombin, transforming growth factor β (TGF-β), PDGF and VEGF, platelet factor 4, TF, IGF, FGF, CXCL12 or stromal cell-derived factor-1 (SDF-1), CD40 ligand, and EGF [3, 5, 24, 25]. Factors secreted by platelets induce and increase the activity of fibroblasts and have chemotactic effects first on neutrophils and then on macrophages, which ultimately leads to removal of dead cells and cell debris [26]. Moreover, platelets synthesize and secrete factors that induce and regulate proliferation and migration of other cell types, such as smooth muscle cells (SMCs) [27] and MSCs [28].

Cell proliferation and extracellular matrix remodeling are particularly important in early stages of regeneration. This response is modulated by the TGFβ signaling pathway in all Smads proteins. Inhibition of this pathway by the TGFβ antagonist SB-431542 leads to a decrease in cell proliferation and prevents regeneration [29]. Thus, in mice with TGF-β3 deficiency, inhibition of tissue inhibitor of metalloproteinases-2 (TIMP2) and matrix metallopeptidase-13 (MMP-13) or collagenase-3 was observed [30]. This

enzyme shows very high degrading activity against collagens of types I, II, III, IV, and XIV during endochondral and intramembranous osteogenesis. In human fibroblasts, it was shown that the addition of TGF-β1 led to an increase in the levels of mRNA and matrix metalloproteinase-2 (MMP-2) and a decrease in the level of collagenase mRNA [31]. TGF-β1 also regulated synthesis of TIMPs, which inhibit matrix metallopeptidases (MMPs) via the mitogen-activated pathway [32].

Activated platelets trigger recruitment, adhesion, and proliferation of adult stem cells, including CD34-positive progenitor cells [33], MSCs [28], SMS precursors [27, 34], and endothelial cell precursors [35]. Stromal cell-derived factor-1 (SDF-1) [36] and IGF-1 [37], which are also released by platelets, act as "homing beacons" for progenitor cells at the site of damage.

Activated platelets form extracellular vesicles by releasing their plasma membrane - platelet extracellular vesicles (PEV), through which intercellular communication with leukocytes is carried out. Although PEV can be produced in healthy individuals, their increased level is detected in injuries. The ability of PEV to bind to granulocytes, lymphocytes, and monocytes to form leukocyte vesicular complexes (LVCs) was shown [38–40].

Platelets are involved in the regulation of apoptosis and interaction of regenerating cells [41]. They secrete both proapoptotic (Fas-L [42], CD40L [43], tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) [44], TNF-like weak inducer of apoptosis (TWEAK) [45], and tumor necrosis factor superfamily element 14, also known as LIGHT [46]) and anti-apoptotic (HGF [47], SDF-1 [22], serotonin [27, 48], ADP [27] and S-1-P [49]) mediators. In addition, platelet-derived microparticles can regulate apoptosis in endothelial cells and SMCs and transmit survival signals to monocytes, endothelial cells, and neural stem cells. In the spleen and lungs, granzyme B acts as a meditator for platelet-induced apoptosis. Amphoterin, which is exported on the cell surface of platelets upon their activation, also regulates apoptosis and autophagy of tumor cells, which depends on redox processes (redox status). Therefore, platelets regulate a complex set of tissue repair mechanisms [50].

Along with matrix remodeling, proliferation, and differentiation of specific cells, restoration of microcirculation and innervation plays an essential role in reproducing the structure the damaged tissue.

There is growing evidence that platelets are a necessary condition for angiogenesis in wound healing / tissue regeneration [51, 52]. At the sites of platelet aggregation, regeneration of damaged vascular intima begins [53].

An injection of own platelets and leukocytes in a rat with hind limb ischemia initiated angiogenesis in it [54]. There was a significant decrease in neovascularization with a fall in the number of platelets *in vivo* [55, 56]

Platelets secrete various promoters of angiogenesis, such as VEGF, basic fibroblast growth factor (bFGF), EGF, and PDGFs or angiopoietin-1 [57].

VEGF is a very powerful angiogenic factor [58, 59]. Changes in the proliferative activity of the endothelium and apoptosis of endothelial cells are caused by release of VEGF and endostatin by platelets [7]. The VEGF-C and VEGF types are contained in α-granules of platelets and are released upon platelet activation [60]. Platelets not only synthesize VEGF but also act as carriers of this factor from other sources of its formation [60].

In platelets and megakaryocytes, angiopoietin-1 (which provides stabilization of proliferating endothelial cells and vessels) was found in vascularized tissues, while it was absent in these cells in non-vascular zones [61]. Angiopoietin-1 is released from platelets after their activation, for example, by thrombin [61].

Redistribution of endogenous growth factors from the cytoplasm of intact platelets to the periphery of filopodia and laminopodia in activated platelets may be associated, at least to some extent, with the regulation of angiogenesis [62, 63]. In addition to angiogenesis stimulators, platelets secrete a number of its inhibitors, such as angiostatin, endostatin, platelet factor (PF)-4, or thrombospondin (TSP)-1. Angiostatin is an example of an angiogenesis inhibitor formed by platelets, which is released during the aggregation of blood plates [64].

Endostatin specifically inhibits endothelial cell proliferation and powerfully suppresses angiogenesis and tumor growth [65]. PF-4 was the first hemostatic protein to show an angiogenesis-inhibiting effect *in vivo* [66]. At least partially, the antiangiogenic activity of PF-4 is due to interference with FGF-2, which causes inhibition of its dimerization following interaction with the FGF receptor and internalization.

Platelet thrombospondin (TSP) is also an inhibitor of angiogenesis; it destabilizes local contacts of endothelial cells and inhibits proliferation of the latter [67]. Moreover, thrombospondins, megakaryocytes, and

platelets act as the main antiangiogenic switches and determine the degree of revascularization *in vivo* [68].

Interestingly, platelet-induced angiogenesis requires the physical presence of platelets, because their secretion (supernatant) alone does not have a noticeable effect on tube formation *in vitro* [69]. Thus, the cell-cell interaction between platelets and endothelial cells appears to play an important role in neovascularization. In another study, adding platelets to a solution for infusions before injecting animals induced dose-dependent angiogenesis [70].

To initiate angiogenesis, destabilization is necessary – weakening of intercellular contacts between endothelial cells, destruction of the basement membrane, as well as local proteolysis of matrix proteins for endothelial cells or their precursors from the circulating blood to migrate and form new vessels [71, 72].

Urokinase is most often considered as a key regulator of vascular wall remodeling after mechanical damage [73]. Activated platelets release neurotransmitters, serotonin, dopamine, histamine, and glutamate, and can also alter the activity of neuronal cells [74]. The presence of platelets in the area of damage to the nervous system accelerates restoration of function and enhances not only angiogenesis but also neuronal regeneration [75].

In small experimental animals, it was shown that when the central and peripheral sections of the cut nerve are connected with a collagen tube filled with PRP, it induces axon regeneration. Thus, it is possible to compensate for the defect of the sciatic nerve in rats up to 1 cm long [76–79]. In this case, a thicker myelin sheath is formed, the rate of regeneration increases and recovery goes over a greater distance [10]. For PRP fibrin to promote more neurons to regenerate over a greater distance, it must bind and interact with neurotrophic cells. In this interaction, an important role is attributed to such factors as nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), glial cell line-derived neurotrophic factor (GDNF), neurotrophin-3 (NT-3), and PDGF [80–85]. These factors, which, in turn, are associated with platelets and mesenchymal cells, transform matrix fibrin to actively promote axonal regeneration [86].

The noted phenomenon was shown during transection of the facial nerve in the guinea pig [87], the facial nerve in the rat [76], the sciatic nerve in the rat [77, 88–90], and the cavernous nerve in the rat [87, 91].

It is generally accepted that macrophages are an important link in the regeneration process, acting as coordinators of actions aimed at restoring the original tissue structure or scarring. Macrophages act as a source of proinflammatory cytokines in the damaged area, such as interleukin (IL) 1, IL-6, and tumor necrosis factor (TNF). They are responsible for controlling adhesion and migration of inflammatory cells, as well as proliferation of fibroblasts and keratinocytes [3].

The depletion of macrophages in axolotl by injecting clodronate encapsulated in liposomes leads to impaired regeneration of the amputated limb [92]. Clodronate is unable to penetrate into the cell membrane. However, being encapsulated in liposomes, it is phagocytosed by macrophages. This drug is metabolized by macrophages *in vitro* to adenosine-5- [B, γ-dichloromethylene] triphosphate (AppCCl2p). AppCCl2p (ATP analogue), inhibiting the mitochondrial electrogenic ADP / ATP translocase, causes depolarization of the mitochondrial membrane and subsequent cytochrome C release and caspase activation, which leads to specific apoptosis of macrophages [93]. Therefore, we can talk about a relationship between the lack of regeneration of the extremities and depletion of macrophages in this experiment.

Selective macrophage depletion using clodronate in modeling myocardial infarction leads to a serious violation of the myocardial architecture, increased collagen deposition, and increased mortality in mice [94, 95].

The use of a transgenic mouse (lysM-Cre / DTR mouse strain) containing macrophages sensitive to diphtheria toxin (DTox) showed delayed wound healing with strong morphological disturbances [96]. This is due to a decrease in TGF-β1 expression, a dysregulated VEGF pattern, and an almost complete loss of wound contraction in the absence of myofibroblast differentiation. Macrophage depletion was detected by decreased mRNA expression of EGF-like module-containing mucin-like hormone receptor-1 (Emr-1) and lysozyme of macrophages (LysM), which is a macrophage-specific marker F4/80.

All of the above-mentioned studies suggest that macrophages play a significant role in the regeneration process. Without these cells, regeneration fails, a hypertrophic scar or a non-healing wound is formed.

The macrophage population can be divided into two functional phenotypes. They are named M1 (classically activated) and M2 (alternatively activated) macrophages [3]. M1 macrophages are activated in response to damage to lipopolysaccharide (LPS), TNF-α, and interferon (INF)γ [97]. These ligands act on macrophages via LPS / IFNγ or TLR-2, -3, -4, and -9

and cause the release of IL-1B, TNFα, and IL-6, mediated by the signaling factors NF-kB, STAT1, IRF5, and AP-1 [98]. These cytokines amplify the inflammatory and antimicrobial responses [3, 99, 100].

IL-4 and IL-13 (M2a), which trigger Fcγ receptors in the presence of a Toll-like receptor (M2b) or IL-10 (M2c), can stimulate macrophages to differentiate into M2 macrophages [97, 98]. These three macrophage phenotypes are not activated, for example, M1, and exhibit properties different from them.

M1 and M2a macrophages are present together at the site of damage. Macrophages migrating to the wound on the 1st day after injury mainly (85%) show the M1 phenotype [101], but on the 5th day, the M2 phenotype is the dominant macrophage population in the wound [98]. Compared to the axolotl, the mammalian has an increase in proinflammatory cytokines over anti-inflammatory ones after injury [92], which may be a key reason for the decrease in the regenerative properties of mammalian tissues.

The activation of both Toll-like and Fcγ receptors in macrophages results in the M2b phenotype. The M2b phenotype, as compared to M2a, produces much higher levels of IL-10 along with the proinflammatory cytokines TNFα, IL-1β, and IL-6 [97]. During a later proliferative phase, the M2b-mediated release of IL-10 appears to stimulate the activation of M2c macrophages [98]. IL-10 inhibits production of proinflammatory cytokines, such as TNFα, IL-6, and IL-12, and antigen presentation by macrophages through the downregulation of major histocompatibility complex (MHC) class II molecules [97]. Activation of the STAT3 signaling pathway results in the release of TGFβ.

Since platelets and macrophages play the key role in regeneration, the mechanisms of interaction of these cells in the dynamics of repairing damaged tissues are of particular importance.

During regeneration, platelets and macrophages interact with one other both directly and indirectly through other cells (for example, endothelial cells), exerting a reciprocal effect. Platelets and platelet factors (mediators) activate and modulate apoptosis in monocytes, and platelet phagocytosis is essential in pro- and anti-inflammatory processes [102].

With the direct interaction of activated platelets with blood monocytes, platelet-monocyte complexes form. Aggregates of platelets with monocytes form more easily (i.e. at lower concentrations of platelet agonists) and faster and are more stable than platelet-neutrophil and platelet-lymphocyte complexes [103]. Sialidase treatment of platelets leads to an increase in their binding to homologous peritoneal macrophages but does not affect the rate of phagocytosis. The interaction of platelets with macrophages is mediated by a galactose-specific receptor on the surface of macrophages [104].

Infection-induced thrombocytosis is a clinically significant complication of tuberculosis, accompanied by impaired immunity. Inhibition of platelets with aspirin or treatment of the platelet-specific receptor, glycoprotein IIb / IIIa, with inhibitors leads to a decrease in platelet-macrophage interactions and restoration of macrophage-mediated immunity to mycobacterial infection [105, 106].

When interacting with activated platelets through PSGL-1 / P-selectin, as well as when binding products of activated platelets (RANTES, IL-1β, and PAF), NF-kB-dependent inflammatory genes are induced in monocytes [107]. Binding of PSGL-1 leads to the activation of the MAP kinase and the mTOR pathways [107]. A signal triggered in monocytes upon contact with platelets and binding of endogenous IL-1 induces expression of cyclooxygenase-2 (COX-2) and formation of prostaglandin E2 (PGE2) dependent on it [107]. The latter, in turn, reduces the activity of platelets [108, 109].

Upon contact with platelets, monocytes acquire an inflammatory phenotype and increase the affinity of adhesion to the endothelium [110, 111]. Platelets are captured by monocytes and macrophages, which causes an increase in the release of cytokines from monocytes [112]. Thus, activated platelets affect the survival and differentiation of monocytes, after which the complexes of monocytes with activated platelets disintegrate [113].

Platelet-macrophage communication is also carried out through PEVs, which, after binding to the monocyte and formation of a platelet-monocyte complex, is absorbed by the latter within 30–60 minutes [110]. Thus, PEVs can deliver, in particular, the RANTES chemokine (CCL5) to monocytes and endothelial cells, promoting the attraction of monocytes to the subendothelium [114]

CCL5 is one of the most important monocyte chemoattractants released by platelets after injury. CCL5 interacts with the endothelial surface in the presence of the cytokine IL-1β and acts as a cell-associated signal for monocyte adhesion and migration across the vascular endothelium. IL-1β is also released from platelets [5].

RANTES is also secreted by endothelial cells under the influence of IFNγ and TNFα [115]. TGFβ not

only stimulates the activation of macrophages M2c, but the M2c subtype itself is an important source of TGFβ, which contributes to many aspects of wound healing: inflammation, chemotaxis, wound reduction, angiogenesis, and extracellular matrix (ECM) deposition [3].

The exposure of activated platelets to monocytes causes an increase in the expression of tissue factor (TF) and binding to the coagulation factor Xa and fibrinogen. The resulting thrombin causes not only aggregation and activation of platelets, but also activation of monocytes, directing them to enhanced adhesion and production of chemokines CCL2 and RANTES [116, 117]. Binding of the platelet cytokine CXCL13 to the CXCR5 receptor on monocytes leads to inhibition of TNFα and IL-6 production [118].

Platelets eject microparticles not only upon activation but also upon aging as a result of an apoptosis-like process (apoptosis-induced platelet microparticles). With prolonged incubation with monocytes, they contribute to cell differentiation but suppress their proliferation. Analysis of monocyte membrane receptors shows increased levels of expression of CD11b (integrin aMb2), CD14, and CD31 (platelet / endothelial cell adhesion molecule-1), as well as chemokine receptors CCR5 and CXCR4, but not CCR2, which means that apoptosis-induced platelet microparticles polarize cells towards resident monocyte M2. Cells treated with apoptosis-induced platelet microparticles actively consume oxidized low-density lipoprotein (LDL) and release matrix metalloproteinases and hydrogen peroxide. One more confirmation of differentiation in direction of resident professional phagocytes is that particles stimulate expression of LDL, CD36, and CD68 receptors, as well as production of proinflammatory and immunomodulatory cytokines by monocytes [118].

Therefore, there is no doubt that in the processes of reparative regeneration, one of the leading places is occupied by platelets participating in its regulation at all stages of restoring the structure of damaged tissue. Deciphering the specific mechanisms of their reparative function will allow to develop new effective methods of targeted effect on wound healing.

REFERENCES

1. Chereshnev V.A., Yushkov B.G., Klimin V.G., Butorina E.V. Thrombocytopoiesis. M.: “Izdatelstvo Meditsina”, 2007: 272 (in Russ.).
2. Simpson D.M., Ross R. The neutrophilic leukocyte in wound repair: a study with antineutrophil serum. *The Journal of Clinical Investigation*. 1972; 51 (8): 2009–2023. DOI: 10.1172/JCI107007.
3. Delavary B.M., van der Veer W.M., van Egmond M., Niessen F.B. Beelen R.H.J. Macrophages in skin injury and repair. *Immunobiology.* 2011; 216 (7): 753–762. DOI: 10.1016/j.imbio.2011.01.001.
4. Martin P., Leibovich S.J. Inflammatory cells during wound repair: The good, the bad and the ugly. *Trends in Cell Biology*. 2005; 15 (11): 599–607. DOI: 10.1016/j.tcb.2005.09.002.
5. Nurden A. Platelets, inflammation and tissue regeneration. *Thromb. Haemost*. 2011; 105 (1): 13–33. DOI: 10.1160/THS10-11-0720.
6. Thushara R.M., Hemshekhar M., Basappa Kemparaju K., Rangappa K.S, Girish K.S. Biologicals, platelet apoptosis and human diseases: An outlook. *Crit. Rev. Oncol. Hematol*. 2015; 93 (3): 149–158. DOI: 10.1016/j.critrevonc.2014.11.002.
7. Ma L., Elliott S.N., Cirino G., Buret A., Ignarro L.J., Wallace J.L. Platelets modulate gastric ulcer healing: Role of endostatin and vascular endothelial growth factor release. *PNAS.* 2001; 98 (11): 6470–6475. DOI: 10.1073/pnas.111150798.
8. Su C.Y., Kuo Y.P., Nieh H.L., Tseng Y.H., Burnouf T. Quantitative assessment of the kinetics of growth factors release from platelet gel. *Transfusion*. 2008; 48 (11): 2414. DOI: 10.1111/j.1537-2995.2008.01862.x.
9. Peterson J.E., Zurakowski D., Italiano J.E., Michel L.V., Fox L., Klement G.L., Folkman J. Normal ranges of angiogenesis regulatory proteins in human platelets. *Am. J. Hematol*. 2010; 85 (7): 487. DOI: 10.1002/ajh.21732.
10. Kuffler D.P. Platelet-rich plasma promotes axon regeneration, wound healing and pain reduction: Fact or fiction. *Mol. Neurobiol.* 2015; 52 (2): 990: 1014. DOI: 10.1007/s12035-015-9251-x.
11. Kakudo N., Morimoto N., Kushida S., Ogawa T., Kusumoto K. Platelet-rich plasma releasate promotes angiogenesis in vitro and *in vivo*. *Med. Mol. Morphol*. 2014; 47 (2): 83–89. *DOI:* 10.1007/s00795-013-0045-9.
12. Lacci K.M., Dardik A. Platelet-rich plasma: support for its use in wound healing. *Yale J. Biol. Med*. 2010; 83 (1): 1–9.
13. Flaumenhaft R., Sharda A. Platelet secretion. Platelets. Academic Press, 2019: 349–370. DOI: 10.1016/B978-0-12-813456-6.00019-9.
14. Meyer J., Lejmi E., Fontana P., Morel P., Gonelle-Gispert C., Bühler L. A focus on the role of platelets in liver regeneration: Do platelet-endothelial cell interactions initiate the regenerative process? *Journal of Hepatology.* 2015; 63 (5): 1263–1271. DOI: 10.1016/j.jhep.2015.07.002.
15. Rainys D., Samulėnas G., Kievišas M., Pilipaitytė L., Rimdeika R. Platelet biology and the rationale of PRP therapy in chronic wounds. *Eur. J. Plast. Surg.* 2017; 40 (2): 87–96. DOI: 10.1007/s00238-017-1279-x.
16. Flaumenhaft R. Platelet secretion. In: Platelets; Michelson A.D. (ed.); 3rd ed. Academic Press. Oxford, 2013: 343–366. DOI: 10.1016/B978-0-12-813456-6.00019-9.

17. Rendu F., Brohard-Bohn B. The platelet release reaction: ranules' constituents, secretion and functions. *Platelets.* 2001; 12 (5): 261–273. DOI: 10.1080/09537100120068170.
18. Cheng Y., Austin S.C., Rocca B., Beverly H., Koller B.H., Coffman T.M., Grosser T., Lawson J.A., Fitz Gerald G.A. Role of prostacyclin in the cardiovascular response to thromboxane A2. *Science*. 2002; 296 (5567): 539–554. DOI: 10.1126/science.1068711.
19. Zhang O., Peyruchaud O., French K.J., Magnusson M.K., Mosher D.F. Sphingosine 1-phosphate stimulates fibronectin matrix assembly through a rho-dependent signal pathway. *Blood.* 1999; 93 (9): 2984–2990.
20. Weyrich A.S., Prescott S.M., Zimmerman G.A. Platelets, endothelial cells, inflammatory chemokines, and restenosis: complex signaling in the vascular play book. *Circulation.* 2002; 106 (12): 1433–1435. DOI: 10.1161/01.cir.0000033634.60453.22.
21. Stellos K., Langer H., Daub K., Schoenberger T., Gauss A., Geisler T., Bigalke B., Mueller I., Schumm M., Schaefer I., Seizer P., Kraemer B.F., Siegel-Axel D., May A.E., Lindemann S., Gawaz M. Platelet-derived stromal cell–derived factor-1 regulates adhesion and promotes differentiation of human $CD34^+$ cells to endothelial progenitor cells. *Home Circulation.* 2008; 117 (2): 206–215. DOI: 10.1161/CIRCULATIONAHA.107.714691.
22. Stellos K., Kopf S., Paul A., Marquardt J., Gawaz M., Huard J., Langer H. Platelets in regeneration. Seminars in thrombosis and hemostasis. *Thieme Medical Publishers*. 2010; 36 (02): 175–184. DOI: 10.1055/s-0030-1251502.
23. Gong T., Liu L., Jiang W., Zhou R. DAMP-sensing receptors in sterile inflammation and inflammatory diseases. *Nat. Rev. Immunol*. 2020; 20 (2): 95–112. DOI: 10.1038/s41577-019-0215-7.
24. Petersen F., Brandt E. Platelet-derived chemokines in vascular biology. *Thromb. Haemost.* 2007; 97 (5): 704–713. DOI: 10.1160/th07-01-0066.
25. Stellos K., Sebastian Kopf S., Paul A., Marquardt J.U., Gawaz M., Huard J., Langer H.F. Platelets in regeneration. Seminars in thrombosis and hemostasis. *Thieme Medical Publishers*. 2010; 36 (02): 175–184. DOI: 10.1055/s-0030-1251502.
26. Gurtner G.C., Werner S., Barrandon Y., Longaker M.T. Wound repair and regeneration. *Nature.* 2008; 453 (7193): 314–321. DOI: 10.1038/nature07039.
27. Crowley S.T., Dempsey E.C., Horwitz K.B., Horwitz L.D. Platelet-induced vascular smooth muscle cell proliferation is modulated by the growth amplification factors serotonin and adenosine diphosphate. *Circulation*. 1994; 90 (4): 1908–1918. DOI: 10.1161/01.cir.90.4.1908.
28. Langer H.F., Stellos K., Steingen C. et al. Platelet derived bFGF mediates vascular integrative mechanisms of mesenchymal stem cells *in vitro*. *J. Mol. Cell. Cardiol*. 2009; 47 (2): 315–325. DOI: 10.1016/j.yjmcc.2009.03.011.
29. Denis J.F., Sader F., Gatien S., Villiard E., Anie Philip A., Roy S.. Activation of Smad2 but not Smad3 is required to mediate TGF-β signaling during axolotl limb regeneration. *Development*. 2016; 143 (19): 3481–3490. DOI: 10.1242/dev.131466.
30. Blavie L., Lazaryev A., Groffen J., Heisterkamp N,. DeClerck Y.A., Kaartinen V. TGF-β3-induced palatogenesis requires matrix metalloproteinases. *Molecular Biology of the Cell.* 2001; 12 (5): 1457–1466. DOI: 10.1091/mbc.12.5.1457.
31. Overall C.M., Wrana J.L., Sodek J. Transcriptional and post-transcriptional regulation of 72-kDa gelatinase – type IV collagenase by transforming growth factor-beta l in human fibroblasts Comparisons with collagenase and tissue inhibitor of matrix metalloproteinase gen expression. *J. Biol. Chem.* 1991; 266 (21): 14064–14071.
32. Chen Y., Zhang W., Geng N., Tian K.,Windsor L.J. MMPs, TIMP-2, and TGF-β1 in the cancerization of oral lichen planus. *Head Neck.* 2008; 30 (9): 1237–1245. DOI: 10.1002/hed.20869.
33. De Boer H.C., Verseyden C., Ulfman L.H., Zwaginga J.J., Bot I., Biessen E.A., Rabelink T.J., van Zonneveld A.J. Fibrin and activated platelets cooperatively guide stem cells to a vascular injury and promote differentiation towards an endothelial cell phenotype. *Arterioscler. Thromb. Vasc. Biol.* 2006; 26 (7): 1653–1659. DOI: 10.1161/01.ATV.0000222982.55731.f1.
34. Zernecke A., Schober A., Bot I., von Hundelshausen P., Liehn E.A., Möpps B., Mericskay M., Gierschik P., Biessen E.A., Weber C. SDF-1alpha/ CXCR4 axis is instrumental in neointimal hyperplasia and recruitment of smooth muscle progenitor cells. *Circ. Res.* 2005; 96 (7): 784–791. DOI: 10.1161/01.RES.0000162100.52009.38.
35. Lev E.I., Estrov Z., Aboulfatova K., Harris D., Granada J.F., Alviar C., Kleiman N.S., Dong J.F. Potential role of activated platelets in homing of human endothelial progenitor cells to subendothelial matrix. *Thromb. Haemost.* 2006; 96 (4): 498–504.
36. Askari A.T., Unzek S., Popovic Z.B., Goldman C.K., Forudi F., Kiedrowski M., Rovner A., Ellis S.G., Thomas J.D., Di Corleto P.E., Topol E.J., Penn M.S. Effect of stromal-cell-derived factor-1 on stem cell homing and tissue regeneration in ischemic cardiomyopathy. *Lancet.* 2003; 362 (9385): 697–703. DOI: 10.1016/S0140-6736(03)14232-8.
37. Li Y., Yu X.Y., Lin S.G., Li X.H., Zhang S., Song Y-H. Insulin-like growth factor 1 enhances the migratory capacity of mesenchymal stem cells. *Biochem. Biophys. Res. Commun.* 2007; 356 (3): 780–784. DOI: 10.1016/j.bbrc.2007.03.049.
38. Loyer X., Vion A.C., Tedgui A., Boulanger C.M. Microvesicles as cell–cell messengers in cardiovascular diseases. *Circulation Research*. 2014; 114 (2): 345–353. DOI: 10.1161/CIRCRESAHA.113.300858.

39. Raposo G., Stoorvogel W. Extracellular vesicles: exosomes, microvesicles and friends. *J. Cell Biol*. 2013; 200 (4): 373–383.DOI: 10.1083/jcb.201211138.
40. Forlow S.B., McEver R.P., Nollert M.U. Leukocyte-leukocyte interactions mediated by platelet microparticles under flow. *Blood*. 2000; 95 (4): 1317–1323. DOI: 10.1182/blood.V95.4.1317.004k30_1317_1323.
41. Gawaz M., Vogel S. Platelets in tissue repair: control of apoptosis and interactions with regenerative cells. *Blood.* 2013; 122 (15): 2550–2554. DOI: 10.1182/кровь-2013-05-468694.
42. Ahmad R., Menezes J., Knafo L., Ahmad A. Activated human platelets express Fas-L and induce apoptosis in Fas-positive tumor cells. *J. Leukoc. Biol*. 2001; 69 (1): 123–128.
43. André P., Nannizzi-Alaimo L., Prasad S. K., Phillips D.R. Platelet-derived CD40L: the switch-hitting player of cardiovascular disease. *Circulation.* 2002; 106 (8): 896–899. DOI: 10.1161/01.cir.0000028962.04520.01.
44. Crist S.A., Elzey B.D., Ludwig A.T., Griffith T.S., Stack J.B., Lentz S.R., Ratliff T.L. Expression of TNF-related apoptosis-inducing ligand (TRAIL) in megakaryocytes and platelets. *Exp. Hematol*. 2004; 32 (11): 1073–1081. DOI: 10.1016/j.exphem.2004.07.022.
45. Meyer T., Amaya M., Desai H., Robles-Carrillo L., Hatfield M., Francis J.L., Amirkhosravi A. Human platelets contain and release TWEAK. *Platelets*. 2010; 21 (7): 571–574. DOI: 10.3109/09537104.2010.512403.
46. Otterdal K., Smith C., Oie E., Pedersen T.M., Yndestad A., Stang E., Endresen K., Solum N.O., Aukrust P., Damås J.K. Platelet-derived LIGHT induces inflammatory responses in endothelial cells and monocytes. *Blood*. 2006; 108 (3): 928–935. DOI: 10.1182/кровь-2005-09-010629.
47. Nakamura T., Teramoto H., Ichihara A. Purification and characterization of a growth factor from rat platelets for mature parenchymal hepatocytes in primary cultures. *Proc. Natl. Acad. Sci. USA*. 1986; 83 (17): 6489–6493. DOI: 10.1073/pnas.83.17.6489.
48. Pakala R., Willerson J.T., Benedict C.R. Mitogenic effect of serotonin on vascular endothelial cells. *Circulation*. 1994; 90 (4): 1919–1926. DOI: 10.1161/01.cir.90.4.1919.
49. Hisano N., Yatomi Y., Satoh K. Akimoto S., Mitsumata M., Fujino M.A., Ozaki Y. Induction and suppression of endothelial cell apoptosis by sphingolipids: a possible *in vitro* model for cell-cell interactions between platelets and endothelial cells. *Blood.* 1999; 93 (12): 4293–4299.
50. Yushkov B.G., Klimin V.G., Tkachenko A.E., Dugina E.A. Structural homeostasis. M.: Kommentariy, 2019: 200 (in Russ.).
51. Blair P., Flaumenhaft R. Platelet alpha-granules: basic biology and clinical correlates. *Blood Rev*. 2009; 23 (4): 177–189. DOI: 10.1016/j.blre.2009.04.001.
52. Langer H.F., Gawaz M. Platelets in regenerative medicine. *Basic Res. Cardiol*. 2008; 103 (4): 299–307. DOI: 10.1007/s00395-008-0721-4.
53. Maloney J.P., Silliman C.C., Ambruso D.R., Wang J., Tuder R.M., Voelkel N.F. In vitro release of vascular endothelial growth factor during platelet aggregation. *Am. J. Physiol.* 1998; 275 (3 Pt 2): 1054–1061. DOI: 10.1152/ajpheart.1998.275.3.H1054.
54. Kobayashi T., Hamano K., Li T.S., Nishida M., Ikenaga S., Hirata K., Zempo N., Esato K. Angiogenesis induced by the injection of peripheral leukocytes and platelets. *J. Surg. Res*. 2002; 103 (2): 279–286. DOI: 10.1006/jsre.2001.6309.
55. Kisucka J., Butterfield C.E., Duda D.G., Eichenberger S.C., Saffaripour S., Ware J., Ruggeri Z.M., Jain R.K., Folkman J., Wagner D.D. Platelets and platelet adhesion support angiogenesis while preventing excessive hemorrhage. *Proc. Natl. Acad .Sci. USA.* 2006; 103 (4): 855–860. DOI: 10.1073/pnas.0510412103.
56. Rhee J.S., Black M., Schubert U., Fischer S., Morgenstern E., Hammes H.P., Preissner K.T. The functional role of blood platelet components in angiogenesis. *Thromb. Haemos*t. 2004; 92 (2): 394–402. DOI: 10.1160/TH03-04-0213.
57. Martínez C.E., Smith P.C., Alvarado V.A.P. The influence of platelet-derived products on angiogenesis and tissue repair: a concise update. *Front. Physiol*. 2015, 6: 290. DOI: 10.3389/fphys.2015.00290.
58. Banks R.E., Forbes M.A., Kinsey S.E., Stanley A., Ingham E., Walters C., Selby P.J. Release of the angiogenic cytokine vascular endothelial growth factor (VEGF) from platelets: significance for VEGF measurements and cancer biology. *Br. J. Cancer*. 1998; 77 (6): 956–964. DOI: 10.1038/bjc.1998.158.
59. Wartiovaara U., Salven P., Mikkola H., Lassila R., Kaukonen J., Joukov V., Orpana A., Ristimäki A., Heikinheimo M., Joensuu H., Alitalo K., Palotie A. Peripheral blood platelets express VEGF-C and VEGF which are released during platelet activation. *Thromb. Haemost.* 1998; 80 (1): 171–175.
60. Verheul H.M., Hoekman K., Luykx-de Bakker S., Eekman C.A., Folman C.C., Broxterman H.J., Pinedo H.M. Platelet: transporter of vascular endothelial growth factor. *Clin. Cancer Res.* 1997; 3(12 Pt 1): 2187–2190.
61. Li J.J., Huang Y.Q., Basch R., Karpatkin S. Thrombin induces the release of angiopoietin-1 from platelets. *Thromb. Haemost*. 2001; 85 (2): 204–206.
62. Italiano J.E., Richardson J.L., Patel-Hett S., Battinelli E., Zaslavsky A., Short S., Ryeom S., Folkman J., Klement G.L. Angiogenesis is regulated by a novel mechanism: pro- and antiangiogenic proteins are organized into separate platelet alpha granules and differentially released. *Blood*. 2008; 111(3): 1227–1233. DOI: 10.1182/кровь-2007-09-113837.
63. Klement G.L., Yip T.T., Cassiola F, Kikuchi L., Cervi D., Podust V., Italiano J.E., Wheatley E., Abou-Slaybi A.,

Bender E., Almog N., Kieran M.W., Folkman J. Platelets actively sequester angiogenesis regulators. *Blood.* 2009; 113 (12): 2835–2842. DOI: 10.1182/blood-2008-06-159541.
64. Jurasz P., Alonso D., Castro-Blanco S., Murad F., Radomski M.W. Generation and role of angiostatin in human platelets. *Blood.* 2003; 102 (9): 3217–3223. DOI: 10.1182/кровь-2003-02-0378.
65. O'Reilly M.S., Boehm T., Shing Y., Fukai N., Vasios G., Lane W.S., Flynn E., Birkhead J.R., Olsen B.R., Folkman J. Endostatin: an endogenous inhibitor of angiogenesis and tumor growth. *Cell*. 1997; 88 (2): 277–285. DOI: 10.1016/s0092-8674(00)81848-6.
66. Maione T.E., Gray G.S., Petro J., Hunt A.J., Donner A.L., Bauer S.I., Carson H.F., Sharpe R.J. Inhibition of angiogenesis by recombinant human platelet factor-4 and related peptides. *Science.* 1990; 247 (4938): 77–79. DOI: 10.1126/science.1688470.
67. Iruela-Arispe M.L., Bornstein P., Sage H. Thrombospondin exerts an antiangiogenic effect on cord formation by endothelial cells in vitro. *Proc. Natl. Acad Sci. USA*. 1991; 88 (11): 5026–5030. DOI: 10.1073/pnas.88.11.5026.
68. Kopp H.G., Hooper A.T., Broekman M.J., Avecilla S.T., Petit I., Luo M., Milde T., Ramos C.A., Zhang F.,Kopp T., Bornstein P., Jin D.K., Marcus A.J., Rafii S. Thrombospondins deployed by thrombopoietic cells determine angiogenic switch and extent of revascularization. *J. Clin. Invest*. 2006; 116 (12): 3277–3791. DOI: 10.1172/JCI29314.
69. Pipili-Synetos E., Papadimitriou E., Maragoudakis M.E. Evidence that platelets promote tube formation by endothelial cells on matrigel. *Br. J. Pharmacol.* 1998; 125(6): 1252–1257. DOI: 10.1038/sj.bjp.0702191.
70. Brill A., Dashevsky O., Rivo J., Gozal Y., Varon D. Platelet-derived microparticles induce angiogenesis and stimulate post-ischemic revascularization. *Cardiovasc Res.* 2005; 67(1): 30–38. DOI: 10.1016/j.cardiores.2005.04.007.
71. Lamalice L., Le Boeuf F., Huot J. Endothelial cell migration during angiogenesis. *Circ. Res.* 2007; 100(6): 782–794. DOI: 10.1161/01.RES.0000259593.07661.1 e.
72. Van Weel V., van Tongeren R.B., van Hinsbergh V.W., van Bockel J.H., Quax P.H. Vascular growth in ischemic limbs: a review of mechanisms and possible therapeutic stimulation. *Ann. Vasc. Surg*. 2008; 22 (4): 582–597. DOI: 10.1016/j.avsg.2008.02.017.
73. Tkachuk V.A., Plekhanova O.S., Beloglazova I.B., Parfenova E.V. The role of a multidomain structure of urokinase in regulating vascular growth and remodeling. *Ukr. Biochem. Journ.* 2013; 85 (6): 18–45 (in Russ.).
74. Dukhinova M.S., Ponomarev E.D. The role of platelets in neuroinflammatory diseases. Review. *Bulletin of Moscow University.* 2018; 73 (3): 125–131 (in Russ.).
75. Komissarova S.N. Regeneration of cortical neurons in experimental hemorrhagic stroke: the role of platelets and modulated microgravity effects: abstract of a thesis. M.; 2015: 25 (in Russ.).
76. Farrag T.Y., Lehar M., Verhaegen P., Carson K.A., Byrne P.J. Effect of platelet rich plasma and fibrin sealant on facial nerve regeneration in a rat model. *Laryngoscope*. 2007; 117(1): 157. DOI: 10.1097/01.mlg.0000249726.98801.77.
77. Sariguney Y., Yavuzer R., Elmas C., Yenicesu I., Bolay H., Atabay K. Effect of platelet-rich plasma on peripheral nerve regeneration. *J. Reconstr. Microsurg.* 2008; 24 (3): 159. DOI: 10.1055/s-2008-1076752.
78. Piskin A., Kaplan S., Aktas A., Ayyildiz M., Raimondo S., Alic T. et all. Platelet gel does not improve peripheral nerve regeneration: an electrophysiological, stereological, and electron microscopic study. *Microsurgery*. 2009; 29 (2): 144–153. DOI: 10.1002/micr.20599.
79. Kaplan S., Piskin A., Ayyildiz M., Aktas A., Koksal B., Ulkay M.B. et al. The effect of melatonin and platelet gel on sciatic nerve repair: an electrophysiological and stereological study. *Microsurgery.* 2011; 31 (4): 306. DOI: 10.1002/micr.20876.
80. Dagum A.B. Peripheral nerve regeneration, repair, and grafting. *J. Hand. Ther*. 1998; 11 (2): 111–117. DOI: 10.1016/s0894-1130(98)80007-0.
81. Galla T.J., Vedecnik S.V., Halbgewachs J., Steinmann S., Friedrich C., Stark G.B. Fibrin/Schwann cell matrix in poly-epsiloncaprolactone conduits enhances guided nerve regeneration. *Int. J. Artif. Organs*. 2004; 27 (2): 127–136. DOI: 10.1177/039139880402700208.
82. Povlsen B. A new fibrin seal in primary repair of peripheral nerves. *J. Hand. Surg*. (*Br.).* 1994; 19 (1): 43–47. DOI: 10.1016/0266-7681(94)90048-5.
83. Gao C., Ma S., Ji Y., Wang J.E., Li J. Siatic nerve regeneration in rats stimulated by fibrin glue containing nerve growth factor: an experimental study. *Injury*. 2008; 39 (12): 1414–1420. DOI: 10.1016/j.injury.2008.05.010.
84. Johnson P.J., Tatara A., Shiu A., Sakiyama-Elbert S.E. Controlled release of neurotrophin-3 and platelet-derived growth factor from fibrin scaffolds containing neural progenitor cells enhances survival and differentiation into neurons in a subacute model of SCI. *Cell Transplant*. 2010; 19 (1): 89–101. DOI: 10.3727/096368909X477273.
85. Willerth S.M., Rader A., Sakiyama-Elbert S.E. The effect of controlled growth factor delivery on embryonic stem cell differentiation inside fibrin scaffolds. *Stem. Cell. Res*. 2008; 1 (3): 205–218. DOI: 10.1016/j.scr.2008.05.006.
86. Wood M.D., Moore A.M., Hunter D.A., Tuffaha S., Borschel G.H., Mackinnon S.E. et al. Affinity-based release of glialderived neurotrophic factor from fibrin matrices enhances sciatic nerve regeneration. *Acta Biomater.* 2009; 5 (4): 959–968. DOI: 10.1016/j.actbio.2008.11.008.
87. Cho H.H., Jang S., Lee S.C., Jeong H.S., Park J.S., Han J.Y. et al Effect of neural-induced mesenchymal

stem cells and platelet-rich plasma on facial nerve regeneration in an acute nerve injury model. *Laryngoscope*. 2010; 120 (5): 907–913. DOI: 10.1002/lary.20860.
88. Lichtenfels M., Colome L., Sebben A.D., Braga-Silva J. Effect of platelet rich plasma and platelet rich fibrin on sciatic nerve regeneration in a rat model. *Microsurgery*. 2013; 33 (5): 383–390. DOI: 10.1002/micr.22105.
89. Elgazzar R.F., Mutabagani M.A., Abdelaal S.E., Sadakah A.A. Platelet rich plasma may enhance peripheral nerve regeneration after cyanoacrylate reanastomosis: a controlled blind study on rats. *Int. J. Oral. Maxillofac Surg*. 2008; 37 (8): 748–755. DOI: 10.1016/j.ijom.2008.05.010.
90. Emel E., Ergun S.S., Kotan D., Gursoy E.B., Parman Y., Zengin A. et al. Effects of insulin-like growth factor-I and platelet-rich plasma on sciatic nerve crush injury in a rat model. *J. Neurosurg*. 2011;114 (2): 522–528. DOI: 10.3171/2010.9.JNS091928.
91. Ding X.G., Li S.W., Zheng X.M., Hu L.Q., Hu W.L., Luo Y. The effect of platelet-rich plasma on cavernous nerve regeneration in a rat model. *Asian. J. Androl*. 2009; 11 (2): 215–221. DOI: 10.1038/aja.2008.37.
92. Godwin J.W., Pinto A.R., Rosenthal N.A. Macrophages are required for adult salamander limb regeneration. *Proceedings of the National Academy of Sciences.* 2013; 110 (23): 9415–9420. DOI: 10.1073/pnas.1300290110.
93. Lehenkari P.P., Kellinsalmi M., Näpänkangas J.P., Ylitalo K.V., Mönkkönen J.,Rogers, M.J., Azhayev A., Väänänen H.K., Hassinen I.E. Further Insight into Mechanism of Action of Clodronate: Inhibition of Mitochondrial ADP/ATP Translocase by a Nonhydrolyzable, Adenine-Containing Metabolite. *Molecular Pharmacology*. 2002; 61 (5): 1255–1262. DOI: 10.1124/mol.61.5.1255.
94. Frantz S., Hofmann U., Fraccarollo D., Schäfer A., Kranepuhl S., Hagedorn I., Nieswandt B., Nahrendor f M., Wagner H. Bayer B., Pachel C., Schön M., Kneitz S., Bobinger T., Weidemann F., Ertl G., Bauersachs J. Monocytes/macrophages prevent healing defects and left ventricular thrombus formation after myocardial infarction. *The FASEB Journal*. 2013; 27 (3): 871–881. DOI: 10.1096/fj.12-214049.
95. Van Amerongen M.J., Harmsen M.C., Rooijen N., Petersen A.H., van Luyn M.J.A. Macrophage depletion impairs wound healing and increases left ventricular remodeling after myocardial injury in mice. *The American Journal of Pathology*. 2007; 170 (3): 818–829. DOI: 10.2353/ajpath.2007.060547.
96. Goren I., Allmann N., Yogev N., Schürmann C., Linke A., Holdene M., Waisman A., Pfeilschifter J., Frank S. A transgenic mouse model of inducible macrophage depletion: effects of diphtheria toxin-driven lysozyme M-specific cell lineage ablation on wound inflammatory, angiogenic, and contractive processes. *The American Journal of Pathology*. 2009; 175 (1): 132–147. DOI: 10.2353/ajpath.2009.081002.
97. Martinez F.O., Sica A., Mantovani A., Locati M. Macrophage activation and polarization. *Front. Biosci*. 2008; 13: 453–461. DOI: 10.2741/2692.
98. Gensel J.C., Zhang B. Macrophage activation and its role in repair and pathology after spinal cord injury. *Brain Research.* 2015; 1619: 1–11. DOI: 10.1016/j.brainres.2014.12.045.
99. Wynn T.A., Chawla A., Pollard J.W. Macrophage biology in development, homeostasis and disease. *Nature*. 2013; 496 (7446): 445–455. DOI: 10.1038/nature12034.
100. Nickaeen N., Ghaisari J., Heiner M., Moein S., Gheisari Y. Agent-based modeling and bifurcation analysis reveal mechanisms of macrophage polarization and phenotype pattern distribution. *Sci. Rep*. 2019; 9 (1): 12764. DOI: 10.1038/s41598-019-48865-z.
101. Daley J.M., Brancato S.K., Thomay A.A., Reichner J.S., Albina J.E. The phenotype of murine wound macrophages. *J. Leukoc. Biol.* 2010; 87 (1): 59–67. DOI: 10.1189/jlb.0409236.
102. Pawelski H., Lang D., Reuter S. Interactions of monocytes and platelets: implication for life. *Frontiers in Bioscience*.2014; 6: 75–91. DOI: 10.2741/s416.
103. Mei J., Liu Y., Dai N., Favara M., Greene T., Jeyaseelan S., Poncz M., Lee J.S., Worthen G.S. CXCL5 regulates chemokine scavenging and pulmonary host defense to bacterial infection. *Immunity.* 2010; 33 (1): 106–117. DOI: 10.1016/j.immuni.2010.07.009.
104. Kluge A., Reuter G., Lee H., Ruch-Heeger B., Schauer R. Interaction of rat peritoneal macrophages with homologous sialidase-treated thrombocytes in vitro: biochemical and morphological studies. Detection of N-(O-acetyl) glycoloylneuraminic acid. *European Journal of* Cell Biology. 1992; 59 (1): 12–20.
105. Hortle E., Johnson K.E., Johansen M.D., Nguyen T., Shavit J.A., Britton W.J., Tobin D.M., Stefan H Oehlers S.H. Thrombocyte inhibition restores protective immunity to mycobacterial infection in Zebrafish. *The Journal of Infectious Diseases*. 2019; 220 (3): 524–534. DOI: 10.1093/infdis/jiz110.
106. Dixon D.A., Tolley N.D., Bemis-Standoli K., Martinez M.L., Weyrich A.S., Morrow J.D., Prescott S.M., Zimmerman G.A. Expression of COX-2 in platelet-monocyte interactions occurs via combinatorial regulation involving adhesion and cytokine signaling. *J. Clin. Invest*. 2006; 116 (10): 2727–2738. DOI: 10.1172/JCI27209.
107. Boehlen F., Clemetson K.J. Platelet chemokines and their receptors: what is their relevance to platelet storage and transfusion practice? *Transfus. Med*. 2001; 11 (6): 403–417. DOI: 10.1046/j.1365-3148.2001.00340.x.
108. Petrucci G., de Cristofaro R., Rutella S., Ranelletti F.O., Pocaterra D., Lancellotti S., Habib A., Patrono C., Rocca B. Prostaglandin E2 differentially modulates human platelet function through the prostanoid EP2 and EP3 receptors. *J. Pharmacol. Exp. Ther*. 2011; 336 (2): 391–402. DOI: 10.1124/jpet.110.174821.

109. Passacquale G., Vamadevan P., Pereira L., Hamid C., Corrigall V., Ferro A. Monocyte-platelet interaction induces a pro-inflammatory phenotype in circulating monocytes. *PLoS One*. 2011; 6 (10): 25595. DOI: 10.1371/journal.pone.0025595.
110. Stephen J., Emerson B., Fox K.A., Dransfield I. The uncoupling of monocyte-platelet interactions from the induction of proinflammatory signaling in monocytes. *J. Immunol*. 2013; 191 (11): 5677–5683. DOI: 10.4049/jimmunol.1301250.
111. Scull C.M., Hays W.D., Fischer T.H. Macrophage pro-inflammatory cytokine secretion is enhanced following interaction with autologous platelets. *J. Inflamm. (Lond*.). 2010; 7: 53–58. DOI: 10.1186/1476-9255-7-53.
112. Vieira-de-Abreu A., Campbell R.A., Weyrich A.S., Zimmerman G.A. Platelets: versatile effector cells in hemostasis, inflammation, and the immune continuum. *Semin. Immunopathol*. 2012; 34 (1): 5–30. DOI: 10.1007/s00281-011-0286-4.
113. Mause S.F., von Hundelshausen P., Zernecke A., Koenen R.R., Weber C. Platelet microparticles: a transcellular delivery system for RANTES promoting monocyte recruitment on endothelium. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2005; 25 (7): 1512–1518. DOI: 10.1161/01.ATV.0000170133.43608.37.
114. Marfaing-Koka A., Maravic M., Humbert M., Galanaud P., Emllie D. Contrasting effects of IL-4, IL-10 and corticosteroids on RANTES production by human monocytes. *International Immunology*. 1996; 8 (10): 1587–1594. DOI: 10.1093/intimm/8.10.1587.
115. Corken A., Russell S., Dent J., Post S.R., Ware J. Platelet glycoprotein Ib-IX as a regulator of systemic inflammation. *Arterioscler. Thromb. Vasc. Biol.* 2014; 34 (5): 996–1001. DOI: 10.1161/ATVBAHA.113.303113.
116. Langer H.F., Daub K., Braun G., Schönberger T., May A.E., Schaller M., Stein G.M., Stellos K., Bueltmann A., Siegel-Axel D., Wendel H.P., Aebert H., Roecken M., Seizer P., Santoso S., Wesselborg S., Brossart P., Gawaz M. Platelets recruit human dendritic cells via Mac-1/JAM-C interaction and modulate dendritic cell function *in vitro*. *Arterioscler. Thromb. Vasc. Biol.* 2007; 27 (6): 1463–1470. DOI: 10.1161/ATVBAHA.107.141515.
117. Hagihara M., Higuchi A., Tamura N., Ueda Y., Hirabayashi K., Ikeda Y., Kato S., Sakamoto S., Hotta T., Handa S., Goto S. Platelets, after exposure to a high shear stress, induce IL-10-producing, mature dendritic cells *in vitro*. *J. Immunol.* 2004; 172 (9): 5297–5303. DOI: 10.4049/jimmunol.172.9.5297.
118. Vasina E., Cauwenberghs S., Feijge M., Heemskerk J., Weber C., Koenen R. Microparticles from apoptotic platelets promoter sident macrophage differentiation. *Cell Death Dis.* 2011; 2 (9): 211. DOI: 10.1038/cddis.2011.94.

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Pulmonary embolism with comorbid acute myocardial infarction: a clinical case

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ABSTRACT

The article presents a case of pulmonary embolism with comorbid acute inferior myocardial infarction in the 54-year-old patient who was admitted to the Regional Center for Percutaneous Coronary Interventions. Coronary angiography revealed a multivessel lesion with angiographic signs of instability in the proximal third of the right coronary artery. Pulmonary angiography revealed signs of pulmonary embolism with moderate impairment of pulmonary perfusion.

The described combination is challenging in terms of both diagnosis and subsequent treatment strategy. A feature of this case is the use of a double surgery, consisting of revascularization of the infarct-related artery and fragmentation of thrombotic masses in the pulmonary artery, in combination with thrombolytic therapy.

Key words: acute myocardial infarction, pulmonary embolism, thrombolysis, coronary angiography, pulmonary angiography.

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Тромбоэмболия легочной артерии в сочетании с острым инфарктом миокарда: клинический случай

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РЕЗЮМЕ

Представлен случай сочетания тромбоэмболии легочной артерии и острого нижнего инфаркта миокарда у 54-летнего пациента, который поступил в региональный центр по проведению чрескожных коронарных вмешательств. При выполнении коронароангиографии обнаружено многососудистое поражение с ангиографическими признаками нестабильности в проксимальной трети правой коронарной артерии, при ангиопульмонографии – картина тромбоэмболии легочной артерии с нарушением перфузии легких средней степени тяжести.

Описанное сочетание представляет собой трудную задачу как с точки зрения диагностики, так и последующей лечебной тактики. Особенностью данного случая является применение двойного интервенционного вмешательства, состоящего из реваскуляризации инфаркт-зависимой артерии и фрагментации тромботических масс в легочной артерии в сочетании с тромболитической терапией.

Ключевые слова: острый инфаркт миокарда, тромбоэмболия легочной артерии, тромболизис, ангиография коронарных сосудов, ангиопульмонография.

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

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

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INTRODUCTION

Pulmonary embolism (PE), having no clinical specificity, is one of the diseases that require differential diagnosis, including differential diagnosis with acute myocardial infarction (AMI). Treatment of patients with a combination of these diseases is particularly difficult [1]. An example of successful application of the interventional strategy in managing a patient with pulmonary embolism with comorbid AMI is described below.

CLINICAL CASE

A 54-year-old patient was delivered by an ambulance team to the Regional Center for Percutaneous Coronary Interventions with the diagnosis of acute coronary syndrome with ST-segment elevation. He had considered himself sick for 1.5 hours, when severe weakness, shortness of breath, and tightness in the chest with loss of consciousness appeared for the first time in his life.

He had a history of smoking up to one pack of cigarettes a day and did not measure blood pressure. There were no other risk factors for cardiovascular diseases. Consciousness was clear. The skin was of a normal color. Body mass index (BMI) was 26 kg / m^2. There was no edema on the lower extremities. Breathing was adequate, with a rate of 16 breaths per minute. Upon auscultation, vesicular breathing was noted, both lungs were clear, SpO_2 90%. The heart tones were rhythmic and muffled; no heart murmurs were detected. Blood pressure in both arms was 120 / 80 mm Hg, the heart rate was 100 beats per minute (against the background of a dopamine infusion at a dose of 3 mcg / kg / min). The abdomen was soft and painless. The lower edge of the liver was at the border of the costal arch. When examining other organs and systems, no pathological abnormalities were detected.

Laboratory findings: troponin I and creatine phosphokinase-MB (CPK-MB) were negative upon admission to the hospital (upon repeated examination after 12 hours, the upper limit of normal values was exceeded by 10 times), D-dimer upon admission was > 8,000 ng / ml.

The electrocardiogram showed ST segment elevation in aVL of 1 mm, ST segment depression in II, III, and V_{3-6} of up to 2 mm, and right bundle branch block (Fig. 1). Coronary angiography revealed arteriosclerosis, calcification of the coronary arteries; stenosis

of the left main coronary artery before bifurcation of more than 65%; stenosis of the proximal third of the anterior interventricular branch of more than 70%; stenosis of the middle third of the anterior interventricular branch of about 60%; stenosis of the orifice of the diagonal branch of more than 40%, stenosis of the orifice and the proximal third of the intermediate artery of about 50%; stenosis of the proximal third of the right coronary artery (RCA) of more than 85% with angiographic signs of instability; and angiographic signs of mural thrombosis of the middle third of the RCA (Fig. 2).

Stenting of critical stenosis of the proximal third and thrombosis-affected area of the middle third of the RCA was performed with a good angiographic result (Fig. 3).

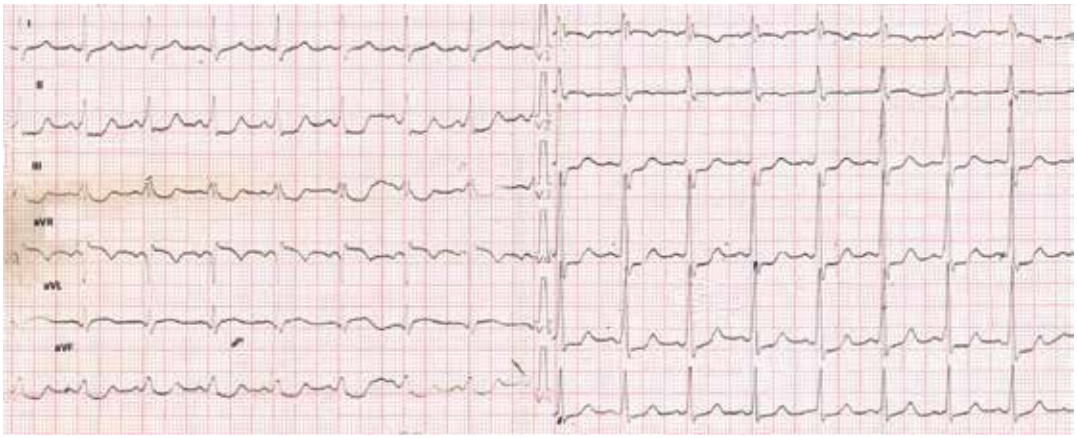

Fig. 1. Electrocardiogram of the patient upon admission: speed – 25 mm / sec, voltage – 10 mm

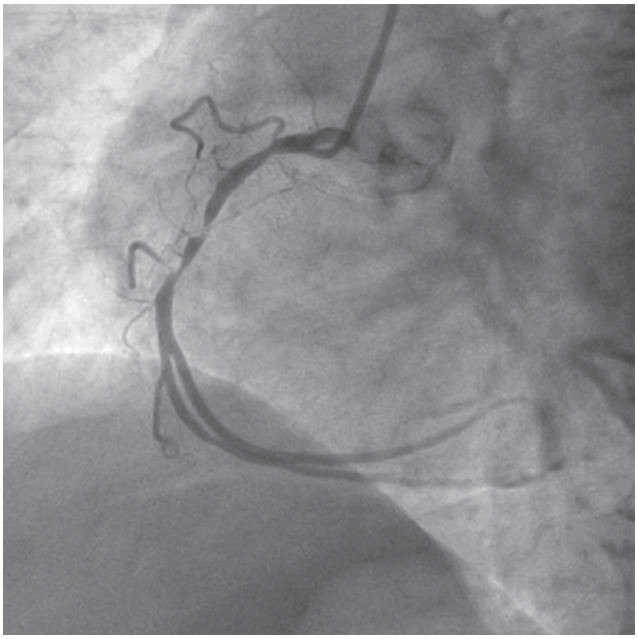

Fig. 2. Image of the RCA obtained during coronary angiography

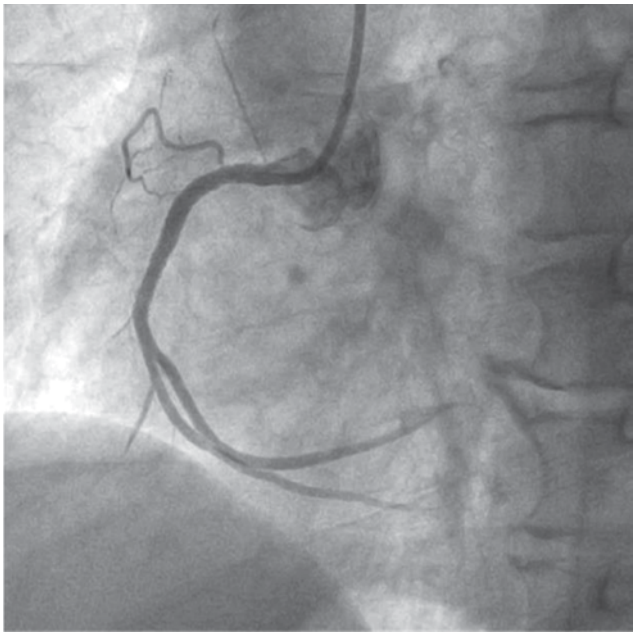

Fig. 3. Image of the RCA after stenting

After stenting, taking into account the features of the clinical presentation and the results of D-dimer determination, it was decided to perform pulmonary angiography, which revealed signs of pulmonary embolism (mainly on the right) of moderate severity (Miller index 19) (Fig. 4). Mechanical fragmentation of the thrombus with selective thrombolysis (alteplase – 15 mg in 15 minutes) and subsequent systemic administration of a thrombolytic (alteplase – 85 mg in 1.5 hours) were performed (Fig. 5).

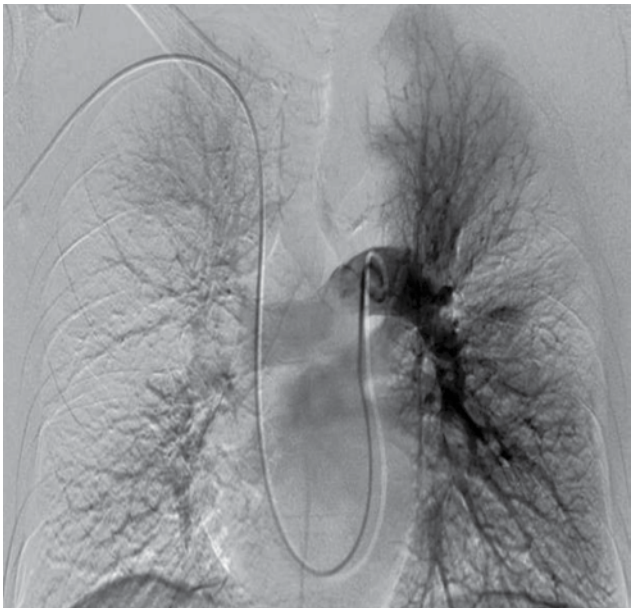

Fig.4. Pulmonary angiography image

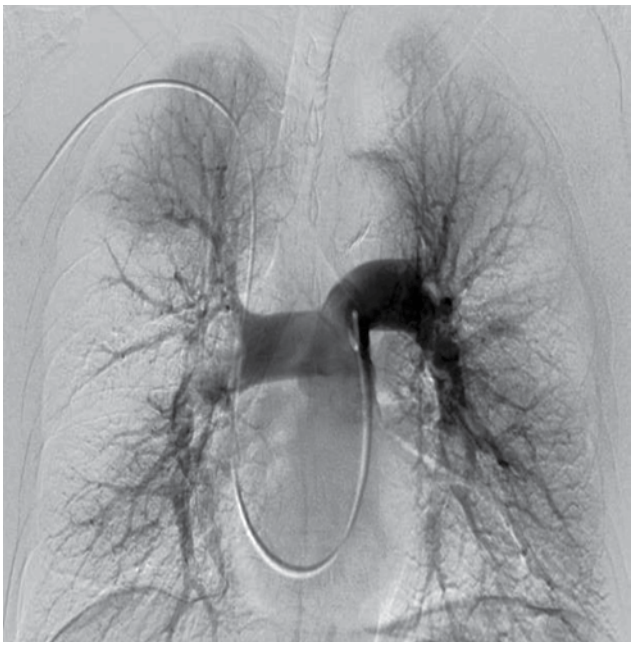

Fig. 5. Pulmonary arteries after fragmentation of thrombotic masses and selective thrombolysis

In echocardiography (after percutaneous coronary intervention (PCI)), dilation of the right ventricle (RV) and left ventricle (LV) cavities, aortic dilation at the level of the sinus of Valsalva, asymmetric hypertrophy of the LV myocardium (posterior wall – 0.9–1.3 cm, interventricular septum – 1.2–1.5 cm), hypokinesis of the posterior LV wall, and LV ejection fraction of 47% were detected.

Ultrasound examination of the lower extremities (after PCI) revealed thrombotic masses with signs of recanalization of up to 20% in one of the posterior tibial veins on the right side; mural thrombotic masses with signs of recanalization of up to 50% in the deep femoral vein; and thrombotic masses with signs of recanalization of up to 50% in the sural veins on the left side.

The postoperative period was uneventful. On the 14th day, the patient was discharged for further treatment in an outpatient setting with a recommendation to continue double antiplatelet therapy in combination with dabigatran under the supervision of a cardiologist and vascular surgeon.

DISCUSSION

Three types of a combination of AMI and PE are described in the literature: PE as a complication of AMI; AMI resulting from paradoxical embolism with a functioning foramen ovale, combined with PE; and AMI as a complication of PE [2–4]. We believe that in the case under discussion, the third variant is most likely to develop against the background of critical stenosis of RCA, which is consistent with the 4th Universal definition of myocardial infarction [5].

The following reasons for ST segment elevation in PE are suggested: 1) true myocardial ischemia (occlusion, embolism, atherosclerotic plaque); 2) insufficiency of coronary artery blood flow due to an acute increase in the right ventricular afterload [6]; 3) transmural ischemia of the right ventricle due to hypotension, hypoxemia, pulmonary arterial hypertension, and hypercatecholaminemia [7]; 4) compression of the coronary arteries by a dilated pulmonary artery due to developmental abnormalities [8, 9].

In addition to the difficulty in diagnosing such a combination of diseases, there are also difficulties in determining the most optimal treatment strategy. In most of the described cases, systemic thrombolysis was used, which was performed at various time intervals, due to delayed diagnosis of PE by contrast enhanced computed tomography (CT). There is a description of a clinical case using a double intervention [10].

According to the latest European recommendations for the diagnosis and management of patients with acute pulmonary embolism, pulmonary angiography is indicated for elective invasive percutaneous treatment of PE [11]. In our case, taking into account the patient's being in the catheter laboratory, in order to minimize time loss associated with patient's

transportation, it was decided to perform an emergency pulmonary angiography with subsequent intervention.

CONCLUSION

The given clinical example demonstrates the complexity of diagnosing and treating a patient with a combination of life-threatening conditions. In the presence of initial hypotension (shock) with an impossibility to perform immediate CT angiography, bedside echocardiography is a method for verifying the diagnosis of high-risk PE, the results of which will allow to differentiate PE and AMI in the shortest possible time [11].

REFERENCES

1. Omar H.R., Mangar D., Camporesi E.M. Acute pulmonary embolism masquerading as inferior myocardial infarction. *Am. J. Emerg. Med*. 2015; 33 (4): 580–581. DOI: 10.1016/j.ajem.2014.11.036.
2. O'Sullivan C.J., Magarzo J.G.A., Bernheim A.M. et al. Paradoxical embolism via a sinus venosus atrial septal defect causing an inferior ST-segment elevation myocardial infarction in a 23-year-old woman. *BMJ CaseRep*. 2016; 2016: bcr2016215184. DOI: 10.1136/bcr-2016-215184.
3. Tukayea D.N., Cavallazzib R.S. Paradoxical thromboembolism/ST-elevation myocardial infarction via a patent foramen ovale in sub-massive pulmonary embolism following an upper extremity deep venous thrombosis: is it time for a change in the standard of care? *Cardiol. Res*. 2014; 5 (3–4): 112–117. DOI: 10.14740/cr335w.
4. Jolobe O.M.P. The differential diagnosis of acute pulmonary embolism and ST segment inferior myocardial infarction. *Am. J. Emerg. Med*. 2015; 33 (5):723. DOI: 10.1016/j.ajem.2015.02.016.
5. Thygesen K., Alpert J.S., Jaffe A.S., Chaitman B.R., Bax J.J., Morrow D.A., White H.D., ESC Scientific Document Group. Fourth universal definition of myocardial infarction (2018). *European Heart Journal*. 2018; 40 (3): 237–269. DOI: 10.1093/eurheartj/ehy462.
6. Lu Y.-W., Tsai Y.-L., Chang C.-C., Huang P.-H.. A potential diagnostic pitfall in acute chest pain: Massive pulmonary embolism mimicking acute STEMI. *Am. J. Emerg. Med.* 2018; 36 (3): 461–463. DOI: 10.1016/j.ajem.2017.11.046.
7. Zhan Z.-Q., Wang C.-Q., Baranchuk A. Acute pulmonary embolism with ST-segment elevation in the inferior leads. *Int. J. Cardiol.* 2014; 177 (2): 718–719. DOI: 10.1016/j.ijcard.2014.10.017.
8. Shishido K., Moriyama N., Shimizu T., Saito Sh. Anomalous acute left main myocardial infarction due to compression between pulmonary artery and aorta by acute pulmonary thromboembolism. *JACC: Cardiovascular interventions.* 2016; 9 (23): e227–228. DOI: 10.1016/j.jcin.2016.09.045.
9. Takeda N., Ohtaki E., Kitahara K. et al. Pulmonary thromboembolism in a patient with an anomalous right coronary artery as a cause of unusual biventricular myocardial infarction. *Circ. J.* 2004; 68 (9): 883–886. DOI: 10.1253/circj.68.883.
10. Smith G.J, Koul S., Roijer A. et al. Acute right ventricular failure caused by concomitant coronary and pulmonary embolism: successful treatment with endovascular coronary and pulmonary thrombectomy. *European Heart Journal: Acute Cardiovascular Care*. 2013; 2 (2): 131–136. DOI: 10.1177/2048872613486336.
12. Konstantinides S.V., Meyer G., Becattini C. et al. 2019 ESC Guidelines for the diagnosis and management of acute pulmonary embolism developed in collaboration with the European Respiratory Society (ERS). *Eur. Heart J.* 2020; 41(4): 543–603. DOI: 10.1093/eurheartj/ehz405.

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A rare case of a metastatic neuroendocrine tumor of the pancreas

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ABSTRACT

Aim. To study a rare sporadic case of metastatic gastrinoma associated with mutations in the *MEN1* and *TSC2* genes in a 25-year-old male.

Materials and methods. A retrospective analysis of the history of a 25-year-old patient with sporadic gastrinoma with a highly aggressive clinical course and high metastatic potential was performed. Sequencing of the DNA extracted from the surgical tumor biopsy was performed on the Illumina NextSeq 550 sequencer (Illumina Inc., USA) with the mean coverage of at least 100× using the AmpliSeq target panel for Illumina Comprehensive Cancer Panel for studying exons of 409 genes, mutations in which are associated with oncopathology.

Results. The article presents the results of complex diagnosis and treatment of metastatic gastrinoma using modern locoregional therapy and drugs from the group of somatostatin analogues. Using next generation sequencing and Sanger sequencing, sporadic mutations in the *MEN1* and *TSC2* genes with pronounced clinical significance were identified in the extracted DNA.

Conclusion. The identified mutations, being the drivers of the tumor process, apparently determined the atypical development of the presented clinical case – the sporadic Zollinger – Ellison syndrome. Complete morphological and immunohistochemical validation of the neuroendocrine tumor before treatment determined a successful treatment strategy, including the use of somatostatin analogues in adjuvant and neoadjuvant therapies in combination with chemoembolization of hepatic metastases.

Key words: neuroendocrine tumor, Zollinger – Ellison syndrome, next generation sequencing, metastases.

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Редкий случай метастатической нейроэндокринной опухоли поджелудочной железы

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РЕЗЮМЕ

Цель. Изучение редкого спорадического случая метастатической гастриномы, ассоциированной с мутациями в генах *MEN1* и *TSC2*, у мужчины 25 лет.

Материалы и методы. Был проведен ретроспективный анализ истории болезни пациента 25 лет с наличием спорадической гастриномы с высоко агрессивным клиническим течением и высоким метастатическим потенциалом. Секвенирование ДНК, экстрагированной из операционного биоптата опухоли, проводили на секвенаторе Illumina NextSeq 550 (Illumina Inc., США) со средним покрытием не менее 100× с применением таргетной панели AmpliSeq Comprehensive Cancer Panel for Illumina для исследования экзонных регионов 409 генов, мутации в которых ассоциированы с онкопатологией.

Результаты. Представлен результат комплексной диагностики и успешного лечения метастатической гастриномы с применением современных локорегиональных методов лечения и препаратов из группы аналогов соматостатина. С помощью секвенирования нового поколения и секвенирования по Сэнгеру в экстрагированной ДНК были выделены спорадические мутации в генах *MEN1* и *TSC2* с выраженным клиническим значением.

Заключение. Идентифицированные мутации, являясь драйверами опухолевого процесса, очевидно, определили нетипичное развитие представленного клинического случая – спорадического синдрома Золлингера – Эллисона. Полная морфологическая и иммуногистохимическая верификация нейроэндокринной опухоли до начала лечения определила его успешную тактику, включавшую применение аналогов соматостатина в адъювантном и неоадъювантном режимах в сочетании с химиоэмболизацией печеночных метастазов.

Ключевые слова: нейроэндокринная опухоль, синдром Золлингера – Эллисона, секвенирование нового поколения, метастазы.

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

At the annual meeting of the American Surgical Association in Philadelphia on April 29, 1955, Robert Zollinger and Edwin Ellison were the first to present the relationship between non-insulin-secreting tumors of the pancreas and hypersecretion of hydrochloric acid in the stomach. Due to the ability to produce a large amount of gastrin, the tumor became known as gastrinoma. Clinical manifestations of the disease are included in the Zollinger – Ellison syndrome (ZES), although these terms are currently used as synonyms. It has been only 65 years since the first description of the syndrome, but during this time, more than 3,300 articles have been published, and unique clinical data of more than 1,000 patients have been presented. It has been established that the annual incidence of the disease is 1 case per 1 million population [1].

The initial manifestations of the disease usually appear at the age of 30–60 years. The onset of manifestations is associated with the tumor status – sporadic or hereditary cancer [2]. ZES includes ectopic secretion of gastrin by a neuroendocrine tumor, gastrinoma, primarily localized in the duodenum (60–80%) or pancreas (10–40%). The result is hypersecretion of hydrochloric acid and subsequent development of gastroesophageal reflux disease and ulcer, which are normally resistant to classical drug therapy [3].

In 30% of patients with multiple neuroendocrine neoplasia type 1 (*MEN1*), a pancreatic neuroendocrine tumor (pancreatic NET) is diagnosed, and gastrinoma in this case develops exclusively before the age of 20 and is more often localized in the duodenum [4, 5]. The treatment strategy and the outcome for sporadic gastrinomas and gastrinomas associated with *MEN1* are different. Distinctive features of the latter include their small size, multifocal growth, and high metastatic potential. The main method of treatment is surgical, but its results are still characterized by a low rate of favorable outcomes. Signs of biochemical recurrence are present in more than 95% of patients within 3 years after surgery [6].

It should be noted that a combination of ZES with other hereditary syndromes is an extremely rare phenomenon. The literature describes only one case of gastrinoma in a 34-year-old man who was diagnosed with tuberous sclerosis in childhood without a family history of the condition. At the time of diagnosis, multiple metastases were found in the liver, lungs, and spine. Being inoperable, the patient died 6 months later [7]. Early clinical manifestations and an aggressive course of the disease are not typical of sporadic gastrinomas. In this regard, the case of pancreatic gastrinoma without a family history of the disease with early development of ZES and the presence of sporadic mutations in the *MEN1* and *TSC2* genes is of particular interest.

CLINICAL CASE

In September 2018, patient A., 25 years old, went to a general practitioner in the place of residence with complaints of general weakness, dizziness, abdominal pain in the anticardium arising after eating, and nagging pain in the right hypochondrium. The performed esophagogastroduodenofibroscopy helped to detect an ulcerative lesion in the cardiac region of the stomach and erosive duodenitis. Magnetic resonance imaging (MRI) of the abdominal organs showed a neoplasm in the tail of the pancreas (45 × 55 × 42 mm) and similar lesions in S1 (14 × 20 mm) and S7 (16 × 16 × 10 mm) segments of the liver (Fig. 1).

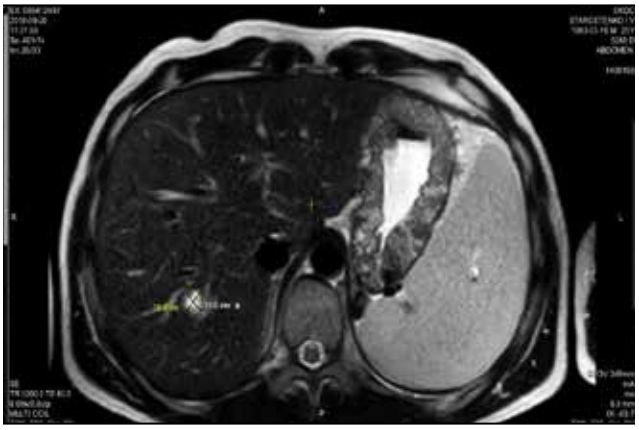

Fig. 1. MRI of the abdominal organs. Visualization of a metastatic focus in the liver parenchyma

For further examination and treatment, patient A. was referred to the National Medical Research Center for Oncology (NMRCO), Rostov-on-Don (former Rostov Research Institute of Oncology – RRIO). The morphological study of the biopsy material from the pancreas and liver made it possible to verify the neuroendocrine tumor in the tail of the pancreas. According to the immunohistochemical study, strong positive reactions were determined with the classical markers of the neuroendocrine phenotype: chromogranin A (CgA), synaptophysin, Ki-67 = 10% (which corresponds to a highly differentiated G2 pancreatic NET, according to the classification of the World Health Organization (2017)). In addition to the mandatory minimum set of markers, an expanded panel of markers to determine expression of a range of hormones was used. It identified high levels of gastrin expression. Besides, somatostatin receptors type 2A were present in large numbers on the surface of primary cancer cells.

Therefore, according to the results of histological and immunohistochemical studies, a functionally active pancreatic NET – gastrinoma – with clinical presentation of ZES was verified. The diagnosis of pT-3N1M1 (hep), stage IV neuroendocrine tumor of the tail of the pancreas with regional lymph node and liver metastases was established.

TREATMENT

The NMRCO board of the NMRCO board of doctors recommended to carry out surgical treatment including corporocaudal resection of the pancreas with

splenectomy and atypical resection of the S1 and S7 segments of the liver.

As preoperative preparation, the patient was prescribed lanreotide, which decreased CgA in the blood serum from 500 to 34 nmol / l. After 1 month of taking lanreotide, the surgery was performed. Laparotomy visualized a tumor in the tail of the pancreas, growing to the splenic hilum up to 1.5 cm in diameter (Fig. 2). The tumor conglomerate included the splenic artery and vein and was tightly adjacent to the left lateral semicircle of the superior mesenteric and portal veins. The splenic artery was tumor-free. Enlarged paracaval lymph nodes were identified. Mobilization of the ligament of Treitz and transsection of the peritoneum above the mesentery of the small intestine visualized the superior mesenteric vein. It was dorsomedial to the tumor. The surgery included corporocaudal resection of the pancreas, splenectomy, resection of the mesentery of the large intestine, perirenal fat, and left adrenal gland. Atypical resection of the S1 and S7 segments of the liver was performed.

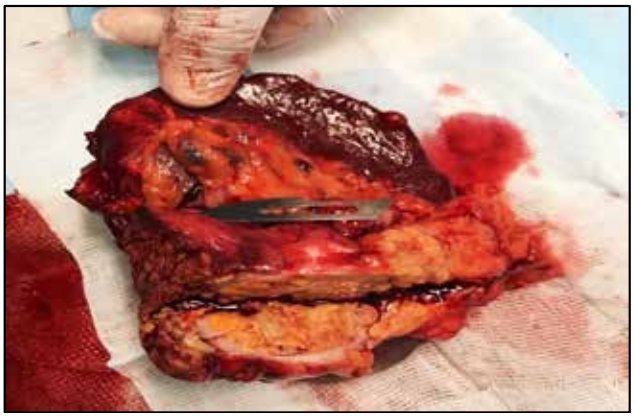

Fig. 2. Gross specimen of the tumor in the tail of the pancreas

In the postoperative period, the patient continued to receive lanreotide, against the background of which the progression of the disease was recorded 3 months after the surgery: elective MRI showed a neoplasm in the liver S7 segment, 12 × 11 × 10 mm in size (Fig. 3).

The NMRCO board of doctors recommended to carry out transarterial chemoembolization (TACE) of the liver using lipiodol-cisplatin emulsion. Considering the progression of the metastatic process in the liver, it was decided to add chemotherapy to the ongoing treatment according to the GEMOX scheme.

The patient was included in the study of the genetic profile of pancreatic NET by the method of massively parallel sequencing (next generation sequencing, NGS). Sequencing of DNA extracted from the surgical biopsy of the tumor was performed on the Illumina NextSeq 550 sequencer (Illumina Inc., USA) with an average coverage of at least 100 × using the AmpliSeq ™ target panel for Illumina Comprehensive Cancer Panel ® to study exon regions of 409 genes, mutations in which are associated with oncopathology. Analytical sensitivity for mutation detection was 5%. The pathogenicity of the identified nucleotide substitutions was assessed according to the recommendations of the American College of Medical Genetics and Genomics (ACMG) and Association of Molecular Pathology (AMP)[8].

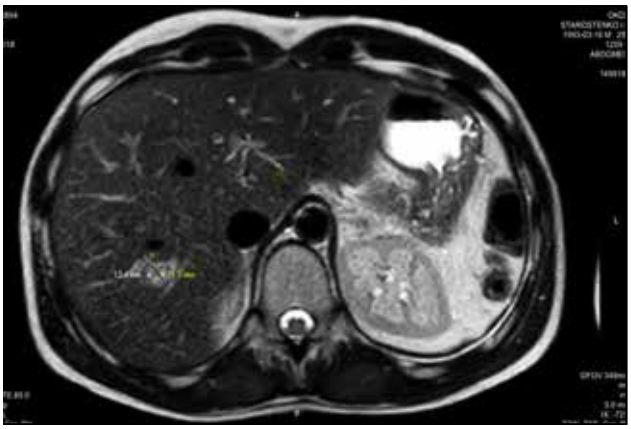

Fig. 3. MRI of the abdominal organs 3 months after the corporoduodenal resection of the pancreas and atypical liver resection with signs of the disease progression in the liver S7 segment

In the studied DNA sample, 1,041 variants of nucleotide sequences were found, two of which, in the *MEN1* and *TSC2* genes, were identified as pathogenic mutations with strong clinical significance according to the ACMG and AMP classification (Fig. 5). A frameshift mutation in the *MEN1* gene at c.248delT (p.Leu83ArgfsTer36) was described in the studies on pancreatic tumors [9, 10]. The detected variant was represented by a mosaic form (variant allele frequency (VAF) was 12.6%). The mutation c.337-1G>A (rs45517105), presented in the mosaic form (VAF = 7.6%) and associated with tuberous sclerosis, was found at the 3' acceptor splice site of intron 4 of the *TSC2* gene [11].

The identified pathogenic mutations were subsequently verified by direct Sanger sequencing. Their presence in the tumor and their absence in the blood leukocytes were confirmed. Thus, the diagnosis of sporadic gastrinoma with ZES was confirmed. Cur-

rently, the patient is under medical supervision with no signs of disease progression.

CONCLUSION

The presented case of sporadic gastrinoma is of high scientific value. The rarity of this type of neuroendocrine pancreatic tumor is combined here with its high malignant and metastatic potential and development of a complete clinical picture of ZES against the background of a fairly young age of the patient. Early development of gastrinoma is usually associated with the presence of hereditary syndromes, the manifestations of which were absent in the patient. However, using NGS and direct Sanger sequencing, sporadic mutations in the *MEN1* and *TSC2* genes with a strong clinical significance were detected, typically associated with development of multiple endocrine neoplasia type 1 (MEN1) and tuberous sclerosis, respectively. Obviously, the combination of two pathogenic mutations predetermined high aggressiveness of the disease.

Emerging new methods of locoregional therapy for hepatic metastases in NET, such as TACE, made it possible to select a personalized treatment strategy and exclude its aggressive impact on the entire liver parenchyma. High efficiency of this method is proven by the absence of disease progression in the described patient within 16 months of follow-up after TACE.

REFERENCES

1. Ellison E.C., Johnson J.A. The Zollinger – Ellison syndrome: A comprehensive review of historical, scientific, and clinical considerations. *Curr. Probl. Surg.* 2009; 46 (1): 13–106. DOI: 10.1067/j.cpsurg.2008.09.001.
2. Shao Q., Zhao B., Dong L., Cao H., Wang H. Surgical management of Zollinger – Ellison syndrome: Classical considerations and current controversies. *World J. Gastroenterol*. 2019; 25 (32): 4673–4681. DOI: 10.3748/wjg.v25.i32.4673.
3. Ito T., Cadiot G., Jensen R.T. Diagnosis of Zollinger – Ellison syndrome: Increasingly difficult. *World J. Gastroenterol.* 2012; 18 (39): 5495–5503. DOI: 10.3748/wjg.v18.i39.5495.
4. Stevenson M., Lines K.E., Thakker R.V. Molecular Genetic Studies of Pancreatic Neuroendocrine Tumors: New Therapeutic Approaches. *Endocrinol. Metab. Clin. N. Am.* 2018; 47 (3): 525–548. DOI: 10.1016/j.ecl.2018.04.007.
5. Albers M.B., Manoharan J., Bartsch D.K. Contemporary surgical management of the Zollinger – Ellison syndrome in multiple endocrine neoplasia type 1. *Best Pract. Res. Clin. Endocrinol. Metab.* 2019; 33 (5): 101–318. DOI: 10.1016/j.beem.2019.101318.
6. Norton J.A., Foster D.S., Ito T., Jensen R.T. Gastrinomas. Medical or surgical treatment. *Endocrinol. Metab. Clin. N. Am.* 2018; 47 (3): 577–601. DOI: 10.1016/j.ecl.2018.04.009.
7. Schwarzkopf G., Pfisterer J. Metastasizing gastrinoma and tuberous sclerosis complex. Association or coincidence? *Zentralbl. Pathol.* 1994; 139 (6): 477–481.
8. Li M.M., Datto M., Duncavage E.J., Kulkarni S., Lindeman N.I., Roy S., Tsimberidou A.M., Vnencak-Jones C.L., Wolff D.J., Younes A., Nikiforova M.N. Standards and guidelines for the interpretation and reporting of sequence variants in cancer: A Joint Consensus Recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists. *J. Mol. Diagn*. 2017; 19 (1): 4–23. DOI: 10.1016/j.jmoldx.2016.10.002.
9. Gleeson F.C., Voss J.S., Kipp B.R., Kerr S.E., van Arnam J.S., Mills J.R., Marcou C.A., Schneider A.R., Tu Z.J., Henry M.R., Levy M.J. Assessment of pancreatic neuroendocrine tumor cytologic genotype diversity to guide personalized medicine using a custom gastroenteropancreatic next-generation sequencing panel. *Oncotarget*. 2017; 8 (55): 93464–93475. DOI: 10.18632/oncotarget.18750.
10. Jiao Y., Yonescu R., Offerhaus G.J., Klimstra D.S., Maitra A., Eshleman J.R., Herman J.G., Poh W., Pelosof L., Wolfgang C.L., Vogelstein B., Kinzler K.W., Hruban R.H., Papadopoulos N., Wood L.D. Whole-exome sequencing of pancreatic neoplasms with acinar differentiation. *J. Pathol*. 2014; 232 (4): 428–435. DOI: 10.1002/path.4310.
11. Niida Y., Wakisaka A., Tsuji T., Yamada H., Kuroda M., Mitani Y., Okumura A., Yokoi A. Mutational analysis of TSC1 and TSC2 in Japanese patients with tuberous sclerosis complex revealed higher incidence of TSC1 patients than previously reported. *J. Hum. Genet.* 2013; 58 (4): 216–225. DOI: 10.1038/jhg.2013.3.

Authors contribution

Kit O.I. – analysis of the scientific work, critical revision of the manuscript for important intellectual content. Trifanov V.S. – treating doctor, conception of the study, drafting of the article. Timoshkina N.N. – drafting of the article, carrying out of the molecular and genetic research. Kolesnikov E.N. – conception of the study, planning of treatment. Gvaldin D.Yu. – carrying out of the molecular and genetic research, bioinformatic analysis of data. Karnaukhov N.S. – analysis of the morphological material. Kutilin D.S. – bioinformatic analysis of the NGS data. Meshcheryakova M.Yu. – drafting of the article, illustrations.

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Cephalalgia against the background of systemic mastocytosis: a clinical case

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ABSTRACT

The article presents a clinical case of comorbid pathology – development of migraine against the background of systemic mastocytosis. The classification and clinical manifestations of systemic mastocytosis, a rare blood disease, are given.

This clinical case illustrates an example of excessive mast cell degranulation (with the release of proinflammatory and vasodilating agents as a result of mast cell pathology). In this regard, in addition to the pathogen-specific therapy for systemic mastocytosis (including prevention of mast cell degranulation), it seems important to describe the effectiveness and the possibility of prescribing preventive and emergency therapy for migraine against the background of the underlying hematological disease – systemic mastocytosis.

Treatment of cephalalgia in patients with mastocytosis is a complex clinical task, in the solution of which it is necessary to take into account serious limitations in prescription of acetylsalicylic acid and other non-steroidal anti-inflammatory drugs.

Key words: cephalalgia, migraine, aura, mast cells, systemic mastocytosis.

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Conformity with the principles of ethics. The patient's written consent was obtained for the publication of this clinical case. The study was approved by the local Ethics Committee (Protocol No. 11-3/19 of 20.11.2019).

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Цефалгический синдром на фоне системного мастоцитоза. Клинический случай

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РЕЗЮМЕ

Приведен клинический случай коморбидной патологии – развития мигрени на фоне системного мастоцитоза. Приводятся классификация, клинические проявления системного мастоцитоза – редкого клонального заболевания крови.

Данный клинический случай иллюстрирует пример избыточной дегрануляции тучных клеток (с выделением провоспалительных и вазодилатирующих агентов в результате патологии мастоцитов). В связи с этим, помимо патогенетической терапии системного мастоцитоза (включающей профилактику дегрануляции тучных клеток), нам представляется также важным отразить эффективность и возможность назначения профилактической и экстренной терапии мигрени на фоне основного гематологического заболевания – системного мастоцитоза.

Терапия головной боли у пациентов с мастоцитозом представляет собой сложную клиническую задачу, в решении которой необходимо учитывать серьезные ограничения в назначении ацетилсалициловой кислоты и других нестероидных противовоспалительных препаратов.

Ключевые слова: цефалгический синдром, мигрень, аура, тучные клетки, системный мастоцитоз.

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

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

Соответствие принципам этики. Для публикации данного клинического случая было получено письменное согласие пациента. Исследование одобрено локальным этическим комитетом (протокол № 11-3/19 от 20.11.2019).

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INTRODUCTION

Cephalgic syndrome often accompanies the course of various pathological processes. The algorithm for examining a patient with headache (according to regional and international clinical guidelines) consists of anamnesis, clinical presentation, instrumental examination, and exclusion of secondary causes of headache. It is especially important to exclude these causes when alarming signs appear – markers indicating the need for additional examination. Such alarming signs include the first onset of headache after 50 years and thunder-like headache, progressively worsening and atypical for the patient, if accompanied by neurological symptoms and signs of intracranial hypertension [1–3].

Cephalalgia is often associated with giant cell arteritis, transient ischemic attacks, stroke, brain tumors, venous sinus thrombosis, subarachnoid hemorrhage, epilepsy, mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS), cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), cervical artery dissection, cervicalgia, and other diseases [2].

This article presents our own experience in treating cephalalgia in a patient with a verified diagnosis of mastocytosis. Mastocytosis is a rare blood disease that involves proliferation of mast cells (MCs) [4]. MCs were first described in 1863 by Frederick Recklinghausen (1833–1910) [5]; they play an important role not only in allergic reactions, but also in the immune response. The prerequisite for mastocytosis development is appearance of clonal MCs with a D816V mutation in the *c-KIT* gene. The product of this gene is transmembrane tyrosine kinase receptor CD117 for mast cell growth factor, and disturbances of the receptor apparatus lead to tumor transformation and inhibition of apoptosis.

The disease occurs among adults and children and is characterized by growth, activation, and accumulation of MCs in various organs and tissues of the body. With excessive accumulation of MCs in the skin (the so-called cutaneous mastocytosis), patients can be diagnosed with atopic dermatitis for many years under the supervision of dermatologists and allergists [6]. Another variant of the course is accumulation of MCs in other areas (skeletal system, gastrointestinal tract, liver, spleen, central nervous system), which is typical of systemic mastocytosis. According to the 2016 World Health Organization (WHO) criteria, mastocytosis can be divided into several subtypes: cutaneous mastocytosis, indolent (sluggish) systemic mastocytosis, smoldering systemic mastocytosis, systemic mastocytosis with associated hematological non-mast cell disease, aggressive systemic mastocytosis, mast cell leukemia, and mast cell sarcoma.

The cutaneous form of mastocytosis in children in 80% of cases independently regresses after puberty without any specific treatment. The aim of this article was not to discuss in detail the criteria for establishing the diagnosis of systemic mastocytosis. We only note that there are major criteria (multifocal dense infiltrates consisting of MCs (≥ 15 in the aggregate) and determined in a biopsy sample of the bone marrow and (or) other organs) and minor criteria (in biopsies of the bone marrow or other organs, > 25% of MCs located in the infiltrate are spindle-shaped or have atypical morphology, or > 25% of all MCs obtained from bone marrow aspirate smears are immature or atypical; D816V mutation of the *c-KIT* gene, mast cells in the bone marrow or organs express CD2 and / or CD25, serum tryptase levels are higher than 20 μg / l). The diagnosis is valid in the presence of one major and one minor or three minor criteria [7].

Typical symptoms of mastocytosis include urticaria, pruritus, rhinorrhea, nausea, dyspepsia, and nonspecific manifestations in the pulmonary system (wheezing, shortness of breath). In the nervous system, the most frequent manifestation of systemic mastocytosis is headache [8, 9]. Highly variable clinical presentation is due to paroxysmal degranulation of MCs in the tissue or in the systemic circulation, resulting in rhinorrhea, itching, syncope, diarrhea, and cephalalgia.

There is a sufficient number of studies describing the relationship between development of migraine in patients and pathology of MCs; hence, correction of cephalalgia remains the most important aspect in the treatment of patients with mastocytosis [10].

The role of MCs in the pathophysiology of migraine is widely discussed [11]. Peptides, such as calcitonin gene-related peptide, chemokine A, neurotensin, pituitary adenylate cyclase-activating peptide, and substance P, activate MCs, releasing vasoactive and proinflammatory mediators, which ultimately lead to the development of cephalalgia. In response to the effect of corticotropin-releasing hormone, MCs secrete proinflammatory and vasodilator molecules (interleukin-6 (IL-6), vascular endothelial growth factor (VEGF), nitric oxide (NO), histamine). Such a pathological chain develops as a result of stress factors, playing an important role in the development of migraine attacks. It is known that vasodilation of extracerebral vessels and cortical spreading depression are also involved in the pathogenesis of migraine, which explains some of the aura symptoms [12].

The Mayo Clinic conducted a retrospective study on the association between headache and MC activity. It included 64 patients with an established diagnosis of systemic mastocytosis (7), of which cephalalgia was observed in 36 patients (56.2%). Of these, 21 patients were carriers of the D816V mutation in the *c-KIT* gene (28 people were examined), headache was determined according to the International Headache Criteria [13]. In patients suffering from migraine and other types of headache in the clinical presentation, the following symptoms were noted (during the attack): redness, local fever, heaviness in the chest, abdominal pain. This symptom complex allowed the authors of the study to suggest an association between activation of mast cells and development of cephalalgia. In this work, the authors also noted a five-fold increase in the prevalence of aura symptoms in comparison with the general population [14].

Treatment of patients with mastocytosis presents certain difficulties, since a sufficiently large number of drugs can be triggers of mast cell activation (including development of anaphylactic shock); therefore, their use is limited in this cohort of patients. These drugs include (among others) non-steroidal anti-inflammatory drugs, antibiotics (vancomycin, polymyxin), and vitamin B1 (thiamine).

CLINICAL CASE

Patient R., 45 years old, had an appointment with a neurologist at the Research Center of Neurology with complaints of headache attacks, mainly in the right side of the head, lasting from several hours to 2–3 days. Before the attack, the patient noted (not always) zigzagging lines, sometimes bright flashes before the eyes. Headache attacks were often triggered by drinking wine. The frequency of attacks varied from 1–2 times a month to 2–3 times a week, depending on the season (more often, in winter). To relieve these symptoms, the patient took metamizole sodium and triptans (no effect). Upon additional questioning, it turned out that triptans were taken during a full-blown painful attack (which may explain their ineffectiveness).

From the anamnesis, it is known that since 1998, the patient has been observed by dermatologists for cutaneous mastocytosis. In 2013, she went to the Research Center of Hematology, where an examination was carried out. A skin biopsy was performed. In the skin biopsy, the morphological pattern and immunophenotype (in the upper layers of the dermis, elongated cells expressed CD117, CD25, and Tryptase) corresponded to the diagnosis of mastocytosis.

A molecular genetic study of peripheral blood revealed a D816V mutation in the *c-KIT* gene. Serum tryptase level was 16.9 μg / ml (reference value is less than 11 μg / l). During the histological examination of the trepanobioptate, no data for the damage to the bone marrow were obtained. According to the ultrasound data, the sizes of the liver, spleen, and lymph nodes were within normal values. Complete blood test parameters were within normal values (hemoglobin – 141 g / l; erythrocytes – 4.63 10^{12} / l; platelets – 235 × 10^9 / l; leukocytes – 8.63 × 10^9 / l). Coagulogram revealed normal coagulation (activated partial thromboplastin time (APTT) – 25.3 sec; prothrombin index according to Quick – 92%; fibrinogen – 3.3 g / l); platelet aggregation with adenosine diphosphate (ADP) – 73%; with ristomycin – 86%; with collagen – 84%. The patient received symptomatic therapy: sodium cromoglycate, ketotifen – in case of itching. During the observation period, the patient noted the appearance of new maculopapular rash of red and brown color on the skin of the thighs, legs, hands, and neck. Darier's sign was positive. Headache was the predominant symptom in the clinical presentation.

Due to the appearance of complaints of headaches and the predominance of cephalalgia in the clinical presentation, it was recommended to contact the Research Center of Neurology. The general somatic status included a reduced body mass index (17 kg / m^2), a tendency to arterial hypotension (BP of 100 / 60 mm Hg), and heart rate of 59 beats per minute).

In the neurological status: conscious, correctly oriented in place, time and self. Emotional background: somewhat emotionally labile. There were no meningeal symptoms. Cranial nerves were intact. No paresis was detected, tendon and periosteal reflexes were lively and symmetric. There were no clear sensitivity disorders. There were no pathological pyramidal signs. The finger-nose test was performed with discoordination on both sides. Slight staggering during the Romberg test was observed. Subjective headache assessment on the Visual Analogue Scale (VAS) – 7 points.

Findings of instrumental and laboratory research methods. Magnetic resonance imaging (MRI) of the brain: a single focal lesion of vascular origin in the right frontal region was determined (migraine focus?); the rest of the departments were without pathology. Electroencephalography (EEG) revealed nonspecific signs of dysfunction of the brain bioelectrical activity and dysfunction of diencephalon structures. Duplex ultrasound of the main arteries of the head revealed no pathology.

Complete blood count and biochemistry within normal limits; the coagulogram showed normal coagulation. According to the data of platelet aggregation, an increase in their aggregation properties was noted: with ADP – 50 (37–43)%; with adrenaline – 48 (40–46)%.

Taking into account the clinical manifestations and the data of instrumental research methods, the diagnosis of migraine with aura was established. It was decided to prescribe tricyclic antidepressants in therapeutic doses (amitriptyline, 50 mg / day). As the background therapy, we did not prescribe calcium and beta-blockers (taking into account the tendency to hypotension and bradycardia), so we selected a caffeine-containing combination drug which also has antiplatelet properties.

Prescription of acetylsalicylic acid for mastocytosis is unacceptable due high risk of anaphylaxis. In addition, we recommended to take triptans to stop seizures (with a need to take it in the first minutes of

an attack or during aura symptoms). After 3 weeks, a positive trend was noted in the form of a decrease in the frequency and intensity of attacks. During the follow-up, a single attack was noted. A repeated blood test (after 3 weeks) of platelet aggregation with adrenaline and ADP showed a significant decrease in the aggregation properties of the latter (34 and 38%, respectively), which indicates an important contribution of antiplatelet therapy to prevention of cephalalgia.

CONCLUSION

An important aspect of neurological practice is management of comorbid patients with cephalalgia. One of the main aspects in treatment of headaches (as well as the underlying disease) against the background of mastocytosis is preventing activation of mast cells (degranulation of cytoplasmic compartments with release of histamine, prostaglandins, interleukins, etc.), which lead to variable clinical presentation, including the development of a migraine attack. Patients should avoid overheating (baths, saunas) and sun exposure and follow a strict diet that excludes seafood (especially shrimps, lobsters, crabs, caviar), alcohol, chocolate, tyramine-containing foods (cheeses, nuts, cereals, legumes), and canned food. Such restrictions are also effective in preventing the frequency of migraine attacks [15].

REFERENCES

1. Steiner T.J., Paemeleire K., Jensen R., Valade D., Savi L., Lainez M., Diener G., Martelletti P. European principles of management of common headache disorders in primary care. *J. Headache Pain*. 2007; 8 (1): S3–47. DOI: 10.1007/s10194-007-0366-y.
2. Osipova V.V., Filatova E.G., Artemenko A.R.,. Lebedeva E.R., Azimova Yu.E., Latysheva N.V., Sergeev A.V., Amelin A.V., Koreshkina M.I., Skorobogatykh K.V., Ekusheva E.V., Naprienko M.A. V., Isagulyan E.D., Rachin A.P., Danilov Al.B., Kurushina O.V., Parfenov V.A., Tabeeva G.R., Gekht A.B., Yakhno N.N. Diagnostics and treatment of migraine: recommendations of Russian experts. *Journal of Neurology and Psychiatry named after S.S. Korsakov*. 2017; 117 (1): 28–42 (in Russ.). DOI: 10.17116/jnevro20171171228-42.
3. Osipova V.V., Azimova Yu.E., Tabeeva G.R., Tarasova S.A., Amelin A.V., Kutsemelov I.V., Moldovanu I.V., Odobescu S.S., Naumova G.I. Diagnostics of headaches in Russia and the countries of the post-Soviet space: the state of the problem and ways to solve it. *Annals of Clinical and Experimental Neurology*. 2012; 6 (2): 16–21 (in Russ.).
4. Akin C., Metcalfe D.D. Systemic mastocytosis. *Annu. Rev. Med*. 2004; 55 (1): 419–432. DOI: 10.1146/annurev.med.55.091902.103822.
5. Recklinghausen F von. Über Eiter und Bindegewebskörperchen. Virchows *Arch. Pathol. Anat. Physiol. Klin. Med*. 1863; (28): 157–197.
6. Castells M., Austen K.F. Mastocytosis: mediator-related signs and symptoms. *Int. Arch. Allergy Immunol*. 2002; 127 (2): 147–152. DOI: 10.1159/000048188.
7. Valent P., Akin C., Metcalfe D.D. Mastocytosis: 2016 updated WHO classification and novel emerging treatment concepts. *Blood.* 2017; 129 (11): 1420–7. DOI: 10.1182/blood-2016-09-731893.
8. Scully R.E., Mark E.J., McNeely W.F., Ebeling S.H., Phillips L.D. Case records of the Massachusetts General Hospital. Weekly clinicopathological exercises. Case 20-1997. A 74-year-old man with progressive cough, dyspnea, and pleural thickening. *N. Engl. J. Med*. 1997; 336 (26): 1895–1903. DOI: 10.1056/NEJM199706263362608.
9. Georgin-Lavialle S., Gaillard R., Moura D., Hermine O. Mastocytosis in adulthood and neuropsychiatric disorders. *Transl. Res*. 2016; 174: 77–85.e1. DOI: 10.1016/j.trsl.2016.03.013.
10. Hermine O., Lortholary O., Leventhal P.S., Catteau A., Soppelsa F., Baude C., Lortholary O., Leventhal P.S., Catteau A., Soppelsa F., Cohen-Akenine A., Palmérini F., Hanssens K., Yang Y., Sobol H., Fraytag S., Ghez D. et al. Case-control cohort study of patients' perceptions of disability in mastocytosis. *PLoS One*. 2008; 3 (5): e2266. DOI: 10.1371/journal.pone.0002266.
11. Theoharides T.C., Donelan J., Kandere-Grzybowska K., Konstantinidou A. The role of mast cells in migraine pathophysiology. *Brain Res. Rev*. 2005; 49 (1): 65–76. DOI: 10.1016/j.brainresrev.2004.11.006.
12. Spierings E.L.H. Pathogenesis of the migraine attack. *Clin. J. Pain*. 2003; 19 (4): 255–262. DOI: 10.1097/00002508-200307000-00009.
13. The International classification of headache disorders; 2nd ed. *Cephalalgia.* 2004; 24 (1): 9–160. DOI: 10.1111/j.1468-2982.2003.00824.x.
14. Smith J.H., Butterfield J.H., Cutrer F.M. Primary headache syndromes in systemic mastocytosis. *Cephalalgia*. 2011; 31 (15): 1522–1531. DOI: 10.1177/0333102411421683.
15. Melikian A.L., Subortseva I.N., Goriacheva S.R., Kolosheinova T.I., Vakhrusheva M.V., Kovrigina A.M. et al. Mastocytosis. Review of the literature and description of clinical cases. *Ter. Arkh.* 2014; 86 (12): 127–134. DOI: 10.17116/terarkh20148612127-134.

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The 80th birthday of Academician Leonid Barbarash

Dedicated to the 80th birthday of Academician Leonid Barbarash, the founder of Kuzbass Cardiology Center (Kemerovo) and Research Institute for Complex Issues of Cardiovascular Diseases.

Leonid S. Barbarash (born June 22, 1941) – Dr. Sci. (Med.), Professor, Academician of the Russian Academy of Sciences, Honored Doctor of the Russian Federation, a top-class cardiac surgeon, the founder of Kuzbass Cardiology Center (Kemerovo), a teacher, a scientist, and a director. The diversity of his knowledge and areas of research became the basis for progressive development of cardiology and cardiac surgery in Kuzbass. The principle of organization of the center that combines several clinical and research areas has proven its viability and effectiveness.

Thanks to the organizational talent of Leonid Barbarash, Kuzbass developed a unique and innovative closed model of medical care delivery for patients with circulatory system diseases, which encompasses all levels of medical care delivery (from outpatient and inpatient care to high-tech medical care and rehabilitation) with a unified approach to strategic management. His outstanding qualities of a scientist, specialist, and professor helped to build a team of highly qualified doctors, scientists, cardiologists, cardiac surgeons, anesthetists and resuscitators, specialists in functional diagnostics, engineers, chemists, mathematicians, and biologists.

In 1987, Leonid Barbarash was the first surgeon behind the Urals to perform aortic valve replacement with combined coronary bypass grafting, followed by the first ascending aortic aneurysm surgery. He streamlined provision of high-tech medical care for patients with circulatory system diseases. For the first time in the world, Leonid Barbarash and his students developed and introduced to the market a fundamentally new generation of epoxy-treated bioprostheses, later implementing them into clinical practice. Research in the field of bioprosthetic heart valves resulted in establishing NeoKor closed joint-stock company in 1987. Under the leadership of Leonid Barbarash, the Laboratory of Reconstructive Cardiovascular Surgery was established in 2001, which was transformed into Research Institute for Complex Issues of Cardiovascular Diseases in 2009.

Leonid Barbarash has received many honorary awards and titles for his outstanding contribution to healthcare. The European Association for Cardiothoracic Surgeons awarded the team led by Leonid Barbarash the C. Walton Lillehei Award in 1997. In 2004, Leonid Barbarash was given the A.N. Bakulev Award for establishment of the cardiac surgery center and personal contribution to the development of bioprosthetic heart valves. Moreover, he received the 2001 Russian Federation National Award in Science and Technology.

In 2005, Leonid Barbarash was awarded the "Prizvanie" (Vocation) National Award of the

Ministry of Healthcare and Social Development of the Russian Federation for his contribution to the development of bioprosthetic heart valves and cardiovascular surgery. In 2007, he received the same award in the nomination "For developing a new method of treatment" for developing unique domestic bioprosthetic heart valves and a special method of their preservation.

In 2007, Leonid Barbarash was awarded the Order of Honor. In 2019, he was awarded the title "Honorary Worker of Science and Technology of the Russian Federation". Moreover, Academician Barbarash was awarded the titles "Hero of Kuzbass" (2013) and "Honorary Citizen of the Kemerovo Region" (2006).

For more than 45 years, Academician Leonid Barbarash has been passing on his knowledge to students, clinical residents, postgraduates, and young practitioners. Leonid Barbarash is the author of more than 700 scientific papers, including 15 monographs and books, as well as more than 70 inventions and utility models. Under his leadership, 14 doctoral and 30 candidate theses have been defended.

Dear Leonid S. Barbarash,
happy 80th birthday!

НАУЧНО-ПРАКТИЧЕСКИЕ КОНФЕРЕНЦИИ СИБГМУ В 2021 г.

Всероссийская научно-практическая конференция

ТИПОВЫЕ ПАТОЛОГИЧЕСКИЕ ПРОЦЕССЫ: СОВРЕМЕННЫЕ ТРЕНДЫ В НАУКЕ

Посвящена 130-летию старейшей в азиатской части России кафедры патофизиологии Императорского (государственного) Томского университета – Томского медицинского института – Сибирского государственного медицинского университета и 75-летию со дня рождения заслуженного деятеля науки РФ, академика РАН Вячеслава Викторовича Новицкого.

МЕРОПРИЯТИЯ В РАМКАХ КОНФЕРЕНЦИИ

- выступления выдающихся российских ученых с академическими лекциями;
- пленарные и устные доклады ученых – ведущих специалистов в области патологии и патологической физиологии;
- обсуждение новые подходов в преподавании патофизиологии в российских вузах и инноваций в высшем образовании;
- музейный час об истории научно-медицинских школ СибГМУ.

Время проведения: 7–8 сентября 2021 г.
Место проведения: СибГМУ, г. Томск, Московский тракт, 2..
e-mail: kaf.pat.fiziolog@ssmu.ru

Информация о доступе к онлайн-трансляции мероприятий конференции будет размещена 15 августа 2021 г. на сайте СибГМУ на странице кафедры патофизиологии по ссылке https://www.ssmu.ru/ru/obrazovanie/departments/patfiz/

Персональная информация о доступе на площадку проведения конференции для удаленных почетных гостей и участников с онлайн-докладами будет разослана дополнительно.

НАУЧНО-ПРАКТИЧЕСКИЕ КОНФЕРЕНЦИИ СИБГМУ В 2021 г.

Международная научно-практическая конференция

РАЗРАБОТКА ЛЕКАРСТВЕННЫХ СРЕДСТВ – ТРАДИЦИИ И ПЕРСПЕКТИВЫ

Посвящена знаковым датам в истории развития ведущей в Сибири школы фармацевтического образования и науки:

- 100 лет со дня рождения выдающегося фармаколога, доктора медицинских наук, профессора Альберта Самойловича Саратикова;
- 100 лет со дня рождения известного ботаника и фармакогноста, доктора биологических наук, профессора Тамары Павловны Березовской;
- 80 лет фармацевтическому факультету;
- 75 лет со дня рождения декана фармацевтического факультета СибГМУ, доктора фармацевтических наук, профессора Степана Евгеньевича Дмитрука, внесшего значительный вклад в развитие фармацевтического образования.

НАУЧНЫЕ НАПРАВЛЕНИЯ КОНФЕРЕНЦИИ

- Фармацевтическая разработка.
- Природные соединения – источник новых лекарственных средств.
- Актуальные вопросы экспериментальной и клинической фармакологии.

К участию приглашаются студенты, аспиранты и молодые ученые российских и зарубежных вузов и академических институтов.
Время проведения: 13–16 сентября 2021 г.
Место проведения: СибГМУ, г. Томск, Московский тракт, 2.
Конференция будет аккредитована в системе НМФО.
Сайт: conf.ssmu.ru/ddtp

Всероссийская научно-практическая конференция

ИННОВАЦИОННЫЕ ТЕХНОЛОГИИ В ОФТАЛЬМОЛОГИИ

Посвящена 130-летию кафедры офтальмологии Сибирского государственного медицинского университета

Время проведения: 16–17 сентября 2021 г.
Место проведения: СибГМУ, г. Томск, Московский тракт, 2.
Конференция проводится под эгидой Общества офтальмологов России.

НАУЧНЫЕ НАПРАВЛЕНИЯ КОНФЕРЕНЦИИ

- Фундаментальные исследования в офтальмологии.
- Современные принципы диагностики в офтальмологии.
- Актуальные тенденции лечения патологии органа зрения.

Конференция будет аккредитована в системе НМФО.
e-mail: Confophthalm2021@mail.ru