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СибГМУ сегодня

«Сибирский государственный медицинский университет, формируя будущее медицинского образования и биомедицины, призван развивать и укреплять Сибирский регион, притягивая таланты и воспитывая лидеров нового поколения в сфере обеспечения здоровья населения»

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- СибГМУ **вступил в ассоциацию биобанков** и специалистов по биобанкированию НАСБИО.
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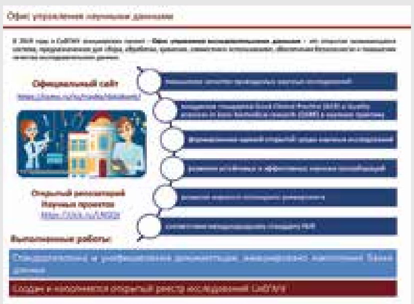

- **Инициирована работа по проекту «Офис управления научными данными»** для создания открытой среды научной коммуникации и обмена полученными результатами научной деятельности.
- Почти в два раза было **увеличено количество статей**, входящих в престижные Q1 и Q2 международные базы цитирования в 2019 году по сравнению с 2018 годом.
- Центром клинических исследований **получено свидетельство об аккредитации** на проведение клинических исследований по клеточным продуктам, а также была начата работа по оценке эффективности и безопасности программного обеспечения медицинского назначения.
- В университете проведена серия Летних студенческих научных школ **SciCamp для студентов и молодых ученых**.
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Association of coronary heart disease and sleep disorders among men in a medium-sized urban city of Western Siberia

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ABSTRACT

The aim of the study was to establish the association of the prevalence of coronary heart disease and sleep disorders among men between the ages of 25 and 64 belonging to an open population of a medium-sized urban city of Western Siberia.

Materials and methods. A cross-sectional epidemiological study was conducted on a representative sample of the population among males of 25–64 years old in Tyumen. The prevalence of coronary heart disease was determined based on standard epidemiological methods. Self-assessments by participants in the study of quality of sleep was determined by the World Health Organization Monitoring Trends and Determinants in Cardiovascular Disease-Psychosocial Program (WHO MONICA-psychosocial). When calculating the odds ratio of developing coronary heart disease (CHD), self-reports of satisfactory, good, or very good sleep were regarded as a lack of an indicator; while very bad and bad sleep were considered positive indicators.

Results. The prevalence of CHD according to the extended epidemiological criteria for men in an open urban population was 12.4%; the detection rate of "definite" and "possible" CHD was almost equal. The age-standardized prevalence rate of sleep disorders was 50.9%. There is a significant risk of developing CHD with extended criteria (5.05), as well as "definite" (5.28) and "possible" (3.13) forms in the male population at 25–64 years of age. In the 55 to 64 age group, there is a significant risk of developing CHD according to the extended criteria (5.57) and the "definite" form of CHD (10.21).

Conclusion. Thus, the findings suggest the importance of further study of sleep disorders in working age men in Siberian populations, its relationships with conventional and non-conventional risk factors of CHD, as well as the feasibility of preventive measures aimed at reducing the influence of psycho-emotional stress factors among the Russian population.

Key words: epidemiological study, coronary heart disease, sleep disorders, open population, men.

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Ассоциации распространенности ишемической болезни сердца и нарушений сна среди мужчин открытой популяции среднеурбанизированного города Западной Сибири

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РЕЗЮМЕ

Целью исследования явилось установление ассоциации распространенности ишемической болезни сердца (ИБС) и нарушений сна среди мужчин открытой популяции 25–64 лет среднеурбанизированного города Западной Сибири.

Материалы и методы. На репрезентативной выборке населения среди лиц мужского пола 25–64 лет было проведено кросс-секционное эпидемиологическое исследование на модели г. Тюмень. Распространенность ИБС определялась на основании стандартных эпидемиологических методов. Самооценка сна определялась по алгоритмам программы ВОЗ «МОНИКА-психосоциальная». При расчете отношения шансов развития ИБС сон удовлетворительный, хороший, очень хороший расценивались как отсутствие признака, сон очень плохой, плохой – как присутствие.

Результаты. Распространенность ИБС по расширенным эпидемиологическим критериям у мужчин открытой городской популяции составила 12,4%, частота выявления «определенной» и «возможной» ИБС была практически одинаковой. Стандартизованный по возрасту показатель распространенности нарушений сна составил 50,9%. В мужской популяции 25–64 лет при нарушении сна установлен существенный риск развития ИБС по расширенным критериям (5,05), а также «определенной» (5,28) и «возможной» (3,13) ее форм. В возрастной категории 55–64 лет установлен существенный риск развития ИБС по расширенным критериям (5,57) и «определенной» формы ИБС (10,21).

Заключение. Полученные данные свидетельствуют о важности дальнейшего изучения нарушения сна у мужчин трудоспособного возраста в сибирских популяциях, его взаимосвязей с конвенционными и неконвенционными факторами риска ИБС, а также о целесообразности превентивных мероприятий, направленных на снижение влияния факторов психоэмоционального напряжения среди российского населения.

Ключевые слова: эпидемиологическое исследование, ИБС, нарушение сна, открытая популяция, мужчины.

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

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INTRODUCTION

In scientific literature, term of sleep is defined, on the one hand, as natural state of body that determines normal functioning of all its systems and organs, and, on the other hand, as one of the important factors affecting the level of population health. The main function of sleep is a recovery process, which, to a large extent, allows body to adapt to changing conditions of internal and external environment. If it is disturbed, adaptation possibilities decrease, which ultimately leads to the development of somatic pathology, and most significantly cardiovascular diseases (CVD) [1]. In scientific literature, dyssomnia is a term for sleep disturbance (SD) combining the following types of sleep disorders: insomnia – difficulty initiating and maintaining sleep; hypersomnia – sleepiness during the day and excessive sleep; parasomnia – periodic night phenomena (heterogeneous group). Much research devoted to the study of sleep disorders indicate dissatisfaction of people with their sleep and shorter duration of sleep in a significant part of the population [2]. The results of a US National Commission study on sleep disorder showed that nearly 40% of America's population has sleep problems, and more than 40 million adults are suffering from chronic sleep disorders, while almost 30 million have periodic insomnia [3].

Recent studies, which transformed the European guidelines for cardiovascular prevention regarding the importance of psychosocial CVD risk factors (RF), indicate that cardiovascular risk and prognosis essentially determine psychosocial RF. These include factors of chronic social stress as well as negative psycho-emotional states (vital exhaustion, trait anxiety, depression), which in turn, are main factors of SD [4–6]. Changes in autonomic cardiovascular regulation, which turn sleep into periods of significant physiological storms characterized by sudden and abrupt changes in heart rate and blood pressure, are associated with cyclical phases of fast and slow sleep. Revealed cardiovascular instability related to phases of sleep served as a basis for studies on the determination of CVD risk and prognosis, primarily, depending on duration and changes in cyclic phases of sleep. There is a number of studies in which SD acts as a possible risk factor for progression and initiation of cardiovascular pathology [7–9]. In accordance with this, the risk of coronary artery disease (CAD) development due to sleep disorders seems relevant and timely in an open population of working age males.

The aim of the study was to establish the association of CAD prevalence and SD among men between the ages of 25 and 64 belonging to an open population of a medium-sized urban city of Western Siberia.

MATERIALS AND METHODS

A cross-sectional epidemiological study was conducted on a randomly selected representative sample of population from voting lists of the Central Administrative District Tyumen (males aged 25–64, n = 1000, n = 250 in every decade of life), the response rate was 85.0%.

Within cardiological screening, a resting 12-lead electrocardiogram (ECG) was performed in the supine position.

According to the advanced epidemiological criteria, CAD prevalence was determined based on standard epidemiological methods with the establishment of CAD according to rigorous epidemiological criteria: a "definite" form of CAD (DCAD) and non-rigorous epidemiological criteria : a "possible" form of CAD (PCAD).

DCAD was established on the basis of a positive answer to the WHO questionnaire (effort angina) and The Minnesota Code Manual of Electrocardiographic Findings, including "definite" myocardial infarction (MI), effort angina and painless form of CAD. The basis for PCAD determination was The Minnesota Code Manual of Electrocardiographic Findings, which included "possible" MI, "possible" ischemia, ischemia with left ventricular hypertrophy, and ARRHYTHIAS (arrhythmic form of CAD).

Study of non-conventional (psychosocial) risk factors for CAD was carried out by means of strictly standardized methods of the WHO MONICA-psychosocial program algorithms, using the standard questionnaire [7].

Self-assessment of sleep was determined by the method of questionnaire and included the following responses: very good sleep, good sleep, fair sleep, poor sleep, very poor sleep. When calculating odds ratio for CAD development in males with SD, fair, good, very good sleep were considered as the absence of a sign; very poor sleep and poor sleep were considered as the presence of a sign.

Statistical data analysis was performed using the standard IBM SPSS Statistics software, application package, version 21.0.

Age-standardization of quantitative rates was carried out in accordance with the last census of the Russian Federation population (urban male population aged 25–64), age-standardized rates (ASR) were determined. The criterion of Pearson chi-square test (χ^2) was used to assess statistical significance of differences between two groups, $p < 0.05$ was taken as critical level of significance in testing statistical hypotheses.

Prevalence associations of SD and CAD were established by calculating the odds ratio with 95% confidence intervals.

RESULTS

Cardiac screening results showed high prevalence of CAD according to the advanced epidemiological criteria – the age-standardized rate (ASR) in the population was 12.4%.

In the population of Tyumen aged 25–64, incidence of DCAD and PCAD detection was almost the same.

According to rigorous epidemiological criteria, the most vulnerable age periods in relation to the increase in CAD prevalence of Tyumen males were the 45–54 and 55–64 age groups, while according to non-rigorous and expanded criteria the entire age range was vulnerable (Fig. 1).

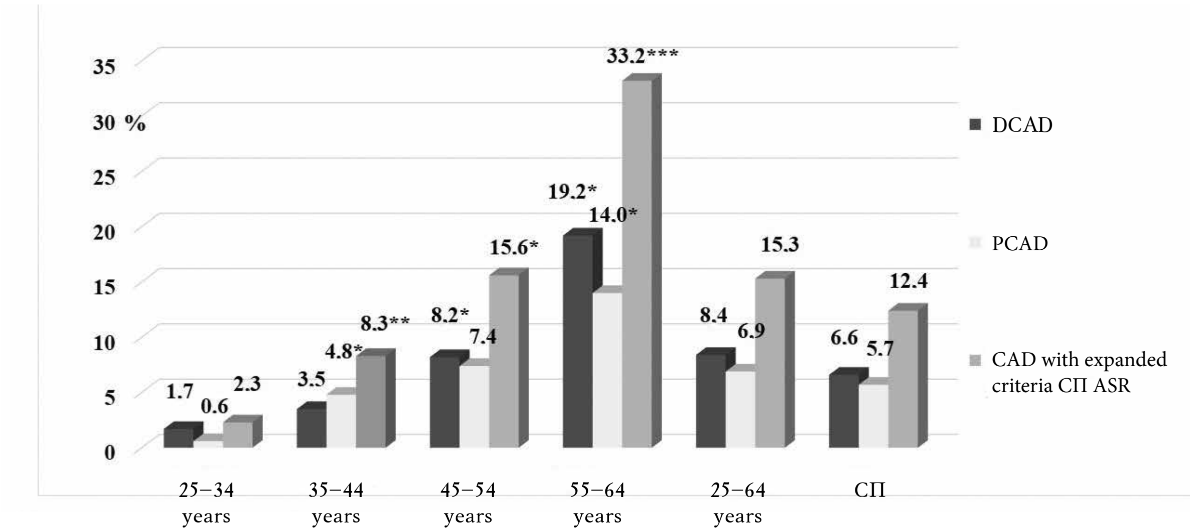

Fig.1. Prevalence of CAD in men aged 25–64 years in an open population; ASR – age-standardized rate; * $p < 0.5$; ** $p < 0.01$; *** $p < 0.001$ – statistical significance of differences between two subsequent age groups

The table shows the results of study on SD in population. 49.2% (ASR) of urban male population identified their sleep as very good or good. The prevalence of “poor sleep” was 8.9%, and “very poor sleep” was 0.6% of male population. Significantly more “very good” answers to the question “How do you sleep?” were received in the youngest (25–34) age group studied compared with the 45–54 age group. Furthermore, the answer “good” was registered more often in young adults aged 25–34 and 35–44, statistically significant differences in these groups and such answers were found in relation to corresponding rates in the mature adults aged 45–54 and 55–64, while in the youngest and oldest age categories, statistically significant differences were also registered at the general population rate.

With regard to fair sleep, in young people in the third decade of life, statistically significant differences were revealed in relation to rates in older age categories and ASR. In the sixth decade of life, fair sleep was registered much more often than in the fourth decade. Poor sleep was also revealed much more often in the 45–54 and

55–64 age categories and in the entire population compared with the rate in young adults aged 25–34; the rate 1.5 times prevailed over ASR in the 55–64 age category. The ASR prevalence rate of respondents with poor and very poor sleep was 9.5% in male population. Good and very good sleep were registered more often in young age categories, fair and poor sleep were in older age categories (Table). Figure 2 shows the odds ratio in the presence-absence of SD and CAD according to rigorous, non-rigorous and the advanced epidemiological criteria in male population aged 25–64. Thus, SD in the population showed the highest risk of CAD (5.28 with 95% CI = 2.67–10.46, $p < 0.05$) and DCAD according to expanded criteria (5.05 with 95% CI = 2, 92–8.74, $p < 0.05$), while the risk of PCAD in the presence of SD was also significant and amounted to 3.13 (95% CI = 1.47–6.65, $p < 0.05$) (fig. 2).

The same trend was not found among different age decades of life, while the rate reached statistical significance only in the 55–64 age category, when the risk of DCAD in the presence of SD almost doubled in this age category compared with the same rate in the general population (10.21 at 95% CI = 3.26–31.93, $p < 0.05$).

According to the advanced epidemiological criteria, risk of CAD in the presence of NS remained basically unchanged in the 55–64 age group compared with the rate of general population (5.57 with 95% CI = 2.30–13.52, $p < 0.05$), and the risk of CAD in the presence of SD was statistically insignificant in the older age group (Fig. 3).

Table

Sleep disturbance in the male population of 25–64 years depending on age

Question/attitude	Age ranges										
	25–34		35–44		45–54		55–64		25–64		СП
	абс.	%	абс.	%	абс.	%	абс.	%	абс.	%	%
How well do you sleep?											
Very well	20	11.3	19	8.3	12	5.2^{*}	13	6.1	64	7.5	8.0
Well	92	52.0	101	44.3	79	34.2^{***}_{*}	59	27.6^{***}_{***}	331	$_{**}38.9^{**}$	41.2
Satisfactorily	59	33.3	90	39.5	110	47.6^{**}	105	49.1^{**}_{*}	364	42.8^{*}	41.4
Badly	6	3.4	17	7.5	28	12.1^{**}	34	15.9^{***}_{**}	85	$_{*}10.0^{**}$	8.9
Very badly	0	0.0	1	0.4	2	0.9	3	1.4	6	0.7	0.6

Notes: statistically significant differences in rates between the 25–34 age group and other age groups are indicated by asterisk (*) in the upper case on the right; between the 35–44 age group and other age groups – in the lower case on the right; between the 45–54 age group and other age groups – in the upper case on the left; between the 55–64 and 25-64 age groups – in the lower case on the left. ASR is the age-standardized rate.
* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

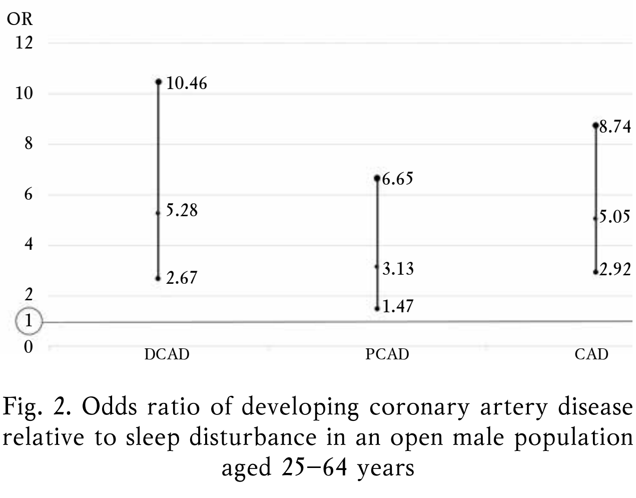

Fig. 2. Odds ratio of developing coronary artery disease relative to sleep disturbance in an open male population aged 25–64 years

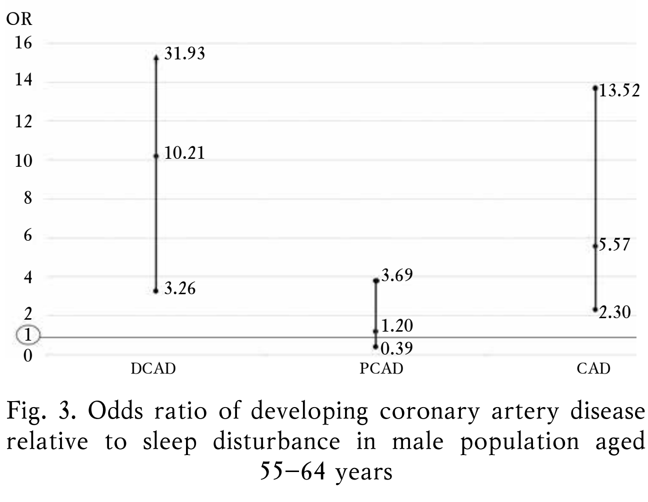

Fig. 3. Odds ratio of developing coronary artery disease relative to sleep disturbance in male population aged 55–64 years

DISCUSSION

In the male population of a medium-sized urban city of Western Siberian (on the model of Tyumen city), ASR prevalence rate of SD was 50.9%. The rate was found to be quite high, but also comparable with the data established on other Siberian populations, namely, the results of Tomsk (61.2%) and Novosibirsk (48.3%) research on working male urban populations [7, 10].

According to T.M. Maximova's study results, among the Russian population, SD rate was on average of 30%, with minimum values of the indicators in young males, and with maximum values in older respondents [11].

At the same time, in the current study among Tyumen males, the incidence rate of SD also prevailed in older age.

According to domestic and foreign researchers, SD appears in presence of psycho-emotional stress factors, such as trait anxiety, vital exhaustion, depression, and, in accordance with this, serves as a predictor of CAD development [2, 3, 5–7, 12].

The study of self-assessment of sleep quality makes it possible to identify SD rate in population, to assess problems associated with SD differentially in various population groups, and to establish groups of population with increased risk of cardiovascular pathology, respectively.

In Tyumen, high SD gradations were five times more likely in males with CAD than in males without CAD, the same tendency was in males with DCAD.

SD with CCAD was detected three times more often compared to ASR in the general population.

In accordance with the current study results concerning other psychosocial factors in Tyumen population [6, 13], general population trends prevailed in the older 55–64 age category, however, alternatively to other factors of psycho-emotional stress, high gradations of SD were significantly more often registered, starting in the 35–44 age category in individuals with DCAD.

This situation is likely to be consequential, because, a significant number of patients with chronic SD associate their complaints with their life situation, and according to studies' data in most cases personal problems predominate. SD arise in connection with problems of identity in the young age, and SD are associated with negative attitudes toward ageing, fear of death and dissatisfaction with overall living in old age. [2].

In Tyumen, the results are confirmed by the data of other studies regarding SD as a predictor of CAD. So, in Novosibirsk male population aged 25–64 with SD and with self-estimation of sleep as "poor", the relative risk of CAD was 2.6 over 10 years of prospective observation compared to males with self-estimation of sleep as "good" [7]. Data analysis of cross-sectional Cardiovascular Health Study in unorganized population showed that daytime sleepiness in males as the only SD was associated with cardiovascular death [12].

According to results of cross-sectional study in Finnish population, the highest prevalence of CAD was found among males whose night sleep was 6 hours or less, and ratio remained after inclusion in multifactor model such parameters as conventional CAD risk factors (smoking, arterial hypertension, alcohol), and in addition, sleep quality, age, taking tranquilizers and sleeping pills, "Coronary" type of personality and presence of psychosocial factors [14].

Thus, findings indicate the importance of further studying in SD of workable male of Siberian populations, its relationships with conventional and non-conventional risk factors for CAD, and expedience of preventive measures aimed to reduce the influence of psycho-emotional stress factors among the Russian population.

CONCLUSION

According to the advanced epidemiological criteria, CAD prevalence was 12.4%, DCAD and PCAD detection rate was almost the same in an open urban male population. ASR prevalence rate for SD was 50.9%. According to the advanced criteria, significant risk of CAD (5.05) was established in male population with SD aged 25–64, as well as DCAD (5.28) and PCAD (3.13). According to expanded criteria, significant risk of CAD (5.57) and DCAD (10.21) was established in the 55–64 age category.

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The role of epidermal growth factor receptor (EGFR) in the efficacy of neoadjuvant chemotherapy in triple-negative breast cancer patients

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ABSTRACT

Background. Identification of predictive molecular markers of triple-negative breast cancer (TNBC) will enable the evaluation of the efficacy of neoadjuvant chemotherapy (NACT) and define optimum approaches for the prognosis of the disease course in TNBC patients.

The aim of the study was to examine the correlation between the expression of the epidermal growth factor receptor (EGFR), its gene's polymorphic variants and the neoadjuvant chemotherapy (NACT) efficacy in triple-negative breast cancer (TNBC) patients.

Materials and methods. The study included 70 patients with triple-negative breast cancer, who had received 2-4 cycles of FAC and CAX regimens. The efficacy of the neoadjuvant chemotherapy was assessed according to the RECIST scale. The EGFR expression level in tumors before and after the NACT was evaluated with the help of immunohistochemistry. Genotypes for E*GFR* (rs2227983 and rs1468727) were detected by a real-time PCR.

Results. It was found that NCT significantly decreases the EGFR expression level in the tumor ($p = 0.000$). The research associates the objective clinical response as well as the pathological complete response with the low EGFR expression level ($p = 0.007$ and $p = 0.000$ respectively). Patients carrying the *EGFR*CC mutant genotype of rs1468727 did not achieve a pathological complete response ($p = 0.042$). In addition, patients with *EGFR*CC mutant genotype are more likely to have tumors with a high EGFR expression compared to *EGFR*TT wild-type genotype patients ($p = 0.047$).

Conclusion. The EGFR expression level in tumor tissue and the polymorphic variants of its gene in the rs1468727 locus can be considered as potential molecular markers with predictive significance in relation to the NACT efficacy in triple-negative breast cancer patients.

Key words: Triple-negative breast cancer, neoadjuvant chemotherapy, epidermal growth factor receptor (EGFR); gene polymorphisms.

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

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Conformity with the principles of ethics. All patients signed an informed consent to participate in the study. The research was carried out according to the principles of voluntariness and confidentiality in

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compliance with the “Fundamentals of the Legislation of the Russian Federation on the Protection of Public Health (Decree of the President of the Russian Federation of December 24, 1993 No. 2288) based on the permission of the local committee on biomedical ethics of the Cancer Research Institute, Tomsk National Research Medical Center.

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Роль рецептора эпидермального фактора роста EGFR в эффективности неоадъювантной химиотерапии у больных тройным негативным раком молочной железы

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РЕЗЮМЕ

Актуальность. Выявление предсказательных молекулярных маркеров тройного негативного рака молочной железы позволит оценить эффективность неоадъювантной химиотерапии (НАХТ) и определить оптимальные подходы к прогнозированию течения заболевания.

Цель исследования. Изучить взаимосвязь экспрессии рецептора эпидермального фактора роста EGFR и полиморфных вариантов его гена с эффективностью неоадъювантной химиотерапии у больных тройным негативным раком молочной железы.

Материалы и методы. В исследование включены 70 пациенток с тройным негативным раком молочной железы, получавших 2–4 курса НАХТ по схеме FAC или CAX. Оценка эффективности НАХТ проводилась по шкале RECIST. Уровень экспрессии EGFR в опухоли до и после НАХТ оценивался иммуногистохимическим методом. Анализ полиморфных вариантов гена *EGFR* в локусах rs2227983 и rs1468727 проведен с помощью полимеразной цепной реакции в режиме реального времени.

Результаты. Выявлено, что в процессе НАХТ уровень экспрессии EGFR в опухоли значимо снижается ($p = 0{,}000$). Показано, что достижение объективного клинического и полного патоморфологического ответа опухоли ассоциировано с низким уровнем экспрессии EGFR ($p = 0{,}007$ и $p = 0{,}000$ соответственно). Отсутствие эффективного ответа на НАХТ у больных тройным негативным раком молочной железы связано с носительством мутантных генотипов EGFRCC в локусе rs1468727 ($p = 0{,}042$). Кроме того, среди пациентов, несущих мутантный вариант гена *EGFR*CC, чаще встречаются опухоли с высокой экспрессией *EGFR* по сравнению с больными, имеющими дикий вариант *EGFRTT* ($p = 0{,}047$).

Заключение. Уровень экспрессии EGFR в опухоли и полиморфные варианты его гена в локусе rs1468727 могут рассматриваться в качестве потенциальных молекулярных маркеров с предсказательной значимостью в отношении эффективности НАХТ у больных тройным негативным раком молочной железы.

Ключевые слова: тройной негативный рак молочной железы, неоадъювантная химиотерапия, рецептор эпидермального фактора роста EGFR, полиморфизм генов.

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

Источник финансирования. Работа выполнена при финансовой поддержке гранта РНФ № 19-75-30016 «Новая технология прогнозирования и профилактики отдаленного метастазирования на основе детекции циркулирующих метастаз-инициирующих и нишеобразующих клеток и их специфических мишеней».

Соответствие принципам этики. Все пациенты подписали информированное согласие на участие в исследовании. Работа проведена согласно принципам добровольности и конфиденциальности в соответствии с «Основами законодательства РФ об охране здоровья граждан (Указ Президента РФ от 24.12.93 № 2288) на основании разрешения локального комитета по биомедицинской этике НИИ онкологии Томского НИМЦ.

Для цитирования: Бабышкина Н.Н., Дронова Т.А., Замбалова Е.А., Завьялова М.В., Слонимская Е.М., Чердынцева Н.В. Роль рецептора эпидермального фактора роста EGFR в эффективности неоадъювантной химиотерапии у больных тройным негативным раком молочной железы. *Бюллетень сибирской медицины.* 2020; 19 (1): 13–20. https://doi.org: 10.20538/1682-0363-2020-1-13–20.

INTRODUCTION

The triple-negative subtype occupies a special place in the structure of breast cancer morbidity, since it is characterized by an aggressive course of the disease and has an unfavorable prognosis for survival [1–3]. The most significant feature of triple-negative breast cancer (TNBC) is the absence of targets for hormone therapy and targeted therapy with Herceptin, which drastically complicates the treatment of this disease. Today, antitumor systemic therapy is one of the main stages of the TNBC complex treatment. When using neoadjuvant chemotherapy (NACT) cytotoxic drugs facilitate tumor shrinkage, which allows clinicians to perform organ-preserving operations and preserve healthy breast tissue to the maximum degree posible. However, a pathologic complete tumor response is observed only in 12–30% of patients [4, 5]. Thus, the search for additional prognostic markers of sensitivity and resistance to various groups of cytostatics, allowing to individualize therapeutic approaches for TNBC patients, remains an urgent task.

One of the molecular markers that has been actively studied in recent years is the epidermal growth factor receptor (EGFR), a transmembrane glycoprotein involved in the regulation of cell growth and malignant transformation. It is generally recognized that the amplification and / or overexpression of EGFR leads to the development of resistance to endocrine therapy in estrogen-dependent tumors [6, 7]. Our recent studies showed the prognostic significance of tamoxifen and demonstrated that patients resistant to tamoxifen therapy had a high level of EGFR expression in tumors [8]. In estrogen-independent tumors, EGFR overexpression is more a result of the increased number of gene copies due to polysomy than due to activating mutations or EGFR amplification and is usually associated with disease progression and low patient survival rates [9, 10]. It is worth noting that a large number of studies have been devoted to the prognostic value of EGFR expression in TNBC patients [11–14]. However, the contribution of functionally significant polymorphic sites of the EGFR gene in comparison with the expression level of its protein product in the mechanisms of resistance to neoadjuvant chemotherapy currently remains under-researched.

The aim of the study was to examine the correlation between the expression of the epidermal growth factor receptor (EGFR), functionally significant polymorphic variants of its gene and the neoadjuvant chemotherapy (NACT) efficacy in triple-negative breast cancer (TNBC) patients.

MATERIALS AND METHODS

The study included 70 patients aged 28–69 with a verified diagnosis of triple-negative breast cancer. The patients were undergoing treatment in the General Oncology Department of the Cancer Research Institute, Tomsk National Research Medical Center (NRMC) in the period from 2007 to 2013. All patients were treated with neoadjuvant polychemotherapy comprised of 2–4 cycles of FAC (5-fluorouracil 500 mg/m^2 on day 1, Adriamycin 50 mg/m^2 on day 1, cyclophosphamide 500 mg/m^2 on day 1, IV; the interval between courses is 21 days) or CAX regimen (cyclophosphamide 100 mg/m^2 IM for 14 days, Adriamycin 30 mg/m^2 IV on days

1, 8; Xeloda 1000 mg/m^2 2 times/day, per os, for 14 days; the interval between courses is 21 days) followed by surgical treatment (in the scope of radical resection, sector resection with axillary lymph node dissection (ALND) or radical mastectomy). Courses of polychemotherapy (FAC) and radiation therapy were given according to the indications in the adjuvant mode.

The NACT efficacy was assessed according to the RECIST scale. An objective clinical response was measured by the sum of complete and partial regressions of the breast tumor. The presence of disease stabilization and disease progression was considered as a lack of efficacy. The severity of drug pathomorphism in breast tissue and regional lymph nodes was evaluated conforming to the classification proposed by E.F. Lushnikov (1977) [15]. Patients were diagnosed with "complete morphological regression" when there were no tumor elements both in the breast tissue and in the lymph nodes under study. The follow-up period was 12–80 months.

In order to study the polymorphic variants of the EGFR gene, DNA was extracted from peripheral blood samples using the QIAamp DNA Mini Kits (50) (Qiagen). Qualitative and quantitative assessment of DNA was carried out on a NanoDrop-1000 spectrophotometer (NanoDrop, USA). Polymorphic variants of the EGFR gene at the rs2227983 and rs1468727 loci were studied using real-time polymerase chain reaction (PCR) using the TaqMan technology.

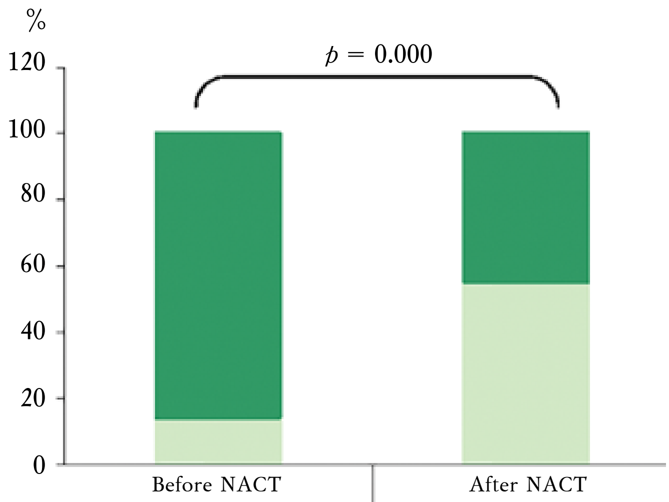
The sequences of primers and samples were selected by the OligoAnalysisVector NTI program using a genetic database (www.ncbi.nlm.nih.gov). The 15 μl PCR reaction mixture included 100 ng of genomic DNA; 0.5–1.5 μl of a specific pair of primers and samples with a concentration of 1 PFU / ml; 200 μm of each deoxynucleotide triphosphate; 1.2–2.0 μl of buffer (60 mM Tris-HCl (pH 8.5 at 25 °C), 1.5 mM MgCl2; 25 mM MKCl; 10 mM 2-Mercaptoethanol; 0.1% Triton X-100) and 0.5–1.0 units Taq DNA polymerase ("Medigen", Novosibirsk). The amplification program included initial denaturation at 95 °C for 2 min, followed by 40 cycles at 95 °C (10 s), annealing at a specific temperature for each pair of primers (30 s) on a CFX96 thermal cycler (Bio-Ra, USA).

The EGFR expression level in the tumor before and after NACT was studied on paraffin sections using the immunohistochemical method. Antibodies to EGFR (clone SP9, working dilution 1: 100) from Novus Biologicals were used. The results of immunohistochemical reactions were evaluated semi-quantitatively, depending on the proportion (%) of positively stained cells and their staining intensity in at least 10 areas of each section at 400x magnification. The staining intensity was evaluated on a scale of 0 to 3, when 0 was defined as negative staining, 1+ as weak staining, 2+ as moderate staining, and 3+ as strong staining. Sections with moderate (2+) or strong (3+) cytoplasmic and / or membrane staining in more than 10% of the cells were considered EGFR-positive, sections with negative staining (0) or weak (1+) expression in less than 10% of the cells were considered EGFR-negative.

SPSS 21.0 (IBM SPSS Statistics, Armonk, NY, USA) was used to analyze the obtained data. The distribution of genotypes of the studied genes was checked for compliance with the Hardy-Weinberg equilibrium. A two-way F-test was used to compare the frequencies of alleles and genotypes of the EGFR gene, to assess their correlation with the level of EGFR expression, as well as to analyze the correlation between the level of EGFR expression and the NACT efficacy. If the number of observations in the contingency table was more than 5, then χ^2 with the Yates correction was taken into account. Differences were considered reliable when at a significance level of $p < 0.05$.

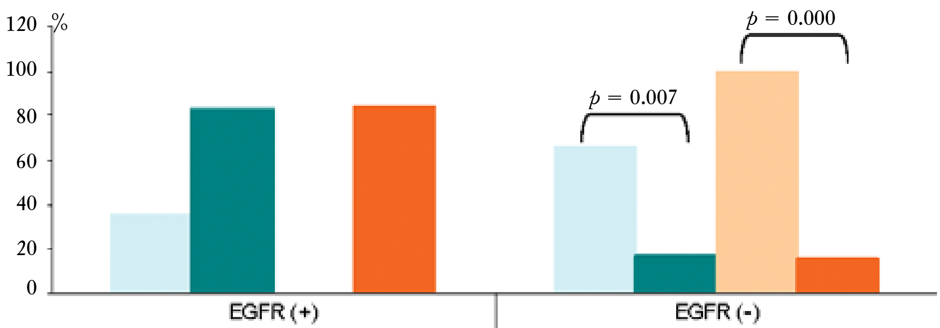
RESULTS

To analyze changes in the EGFR expression level in a tumor, we studied the content of cells with negative and positive expression in biopsy samples before neoadjuvant chemotherapy and in postoperative samples after treatment. It was discovered that the EGFR expression indices had significantly changed during the course of NACT. So, the number of cells with positive EGFR expression decreased from 85.7 to 44.8%; the number of EGFR-negative cells, in contrast, increased from 14.3 to 55.2% ($p = 0.000$, Fig. 1). Since it was found that NACT leads to a decreased EGFR expression level, we analyzed how these expression features were connected with the tumor response to therapy. It was revealed that a high frequency of both objective clinical and pathological complete responses of tumor was observed in patients with a low EGFR expression level ($p = 0.007$ and $p = 0.000$, respectively, Fig. 2).



	Before NACT	After NACT
EGFR (+)	85.7	44.8
EGFR (-)	14.3	55.2

Fig. 1. The EGFR expression level in the tumor tissue before (*a*) and after (*b*) neoadjuvant chemotherapy



	EGFR (+)	EGFR (-)
cOR complete/partial regression	34.8	65.2
NR stabilization/progression	83.3	16.7
pCR complete morphologic response	0	100
nonpCR no response	83.9	16.1

Fig. 2. The correlation between the EGFR expression level in the tumor tissue and the NACT efficacy

Next, in order to assess their possible involvement in triggering sensitivity and resistance mechanisms of the tumor to NACT, polymorphic variants of the EGFR gene were studied at the two loci (rs1468727 and rs2227983) in the peripheral blood samples of TNBC patients. An analysis of objective clinical response revealed that the frequency of occurrence of the mutant EGFRCC genotype at the rs1468727 locus in patients with stabilization or disease progression was more than 2 times higher than that in patients with complete or partial regression, however, without statistical differences (p = 0.114, Table 1). The study of complete morphological regressions made it possible to associate this mutant variant with an inefficacious tumor response (p = 0.042, Table).

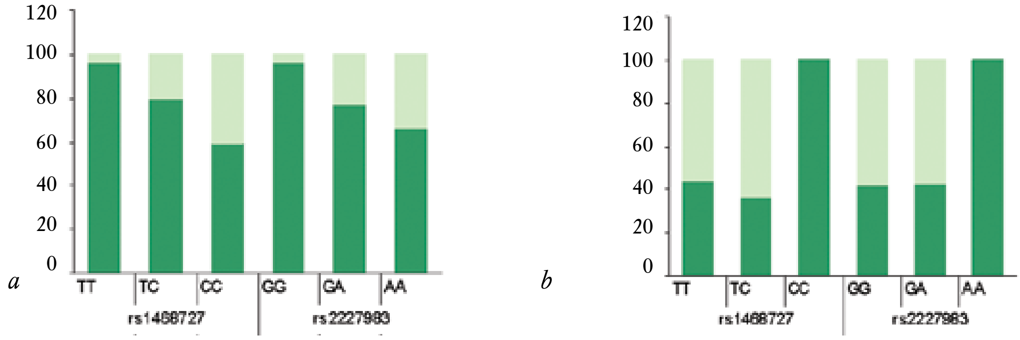
We analyzed the correlation between the EGFR polymorphic variants under study and the expression level of the protein encoded by it. It was discovered that carriers of mutant EGFRCC genotypes of rs1468727 more often had positive expression of EGFR in the tumor before NACT when compared with carriers of the wild-type EGFR gene (p = 0.047, Fig. 3). After NACT, positive expression of EGFR in the tumor was found in all patients (100%) having mutations in this polymorphic locus (p = 0.038, Fig. 3).

Table

The correlation between the ***EGFR*** polymorphisms and NACT efficacy

Genotype/ allele	Objective clinical response, *n* (%)			Pathological complete response, *n* (%)		
	Complete/ partial	Stabilization/ progression	OR (95% CI) *p*	Complete response	No response	OR (95% CI) *p*
			EGFR (rs1468727)			
TT	25 (54.4)	7 (46.7)	1.00	12 (54.5)	20 (51.3)	1.00
TC	17 (36.9)	5 (33.3)	1.00	10 (45.5)	12 (30.8)	1.00
CC	4 (8.7)	3 (20.0)	0.38(0.06–2.54) 0.348	0 (0.0)	7 (17.9)	1.22(1.05–1.44)0.042[1]
T	67 (72.8)	19 (63.3)	1.00	34 (77.3)	52 (66.7)	1.00
C	25 (27.2)	11 (36.7)	0.64(0.25–1.69) 0.322	10 (22.7)	26 (33.3)	0.59(0.23–1.48) 0.217
			EGFR (rs2227983)			
GG	25 (54.4)	7 (46.7)	1.00	12 (54.5)	20 (51.3)	1.00
GA	19 (41.3)	6 (40.0)	1.00	10 (45.5)	15 (38.5)	1.00
AA	2 (4.3)	2 (13.3)	0.30(0.03–3.34) 0.251	0 (0.0)	4 (10.2)	1.11(1.00–1.24) 0.287
G	69 (75.0)	20 (66.6)	1.00	34 (77.3)	55 (70.6)	1.00
A	23 (25.0)	10 (33.4)	0.67(0.25–1.79) 0.372	10 (22.7)	23 (29.4)	0.70(0.27–1.79) 0.420

[1] differences of indices between groups “complete response” and “no response”.



	TT	TC	CC	GG	GA	AA
EGFR (–) before NACT	3.3	20	40	3.3	22.7	33.3
EGFR (+) before NACT	96.7	80	60	96.7	77.3	66.7

	TT	TC	CC	GG	GA	AA
EGFR (–) after NACT	55.6	63.2	0	57.7	57.2	0
EGFR (+) after NACT	44.4	36.8	100	42.3	42.8	100

Fig. 3. The correlation between the EGFR expression level in the tumor tissue before (*a*) and after (*b*) neoadjuvant chemotherapy and the EGFR polymorphisms

DISCUSSION

The study showed a significant decrease in the level of EGFR expression in the tumor during NACT, which is associated with an objective clinical and pathological complete response of the tumor. Literary sources confirm the obtained data. It is known that the use of standard combinations of alkylating agents and taxanes for the treatment of locally advanced or metastatic breast cancer leads to a decrease in the initially high level of EGFR expression, which has prognostic significance [16]. In addition, the EGFR expression level is currently being considered as a potential predictive marker of response to NACT in TNBC patients [17].

Our study associates the lack of an effective response to preoperative chemotherapy in TNBC patients with their mutant EGFR genotypes of rs1468727. It is known that the polymorphic variant of rs1468727 affects the intron region of EGFR and does not directly alter the amino acid sequence of the protein. However, mutations within introns may significantly influence transcription and RNA stability. A mutant variant of the EGFRCC (rs1468727) may be connected with an increase in receptor activity, its expression or stability, which leads to the activation of EGFR-mediated signals and significantly increased proliferative potential of the tumor [18]. Our studies confirm this hypothesis since the mutant EGFRCC genotype is related to no tumor response to NACT. It is important to note that tumors in 60% of patients with this mutation are characterized by positive expression of EGFR. A high level of EGFR expression may contribute to the activation of numerous intracellular messengers, including PI3K / Akt, Ras / MAPK, STAT, which stimulates proliferative processes, increases the invasive potential of the tumor, and eventually contributes to ineffective treatment. It should be noted that the EGFR gene polymorphism under study is scarcely described in the literature. It is only the connection of the rs1468727 mutation and the prognosis of gliomas that has been demonstrated [19].

CONCLUSION

The NACT efficacy in TNBC patients is associated with the genotypic and phenotypic features of EGFR. The mechanisms of ineffectiveness of neoadjuvant chemotherapy can be caused by the EGFR mutation of rs1468727, which leads to the high expression activity of the receptor. This determines the realization of EGFR-mediated signaling cascades providing tumor proliferative potential. The decreased EGFR expression level in tumor tissue after NACT and the polymorphic variants of the EGFR gene (rs1468727) can be considered as potential molecular criteria related to the effectiveness of neoadjuvant chemotherapy in TNBC patients.

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Babyshkina N.N. – conception and design, analysis and interpretation of data. Dronova T.A. – analysis and interpretation of data. Zambalova E.A. – analysis and interpretation of data, literature review. Zavyalova M.V. – analysis and interpretation of data. Slonimskaya E.M. – conception and design. Cherdyntseva N.V. – conception and design.

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Asthenia as a pressing health issue for women with non-psychotic mental disorders: age perspective

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ABSTRACT

The aim of the study was to investigate the clinical phenomena of asthenia and subjective well-being of women aged 40-65 with non-psychotic mental disorders.

Materials and methods. A complete examination of 204 women aged 40–65 who received treatment for non-psychotic mental disorders within 1 year has been conducted. The data on the clinical phenomena of asthenia were supplemented by patients' rank assessment of fatigue and mood, determination of asthenia severity using the Multidimensional Fatigue Inventory (MFI-20), a calculation of the Kupperman menopausal index, and an assessment of the emotional component of well-being on the subjective well-being scale. For statistical processing, the methods of descriptive statistics and non-parametric statistics were used (for comparing independent samples – the Kruskal – Wallis criterion and Mann – Whitney U-criterion were applied; for identifying the connection of signs – Spearman rank correlation was used).

Results. The profile of the identified disorders included neurotic, stress-related and somatoform disorders (F40-49) – 67.7%, organic non-psychotic disorders (F06.4; F06.6) – 27.0%, and affective disorders (F34.1) – 5.4%. The organic asthenic disorder was also the second most frequent diagnosis among 69 patients. 196 women complained of fatigue. Patients with complaints of constant fatigue were significantly more likely to report headache, irritability, low mood, pessimistic thoughts, drowsiness, and asthma attacks. Their low level of subjective well-being correlated with higher rates of asthenia on MFI-20 subscales, except for the "Reduced Motivation" subscale.

The rates for all MFI-20 subscales among women with asthenic syndrome were lower than for patients with the depressive syndrome. A lower level of subjective well-being was revealed in patients with depressive, anxiety-depressive and anxiety-phobic syndromes, which differed by more pronounced manifestations of asthenia.

Conclusion. The conjugation and complementarity of the scales used in the study made it possible to measure both asthenia and the emotional state and subjective well-being of women with non-psychotic mental disorders associated with it.

Key words: asthenia, non-psychotic mental disorders, menopause, women.

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

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Conformity with the principles of ethics. All patients signed a written informed consent. The study was approved by the local Ethics Committee at Mental Health Research Institute of Tomsk NRMC (Protocol No. 99 of 17.04.2017).

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Астения как актуальная проблема здоровья женщин с непсихотическими психическими расстройствами: возрастной аспект

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РЕЗЮМЕ

Цель исследования – изучение клинических проявлений астении и субъективного благополучия у женщин с непсихотическими психическими расстройствами в возрасте 40–65 лет.

Материалы и методы. Проведено сплошное обследование 204 женщин в возрасте 40–65 лет, поступивших в течение 1 года на лечение по поводу непсихотических психических расстройств. Данные о клинических проявлениях астении дополнялись ранговой оценкой пациентами утомляемости и настроения, определением выраженности астении с помощью субъективной Шкалы оценки астении MFI-20; расчетом менопаузального индекса Купермана; оценкой эмоционального компонента благополучия по Шкале субъективного благополучия. При статистической обработке использовались методы описательной статистики, непараметрической статистики (для сравнения независимых выборок – критерий Краскела – Уоллиса, *U*-критерий Манна – Уитни; для выявления взаимосвязи признаков – корреляционный анализ по Спирмену).

Результаты. Структура выявленных расстройств включала невротические, связанные со стрессом и соматоформные расстройства (F40-49) – 67,6%; 27,0% – Органические непсихотические расстройства (F06.4; F06.6); 5,4 % – Аффективные расстройства (F34.1). Органическое астеническое расстройство было также вторым диагнозом у 69 пациенток. Жалобы на утомляемость предъявили 196 женщин. Пациентки с жалобами на постоянную утомляемость значимо чаще отмечали головную боль, раздражительность, сниженное настроение, пессимистические мысли, сонливость, приступы удушья. Выявленный у них низкий уровень субъективного благополучия коррелировал с более высокими показателями астении по субшкалам MFI-20, кроме субшкалы «Снижение мотивации».

У женщин с астеническим синдромом показатели по всем субшкалам MFI-20 были ниже, чем у пациентов с депрессивным синдромом. Выявлен меньший уровень субъективного благополучия у пациенток с депрессивным, тревожно-депрессивным и тревожно-фобическим синдромами, которые отличались и более выраженными проявлениями астении.

Заключение. Сопряженность и взаимодополняемость использованных в исследовании шкал позволяют измерить как астению, так и ассоциированные с ней эмоциональное состояние и субъективное благополучие женщин с непсихотическими психическими расстройствами.

Ключевые слова: астения, непсихотические психические расстройства, менопауза, женщины.

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INTRODUCTION

Asthenia is a non-specific condition widespread both in psychiatric practice and in a clinical picture of internal diseases, which incidence rates in the population are from 12 to 18%, at the reception of general practitioners – in 20–25% of patients [1]. Elevated fatigue is observed in many mental disorders and somatic diseases revealed in women in the period of perimenopause and postmenopause. In the age range of 40–65 years, it is important to estimate the absolute total cardiovascular risk on the European SCORE scale, and to identify diseases and conditions that increase the risk of cardiovascular complications. Russian researchers are studying the risk factors of development of cardiovascular diseases such as obesity, arterial hypertension, dyslipidemia, insulin resistance, diabetes, etc. [2]; biomarkers of cardiovascular diseases [3], and the influence of thyroid hormones [4]. Domestic cardiologists state significant limitations of ability to work due to “combined cardiometabolic problems” of women up to age 60, which become the cause of termination of working activity [5]. The most frequent problems for Russian women aged 45–60 are depressive mood, physical and mental fatigue, and discomfort associated with pains in muscles and joints [6]; while in China women aged 40–60 have physical and mental exhaustion (90.3%), discomfort in joints and muscles (88.5%), irritability (78.1%) and sleep disorders (77.1%) [7]. Polish investigators found depressive symptoms in 25.5% of 815 healthy women aged 45–60 using the Beck Depression Inventory (BDI), in 40.6% – anxiety was identified (according to data of the State-Trait Anxiety Inventory (STAI)). Researchers also determined their interrelationship with insomnia, problems with concentration, fatigue and psychomotor “agitation” [8]. The literature covers multiple studies of prevalence and structure of depressive disorders of women in the perimenopause and postmenopause periods [9]; signs typical of climacteric depression are described [10].

Cross symptoms of asthenia and depression such as weakness, fatigue, sleep disorders often complicate the early diagnosis that is worsened in the presence of concurrent diseases of internal organs [11]. In most cases, psychoemotional disturbances found in women in the “period of menopausal transition” [12] and the postmenopause are associated with symptoms of comorbid diseases of internal organs [13] and psychopathologic disorders caused by coping with the influence of psychosocial factors. Research implemented in this direction is focused on recovery and/or support of women’s energy resources, maintenance of their working efficiency and improvement of the quality of life.

The aim of the study was to investigate the clinical phenomena of asthenia and subjective well-being of women aged 40–65 with non-psychotic mental disorders.

MATERIALS AND METHODS

The study was performed at the 1st Clinical Psychiatric Unit of the Clinic of Mental Health Research Institute of Tomsk NRMC. A complete examination was conducted on 204 women aged 40–65, who received treatment for non-psychotic mental disorders and signed a written informed consent to participate in the study. The diagnosis verification of the current mental disorder was accomplished according to diagnostic criteria of ICD-10. In accordance with inclusion criteria, the study group included patients with “neurotic, stress-related, somatoform disorders, organic non-psychotic disorders, and affective disorders (dysthymia).” Exclusion criteria were cases of verified diagnosis of schizophrenia, presence of signs of dementia, and affective disorders (except for dysthymia).

Clinical psychopathologic, physical, psychological, clinical laboratory, and statistical methods were used in the study. For each patient, a "Score card of complex evaluation of the patient's mental and physical health" was completed.

The data on clinical phenomena of asthenia were supplemented by patients' rating of the fatigue and mood and identification of severity of asthenia with use of the MFI-20 subjective rating scale of asthenia (The Multidimensional Fatigue Inventory; Smets E.M.A., Garssen B.J., Bonke B., De Haes J.C., 1995) [14]. The results covered 5 components (subscales) of asthenia: "General Fatigue", "Physical Fatigue", "Reduced Activity", "Reduced Motivation", and "Mental Fatigue". Indexes higher than 12 points on any of the subscales confirmed the presence of asthenia, a total score on five subscales more than 60 confirmed clinically expressed asthenia.

In the course of study, the comparative analysis of psychopathologic symptoms was conducted in groups of women divided according to the criteria of age evaluation changes of the hypothalamic-pituitary-ovarian system functioning (Stages of ageing of the reproductive system of women according to criteria of Stages of Reproductive Aging Workshop (STRAW) +10 (2011)) [12].

For the quantitative evaluation of the severity of the climacteric syndrome, the Kupperman menopausal index (Kupperman H.S., 1953, modified by E.V. Uvarova, 1982) was measured. The body mass index was calculated from the formula: body weight (kg) / heightI(m).

For measuring the emotional component, the Scale of subjective well-being (Perrudet-Badoux A., Mendelssohn G., Chiche J. (1988), adjusted by M.V. Sokolova (1996)), was used. The mean value was 5.5 ± 2. The higher the index, the lower the subjective well-being of the subject.

The statistical processing was conducted with the use of Statistica 8.0 software. For evaluation of non-parametric data during comparison of independent samples, the Kruskal-Wallis and Mann – Whitney criteria were used. The data were presented as a median, upper and lower quartiles *Me* (Q_1–Q_3). To understand the connection of signs, the Spearmen's correlation analysis (*Rs*) was used. During contingency table analysis, the Fisher's exact test was applied. The critical level of significance during the testing of the hypotheses was accepted as $p = 0.05$.

RESULTS AND DISCUSSION

Currently, only 109 (53.43%) out of 204 examined women addressed the psychiatrist for the first time, 45 (22.05%) entered the treatment for the second time, and 50 (24.5%) had three and more hospitalizations in the medical history. More than a half of patients, 55.39% (113/204), noted stressful situations preceding to this hospitalization, including relations with spouses, parents, children or problems at work.

120 women (58.82%) had secondary vocational education, 75 women (36.6%) – higher education, 8 women (3.92%) – secondary education, 1 person – no higher education. There were 108 married women (52.94%), 88 single women (43.13%) and 8 women were in a relationship. 159 people (77.94%) continued their working activity. Among all 204 women, 48 women (57.14%) were postmenopausal. The structure of non-psychotic mental disorders in the total group included 47.5% (n = 97) of adjustment disorder (F43); 27.0% (55) of organic non-psychotic disorders (F06.4; F06.6); 18.6% (38) of other anxiety disorders (F41); 5.4% (11) of affective disorders (F34.1); and 1.5% (3) of somatoform disorders (F45). The second most frequent diagnosis in 49 women with adjustment disorder and 20 women with anxiety disorders was organic asthenic disorder. The total number of organic disorder cases was 60.8% (124 of 204 women). The concurrent diseases of internal organs were hypertensive illness (predominantly stage II) – 75.98% (155/204), thyroid diseases (more often chronic thyroiditis) – 48.5% (99), diseases of gall-bladder, bile ducts and pancreas – 39.7% (81), diseases of joints (osteoarthritis – 35.8% (73), diseases of esophagus, stomach and duodenum – 20.1% (41), irritable bowel syndrome – 14.7% (30), and liver diseases – 6.4% (13). High combinability of hypertensive illness with metabolic dysfunctions of carbohydrate and fatty metabolisms, type II diabetes mellitus – 5.4% (11), disturbance of glucose tolerance – 12.3% (25), obesity – 37.3% (76), etc. was found.

196 women (96.1%) made complaints of fatigue. Headaches were observed in 74.5% of cases (n = 152), dizziness – in 60.8% (124), sleep disorders – in 82.4% (168), drowsiness – in 48.0% (98), complaints of attention and memory distur-

bances constituted 51.0% (104), mood swings, low mood –74.5% (152), pessimistic thoughts – 41.2% (84), "hot flashes" – 43.6% (89), asthma attacks – 18.6% (38), increased excitability, irritability – 58.8% (120).

Significant differences in the severity of fatigue between groups of working and not working women, married and single women, women with the first or repeated hospitalizations, and women with the presence or absence of menopause were not revealed. Severity of fatigue did not depend on the presence or absence of hypertensive illness. Correlations between the degree of severity of the reported fatigue and age at the moment of evaluation, age of menopause, duration of menopause and the body mass index were not revealed.

During severity ranking of fatigue, 8 patients (average age 55.0 (48.5 to 58.0 years)) denied the presence of the symptom (group 0). 32 patients (average age 53.0 (48.0 to 56.5 years)) noted periodically an arising state of fatigue (group 1); 80 patients (average age 52.0 (45.5 to 57.0 years)) often experienced increased fatigue (group 2); 84 patients (average age 52.0 (45.5 to 57.0 years)) complained of constant severe fatigue (group 3).

Among multiple symptoms in the clinical picture of the patients' current syndrome, intergroup differences on fatigue severity were found depending on the presence/absence of headache (χ^I = 13.98; p = 0.002932). The patients with headache more often complained about constant fatigue (F = 3.245; p = 0.000). In Group 1 complaints of headache were less common (differences with Group 2 were confirmed χ^2=5.78; p = 0.016204; with Group 3 – χ^2=7.37; p = 0.006615) along with pessimistic thoughts (χ^2=6.34; p = 0.042084; and χ^2 = 11.49; p = 0.003204, respectively). Significant differences were revealed between Groups 1 and 3 in frequency of complaints of asthma attacks (χ^2 = 5.26; p = 0.021827; F = 6.555; p = 0.000), drowsiness (χ^2 = 18.33; p = 0.000019, F = 19.378; p = 0.000), low mood (χ^2 = 11.38; p = 0.009859), which also were less common in Group 1. No difference in the frequency of sleep disorders between the groups were found. In Groups 2 and 3, the frequency of headache complaints, low mood, pessimistic thoughts, "hot flashes" and a number of other symptoms did not differ. However, there were differences in the frequency of asthma attacks (χ^2 = 7.27; p = 0.006999; F = 7.622; p = 0.000), elevated excitability and irritability (χ^2 = 6.18; p = 0.012906; F = 6.260; p = 0.000), and drowsiness (χ^2 = 13.96; p = 0.000187; F = 14.372; p = 0.000), which were observed more often in Group 3.

Thus, patients with complaints of constant fatigue (Group 3) were more likely to have such symptoms as irritability, elevated excitability, low mood, pessimistic reflections, drowsiness and asthma attacks.

The comparative analysis of indexes of subjective well-being revealed significant differences between Group 0 (3.5 (2.0–5.0) points) and Group 1 (6.0 (4.0–7.5) points), Group 2 (6.0 (5.0–7.0) points) and Group 3 (7.0 (6.0–8.0) points), that confirmed emotional well-being of patients who did not experience fatigue (p_{0-1} = 0.019645; p_{0-2}=0.004650; p_{0-3}= 0.000222). Significant differences were also found between Groups 1 and 3 (p = 0.007481) Groups 2 and 3 (p = 0.009579) in the absence of differences between Groups 1 and 2. It indirectly indicates similar effects of "occasionally" and "often" arising states of tiredness (likely, not sufficiently differentiated by patients linguistically during completion of the questionnaire) on the level of subjective well-being. According to the results interpretation on the Scale of subjective well-being, higher indexes in Group 3 confirmed a low level of subjective well-being in patients with complaints of constant fatigue.

A positive correlation of the indexes on the Scale of subjective well-being and the value of the menopausal index (Rs = 0.37; p = 0.000000) were calculated, reflecting a decrease in subjective well-being of women as the menopausal index increased. The differences between Groups 0, 1, 2 and 3 were found. Table 1 shows the values of the menopausal index in groups of patients aged 40–65 with non-psychotic mental disorders with different severity of fatigue.

With menopausal index values from 35 to 58 points, which were noted in women in Groups 2 and 3, it was possible to conclude that moderate severity of the climacteric syndrome and vegetative disturbances were predominant.

A direct correlation between indexes on the subjective Scale of asthenia evaluation MFI-20 and on the Scale of subjective well-being in the group of 204 women was found: the more the asthenia was expressed, the lower subjective well-being of the patient was.

Table

Menopausal index values in groups of women aged 40–65 years with different severity of fatigue

Groups	Number of patients	Menopausal Index, Me (Q_1–Q_3)	p	U	Z
0	8	16.0 (13.0–25.0)	(0–1) 0.067869	74.00000	−1.82588
			(0–2) 0.005692	129.500	−2.76503
			(0–3) 0.000432	82.00000	−3.51964
1	32	23.5 (21.0–29.0)	(1–2) 0.000134	687.0000	−3.81932
			(1–3) 0.000000	334.5000	−6.23576
2	80	31.0 (25.0–37.0)	(2–3) 0.000004	1957.500	−4.61389
3	84	38.0 (31.5–44.0)			

Note: p – level of statistical significance of differences between the menopausal indexes in groups.

The indexes of subscales of MFI-20 were compared with the presented complaints of fatigue. Statistically significant correlations were obtained depending on the severity of fatigue on all subscales, that were the most significant for the total indicator of asthenia (Rs = 0.47; p < 0.001) and General Fatigue subscale (Rs = 0.53; p < 0.001). In particular, the average total scores of asthenia on MFI-20 were identified, which made 36.5 (31.0–41.5) points for Group 0, 54.0 (48.0–62.0) points for Group 1, 57.0 (49.0–65.0) points for Group 2 and 66.0 (59.0–72.0) points for Group 3. The obtained quantitative indexes of asthenia confirmed the absence of asthenia in patients in Group 0. In Group 1, the highest severity of asthenia was observed on the subscale "General Fatigue" (tiredness). In Group 2, indexes higher than 12 points were noted on the subscales "General Fatigue", "Physical Fatigue" (flabbiness in muscles and limbs, tiredness, willingness to have a rest), and "Reduced Activity". In Group 3 – along with the indexes listed above, high indexes on the subscale "Mental Asthenia" were obtained (difficulty of keeping attention, worsening of the acumen and memory). In all groups there was no increase in the indexes on the "Reduced Motivation" subscale, that indirectly testified to presence in patients of plans, willingness to implement them and to obtain pleasure from fulfilled businesses.

The subjectively evaluated mood, from mood swings to low mood, apparently correlated with all subscales of MFI-20. Patients not presenting mood complaints (n = 52) had low indexes on the subscales in comparison with the rest of the women. Significant differences were revealed between indexes in patients noting instability, variability of mood during the day (n = 77), and women with complaints of low mood (n = 64) in the total level of asthenia, on the subscales "Reduced Activity" and "Reduced Motivation" as well as patients rating their mood as "full apathy" (n = 11), on the subscales "Physical Fatigue" and "Reduced Motivation". In the meantime, the maximum severity of general, physical and total asthenia on the MFI-20 scale and reduced activity in women with complaints of low mood and apathy was identified. They also had more than 12 points on the "Reduced Motivation" subscale, higher frequency of elevated excitability, irritability and drowsiness during the day in spite of the absence of differences in the frequency of sleep disorders. The peculiarity of mental state of patients with complaints of low mood, in contrast with women without such complaints, was predominantly anxiety-depressive (44.0% vs. 15.4%) and depressive (13.3% vs. 3.9%) syndromes (χ^2 = 21.72; p = 0.000228), which were within adjustment disorder.

In conducting the comparative analysis of the level of asthenia on the subscales of MFI-20 in patients with different psychopathologic syndromes at the moment of the survey, a number of significant differences was revealed. The highest total scores of asthenia were noted in patients with the leading depressive (66.0 (61.0–74.0) points), anxiety-depressive (61.0 (53.0–68.0) points) (which differed them significantly from patients with asthenic syndrome – 55.5 (48.0–65.0) points) and anxiety-phobic (63.0 (53.0–70.0) points) syndromes. This indicator also indicated the significant differences between patients with anxiety and depressive syndrome (p = 0.009441), and depressive and anxiety-depressive syndrome (p = 0.039813). "General Fatigue" reached a higher level than other characteristics of asthenia, irrespective from the leading psychopathologic syndrome.

In women with asthenic syndrome, indexes on all the subscales of MFI-20 were lower than in patients with depressive syndrome (in all cases $p < 0.05$); and the indexes on the subscale "Reduced Activity" were lower than in patients with anxiety-depressive and anxiety-phobic syndromes. Between patients with anxiety and depressive syndromes the significant differences on all the subscales of MFI-20 were found ($p < 0.05$), except on the subscale "Mental Fatigue". Patients with anxiety-depressive and anxiety-phobic syndromes had higher indexes on the scale "Reduced Activity" than patients with anxiety syndrome. "Physical Fatigue" was more severe in patients with depressive syndrome (14.0 (13.0–16.0) points) in comparison with patients with anxiety-depressive syndrome (12.0 (11.0–15.0) points; $p = 0.008555$).

During evaluation of subjective well-being in patients with different psychopathologic syndromes at the moment of the survey, a lower level of well-being was revealed in patients with depressive, anxiety-depressive and anxiety-phobic syndromes which were also notable by more severe manifestations of asthenia.

Thus, elevated fatigue in the overwhelming majority of cases is included in the symptom complex of the current state of women with non-psychotic mental disorders. As a main symptom, asthenia is often observed in adjustment disorder, neurotic and affective disorders, an is an integral sign of organic asthenic disorder. A big number of concurrent diseases of internal organs, that add asthenic manifestations in women in the period of peri- and postmenopause, make the issues of differential diagnosis and appropriate therapeutic strategies topical.

The findings show additional possibilities of use of the subjective Scale of asthenia evaluation MFI-20 in the survey of patients with non-psychotic mental disorders. The subscale "Reduced Motivation", intact in evaluation of different severity of asthenia in subjective complaints of patients, represents a differential sign indicating the presence of low mood. The sensitivity of the MFI-20 subscales to the patient's subjective perception of the variability of their own mood and the revealed differences of asthenia indexes depending on the leading psychopathologic syndrome expand the range of psychometric use of the Scale of evaluation of asthenia MFI-20 and are useful when differential diagnostic difficulties in differentiation of fatigue as a manifestation of asthenia or resource exhaustion arise.

CONCLUSION

The conducted analysis in the group of women with non-psychotic mental disorders allows recommendation of the use of the subjective evaluation scale of asthenia MFI-20, and the Scale of subjective well-being for quantitative confirmation of the dynamics of available symptoms in the mental state of patients along with the use of conventional psychometric scales of rank evaluation of fatigue and mood. The findings show conjugation and complementarity of these scales measuring not only asthenia, but also related emotional state and subjective well-being of the patient.

Early detection of asthenia in women aged 40-65 in view of the risk of cardiovascular complications and interrelationship with depressive and anxiety disorders is aimed at preventing later manifestations and formation of cognitive disorders and senile asthenia. This is the list of the main targets of the current preventive direction and medical examination of the population in primary health care.

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Authors contribution

Belokrylova M.F. – conception and design, carrying out of the practical part of the study, analysis and interpretation of data, critical revision for important intellectual content, final approval of the manuscript for publication. Garganeeva N.P. – conception and design, carrying out of the practical part of the study, analysis and interpretation of data, critical revision for important intellectual content, final approval of the manuscript for publication. Nikitina V.B. – conception and design, carrying out of the practical part of the study, analysis and interpretation of data, critical revision for important intellectual content, final approval of the manuscript for publication. Epanchintseva E.M. – carrying out of the practical part of the study.

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Affective disorders in comorbidity with alcohol addiction: clinical and dynamic features, social adaptation level of patients

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ABSTRACT

The aim of the study was to reveal clinical and dynamic characteristics of affective disorders (AD) in comorbidity with alcohol addiction (AA).

Materials and methods. 65 patients with affective disorders [22 women (34%) and 43 men (66%)] were examined. The main group included 34 patients aged 44.5 [36.0; 51.5] with affective disorders and comorbid alcohol addiction The comparison group included 31 patients aged 45 years [32; 52] with affective disorders without comorbid narcological pathology. Compared groups were matched by sex, age and nosological structure ($p > 0.05$). The following methods were used in the study: clinical and psychopathological, clinical follow-up, psychometric, statistical, as well as these psychometric scales: Clinical Global Impression (CGI), Hamilton Depression Rating Scale (HDRS-17), Hamilton Anxiety Rating Scale (HARS), Social Adaptation Self-evaluation Scale (SASS).

Results. The comparative assessment of clinical and dynamic characteristics of affective disorders and social adaptation level was conducted. Chronology of occurrence of comorbid affective disorders and alcohol addiction was analyzed.

Conclusion. Addition of alcohol addiction to affective disorders worsens the clinical and dynamic indices and social adaptation level of patients.

Key words: affective disorders, alcohol addiction, comorbidity, clinical picture, adaptation.

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Аффективные расстройства при коморбидности с алкогольной зависимостью: клинико-динамические особенности, уровень социальной адаптации больных

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РЕЗЮМЕ

Цель исследования – выявление клинико-динамических характеристик аффективных расстройств (АР) при их коморбидности с алкогольной зависимостью (АЗ).

Материалы и методы. Обследованы 65 пациентов с АР – 22 женщины (34%) и 43 (66%) мужчины. Основная группа – 34 пациента с аффективными расстройствами и коморбидной алкогольной зависимостью в возрасте 44,5 лет [36,0; 51,5]. Группа сравнения – 31 пациент с аффективными расстройствами без коморбидной наркологической патологии в возрасте 45 лет [32; 52]. Сравниваемые группы были сопоставимы по полу, возрасту и нозологической структуре ($p > 0,05$). В исследовании применялись следующие методы: клинико-психопатологический, клинико-катамнестический, психометрический, статистический, а также психометрические шкалы: шкала глобальной клинической оценки CGI, шкала депрессии Гамильтона HDRS-17, шкала тревоги Гамильтона HARS, шкала самооценки социальной адаптации SASS.

Результаты. Проведена сравнительная оценка клинико-динамических характеристик аффективных расстройств и уровня социальной адаптации. Проанализирована хронология возникновения коморбидных аффективных расстройств и алкогольной зависимости. Заключение. Присоединение алкогольной зависимости к аффективным расстройствам ухудшает их клинико-динамические показатели и уровень социальной адаптации пациентов.

Ключевые слова: аффективные расстройства, алкогольная зависимость, коморбидность, клиника, адаптация.

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

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INTRODUCTION

The problem of comorbidity of mental disorders remains relevant and requires further study, despite the huge number of studies devoted to it [1–4]. The high comorbidity level of affective disorders (AD) with other mental disorders is confirmed by the results of epidemiological and clinical studies [5, 4]. Alcohol addiction (AA) is one of the most common comorbid pathologies of individuals with AD along with anxiety and personality disorders [7–10]. The occurrence frequency of alcohol addiction among patients with AD is several times higher than that in the general population. In AD, men are more prone to develop an alcohol addiction, while women more often have anxiety disorders as comorbid pathologies [11]. In bipolar affective disorder (BAD), the risk of developing AA is six to seven times higher than in the general population, and with depression, it occurs in 25–40% [12, 13]. According to the data analysis collected from specialized institutions of eight European countries, among patients aged 18–64 years with alcohol addiction (n = 1767), depression was detected in 43.2% of cases (95% CI: 40.7–45.8) [14]. Patients with AD often use alcohol to alleviate symptoms of depression and anxiety [15]. In narcological pathology, the frequency of mood disorders also reaches a high level. Affective disorders increase the risk of AA and vice versa, but according to individual authors, such a pattern is observed only in males [16].

Numerous studies are devoted to identifying pathogenetic interactions in comorbidity between AA and affective pathology. The results indicating common genetic factors in the development of both disorders [17] and involvement of the same neurohumoral mechanisms in the pathogenesis have been obtained. Such personality traits as alexithymia and social anxiety are common psychological factors of alcoholism and depression [18]. Currently, the problem of correlation between AD and pathological attraction to alcohol remains unresolved and relevant.

It is known that AA can be formed before and after the development of affective pathology. According to some sources, mood disorder manifests itself earlier and alcohol addiction develops during the course of the disorder [19]. The comorbidity of AD with alcohol addiction leads to differentiation difficulties with developed AA and symptomatic alcoholism on the one hand and with depressive disorders (DD) and secondary depression due to ethanol intoxication or a person's reaction to the social consequences of alcoholism on the other hand. An important differential criterion for primary depression is the manifestation of DD before the development of alcoholism.

Affective disorders in comorbidity with AA are characterized by a more frequent recurrence of depressive episodes (DE), a large number of suicidal attempts, a greater degree of maladjustment, and a worse prognosis [20, 21]. The literature data on the effect of comorbid alcoholism on the effectiveness of antidepressant treatment in depressive disorders are mixed. Some authors point out the negative impact of alcoholism on the results of depression treatment with antidepressants [22], while others do not confirm this effect [23]. Also, alcohol abuse complicates the cooperation of the doctor and the patient and reduces patient compliance.

The objective of the study was to reveal the clinical and dynamic characteristics of affective disorders in comorbidity with alcohol addiction. To achieve the objective, a comparative assessment of the clinical and dynamic indicators of AD comorbid with AA and AD occurring in isolation was carried out.

MATERIALS AND METHODS

65 patients with AD were examined 22 women (34%) and 43 (66%) men. [24] The median (ME) age of the female patients was 45.5 years; interquartile range (MDQ) – [35; 56], the median (ME) age of the male patients was 38 years old [31; 57]. A marital status analysis of the patients in the study group revealed a significant proportion of single patients – 39 (60%): widowed – 9 (14%), divorced – 9 (14%), unmarried – 8 (12%). In the study group, patients with higher education accounted for 59% (n = 38), specialized secondary education – 20% (n = 13), and secondary education – 21% (n = 14). Depending on the AD, the patients of the studied sample were distributed as follows: bipolar disorder (BPD), current DE – 18% (n = 12), recurrent depressive disorder (RDD) – 42% (n = 27), DE – 26% (n = 17), dysthymia – 14% (n = 9).

The studied patients were divided into two groups. The main group included 34 patients with AD and comorbid AA (11 women and 23 men), aged 44.5 years [36; 51.5]. The nosological structure of AD was presented: BPD – 24% (n = 8), RDD – 38% (n = 13), DE – 26% (n = 9) and dysthymia – 12% (n = 4). The AA duration of patients was 8 years [3.5; eleven]. In 60% of cases (n = 19), during the period of depression, patients changed their manner of alcohol consumption – they began to drink alone and in smaller portions. In isolated cases (n = 3), intakes of immense amount of alcohol were observed. The main motives for alcohol consumption in the development of depressive symptoms were to distract from painful dark thoughts, block out the feeling of anguish, forget about problems, and cope with anxiety and insomnia.

The comparison group consisted of patients with AD without comorbid narcological pathology: 31 people (11 women and 20 men) aged 45 years [32; 52]. Affective disorders were represented by the following nosologies: BPD – 13% (n = 4); RDD – 45% (n = 14); DE – 26% (n = 8); dysthymia – 16% (n = 5). he compared groups were matched by sex, age, and nosological structure ($p > 0.05$).

The ethical principles of the World Medical Association Declaration of Helsinki (1975), as amended (2008), were observed in working with the patients involved. Of the main research methods the following were used: clinical and psychopathological, clinical follow-up, psychometric and statistical. During the study the following psychometric scales were used: Clinical Global Impression (CGI), Hamilton Depression Rating Scale (HDRS-17) and Hamilton Anxiety Rating Scale (HARS). The levels of life quality and social functioning of patients in various spheres of life were determined by using the Social Adaptation Self-evaluation Scale (SASS), developed in 1997 by M. Bosc, A. Dubini, V. Polin. The questions included in the scale are aimed at assessing satisfaction with certain spheres of life (work, family relations and relationships outside the family, leisure, etc.) and their social functioning.

The following AD characteristics were evaluated in the study groups [25]: age at the onset of AD, syndromic variant of depression, indicators of suicidal behavior of patients, the number of affective episodes per year with BPD and RDD, depression level by HDRS-17, anxiety by HARS, disease severity by CGI-S. The level of social adaptation of patients according to SASS and the chronological sequence of AD and AA development were also evaluated.

Statistical data processing was carried out on a personal computer using the Statistica for Windows (V. 8.0) package of standard applications. For quantitative indicators that do not meet the criteria for a normal distribution, the median and interquartile range Me [Q_1; Q_3] were calculated and the level of statistical significance of differences between the groups was determined by the Mann – Whitney criteria. The analysis of qualitative features was carried out through the study of their frequencies through contingency tables using the χ^2 criterion. When working with small samples, the F-test (Fisher's criterion) was used. The accuracy of the differences between the sample proportions was assessed using the Z-test.

RESULTS

The age at the beginning of AD in the main group was 28.5 years [20.0; 39.5], in the comparison group – 30 years [26; 40]. Groups for this indicator did not have statistically significant differences ($p > 0.05$). The distribution of patients in the studied groups depending on the leading depression syndrome is presented in Table 1. No intergroup differences in the syndromic structure of depression were found ($p > 0.05$).

Table 1

Distribution of patients from the main and control groups depending on the syndromic variant of depression, n (%)

Syndromic variants of depression	Main group	Comparison group
Anxious	13 (38)	9 (29)
Dysphoric	14 (41)	8 (26)
Hypochondriac	3 (9)	4 (13)
Conversion	2 (6)	5 (16)
Adynamic	2 (6)	5 (16)
Total	34 (100)	31 (100)

Next, the following indicators of suicidal behavior were analyzed: suicide ideation in the current episode and suicidal attempts in the past. The distribution of patients depending on the presence of suicidal thoughts in the current state did not have statistically significant intergroup differences ($p > 0.05$): in the main group, suicidal thoughts were present in the clinical picture in 65% of cases (n = 22), in the comparison group – in 48 % (n = 15). On the background of alcohol withdrawal syndrome, suicidal thoughts of the patients from the main group became the most distressing, often obsessive. Analysis of anamnestic and follow-up data indicated a more frequent occurrence of suicidal attempts in medical history of patients from the main group: 27% and 6%, respectively ($p < 0.05$). In the main group, severe depressive experiences and psycho-traumatic circumstances, which were often the social consequences of alcoholization, were the most significant for suicidogenesis.

Estimation of the number of affective episodes per year in patients diagnosed with RDD and BPD showed that in the main group the indicator was higher compared to the comparison group – 1.5 [0.9; 2.0] and 0.9 [0.7; 1.6], respectively, U = 200,000; Z = 2.509, p = 0.012.

According to HDRS-17, the severity of depressive symptoms in the groups did not have statistically significant differences (Table 2).

The Hamilton Anxiety Rating Scale score in the studied patients showed that in the main group there were more patients with a high level of anxiety than in the comparison group ($p < 0.05$). When analyzing the distribution of patients depending on the disease severity according to CGI-S, severe disorder (6 points) was detected more often in the main group than in the comparison group: 35% (n = 12) and 13% (n = 4), respectively ($p < 0.005$).

Table 2

Distribution of patients from the compared groups depending on the severity of depression and anxiety, n (%)

Scale	Main group			Comparison group		
	mild	moderate	severe	mild	moderate	severe
HDRS-17	2 (5.9)	24 (70.6)	8 (23.5)	4 (12.9)	23 (74.2)	4 (12.9)
HARS	1 (2.9)	10 (29.5)	23 (67.6)#	1(3.2)	17 (54.9)	13 (41.9)

# $p < 0.05$.

Depending on the total number of SASS points, patients of the compared groups were divided into three subgroups: with poor social adaptation (0–22 points), with difficult social adaptation (22–35 points) and with good social adaptation (35–52 points). Assessment of the social adaptation level of the studied patients in the main and comparison groups showed that a large proportion were patients with difficult and poor social adaptation (Table 3).

Table 3

Distribution of patients with different levels of social adaptation in the studied groups, n (%)

Indicator	Main group	Comparison group
Poor social adaptation	7 (21)	3 (10)
Difficult social adaptation	23 (68)	17 (55)
Good social adaptation	4 (11)	11 (35) #

# $p < 0.05$.

Also, it turned out that the main group consisted of fewer patients with good social adaptation according to the SASS ($p < 0.05$), as opposed to the comparison group. Assessment of the chronological sequence of the comorbid disorders occurrence in the main group showed that AD in most cases ($p < 0.05$) preceded the development of AA in 74% of cases (n = 25).

DISCUSSION

When analyzing the data, it is worth noting that in patients with AD, both with and without comorbidity with AA, anxiety and dysphoric variants of depression are revealed in more than half of the cases. According to the results of epidemiological and clinical studies, the prevalence of developed anxiety disorders in patients with AD and AA reached high values [9; 26; 27]. Patients with anxiety disorders were not included in the sample, and the existing symptoms of anxiety were an integral part of the AD and AA clinical picture. An assessment of anxiety severity in the groups revealed a higher level of anxiety in patients with a combination of AD with AA compared to patients with AD without comorbid AA. According to the results of the study, the age of patients to the onset of AD with and without comorbidity with AA was not statistically significant. The literature contains data on a younger age of AD manifestation with their comorbidity with other mental disorders [28].

As it is known, AD and AA are often accompanied by suicidal behavior [29, 32], and their comorbidity leads to an even greater risk for suicide [33]. The data on a history of suicidal attempts have confirmed that AD comorbidity with AA increases the risk of suicidal behavior of patients. The obtained indicators confirm the literature data on the negative impact of AD and AA on the social adaptation of patients. The combination of these disorders leads to a more pronounced decrease in this indicator.

In the studied patients, AD preceded the development of AA in most cases, which is consistent with the published data [19]. At the same time, a number of authors indicate that prior to the AD manifestation, alcohol abuse, but not AA, is more common [34].

CONCLUSION

The results of the study indicate that with AD with comorbid AA compared with AD without alcohol addiction, exacerbations of affective pathology are more likely to occur. There is a higher risk of suicidal behavior, anxiety and disease severity. Moreover, patients with comorbidity of these disorders have worse indicators of social adaptation than patients with AD alone. In most cases, alcohol addiction develops on the background of AD. Thus, alcohol addiction in comorbidity with affective disorders negatively affects their clinical and dynamic indicators and the social adaptation level of patients.

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Experimental study of the systemic hemostatic effects of fibrin monomer in inhibition of platelet aggregation

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ABSTRACT

The aim of the study was to examine the hemostatic effects of fibrin monomer in post-traumatic parenchymal hemorrhage against pharmacologically conditioned inhibition of platelet aggregation function.

Materials and Methods. In the *in vivo* experiments in male rabbits, the hemostatic effects of fibrin monomer (FM) (0.25 mg/kg) were evaluated in comparison with tranexamic acid (TXA) (15 mg/kg) in post-traumatic parenchymal hemorrhage against the background of preliminary inhibition of platelet aggregation function with acetylsalicylic acid (2.0 mg/kg) and clopidogrel (8.0 mg/kg). Volume and rate of blood loss as well as the parameters of the hemostatic system were estimated.

Results. In comparison to placebo it has been established that FM when administered intravenously 1 hour before the injury can prevent severe bleeding associated with taking antiplatelets. The volume of blood loss after FM administration decreased by 6.0 times, the rate of blood loss reduced by 5.9 times, and when using TXA it was reduced by 2.4 (P_{FM-TA}<0.02) and 4.8 times, respectively. The hemostatic effects of TXA were realized when the hemostatic balance was shifted towards the increased fibrin formation (an increase in D-dimer plasma level). The use of FM was not accompanied by any significant changes in the blood coagulation system.

Conclusion. The fibrin monomer at a dose of 0.25 mg/kg IV is capable of preventing severe post-traumatic parenchymal bleeding caused by the combined use of drugs exhibiting different antiplatelet action. The phenomenon is not completely clear and needs to be analyzed in further research. The mechanism of hemostatic effects associated with FM is currently being studied.

Key words: fibrin monomer, acetylsalicylic acid, clopidogrel, tranexamic acid, hemostatic effect.

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

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Conformity with the principles of ethics. The study was approved by the local Ethics Committee at Altai State Medical University (Protocol No. 12 of 12.11.2015). Animal studies were carried out in accordance with the Directive 86/609/EEC, the Declaration of Helsinki, and the “Rules of work with experimental animals”.

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Системные гемостатические эффекты фибрин-мономера при ингибировании агрегационной функции тромбоцитов в эксперименте

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РЕЗЮМЕ

Цель. Изучить гемостатические эффекты фибрин-мономера при посттравматическом паренхиматозном кровотечении на фоне фармакологически обусловленного угнетения агрегационной функции тромбоцитов.

Материалы и методы. В экспериментах *in vivo* на кроликах-самцах оценивали гемостатические эффекты фибрин-мономера (ФМ) (0,25 мг/кг) в сравнении с транексамовой кислотой (ТК) (15 мг/кг) при посттравматическом паренхиматозном кровотечении на фоне предварительного угнетения агрегационной функции тромбоцитов ацетилсалициловой кислотой (2,0 мг/кг) и клопидогрелом (8,0 мг/кг). Оценивали объем и темп кровопотери, а также показатели системы гемостаза.

Результаты. Установлено, что ФМ в сравнении с плацебо при внутривенном введении за 1 час до травмы способен профилактировать тяжелое кровотечение, связанное с приемом антиагрегантов. Объем кровопотери после введения ФМ снижался по медиане в 6,0 раз, темп кровопотери – в 5,9 раза, при использовании ТК – в 2,4 ($p_{\text{fM-TA}}$<0.02) и 4,8 раза, соответственно. Гемостатические эффекты ТК реализовывались при смещении гемостатического равновесия в сторону усиления фибринообразования (увеличение уровня D-димера в плазме крови). Применение ФМ не сопровождалось сколько-нибудь значимыми изменениями в системе свертывания крови.

Заключение. Фибрин-мономер в дозе 0,25 мг/кг, способен при в/в введении профилактировать тяжелое посттравматическое паренхиматозное кровотечение, вызываемое сочетанным приемом препаратов, обладающих различными механизмами антиагрегантного действия. Механизм гемостатических эффектов, связанных с ФМ, в настоящее время изучается.

Ключевые слова: фибрин-мономер, ацетилсалициловая кислота, клопидогрел, транексамовая кислота, гемостатический эффект.

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

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Соответствие принципам этики. Работы на животных, одобренные локальным этическим комитетом

ФГБОУ ВО АГМУ МЗ РФ (протокол № 12 от 12.11.2015 г.), проводили в соответствии с Директивой 86/609/EEC, Хельсинкской декларацией и «Правилами проведения работа с использованием экспериментальных животных».

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INTRODUCTION

Hemorrhagic syndrome can be life-threatening and may require emergency measures to preserve the patient's life and health in case of injuries, extensive surgery and iatrogenic exposure [1]. Moreover, reduction of bleeding associated with intensive drug-induced inhibition of platelet aggregation can be a serious problem for cardiac patients among others [2]. In this case, various therapeutic approaches are recommended to reduce blood loss, including donor platelet transfusion, the use of desmopressin (contributing to Von Willebrand factor expression by endotheliocytes) and/or administration of tranexamic acid that inhibits fibrinolytic reactions [3–5].

In the previous studies in our laboratory, when a controlled liver injury *in vivo* was inflicted, the phenomenon of significant hemostatic action of low-dose FM (des-AABB-fibrinogen) was found [6, 7]. This effect was also observed in intravascular thrombin inhibition induced by oral administration of dabigatran etexilate [8]. It should also be noted that in the above-mentioned experiments, FM was administered IV at a dose of 0.25 mg/kg that corresponds to its physiological blood plasma level of healthy people (less than 7.8 μg/ml) [9].

The aim of this study was to examine the hemostatic effects of fibrin monomer in post-traumatic parenchymal bleeding against pharmacologically conditioned inhibition of platelet aggregation function.

MATERIALS AND METHODS

The studies were performed on 49 healthy male rabbits of the Chinchilla breed weighing 3.0-4.5 kg, kept in standard vivarium conditions. Four groups of the animals were formed by block randomization (Fig. 1).

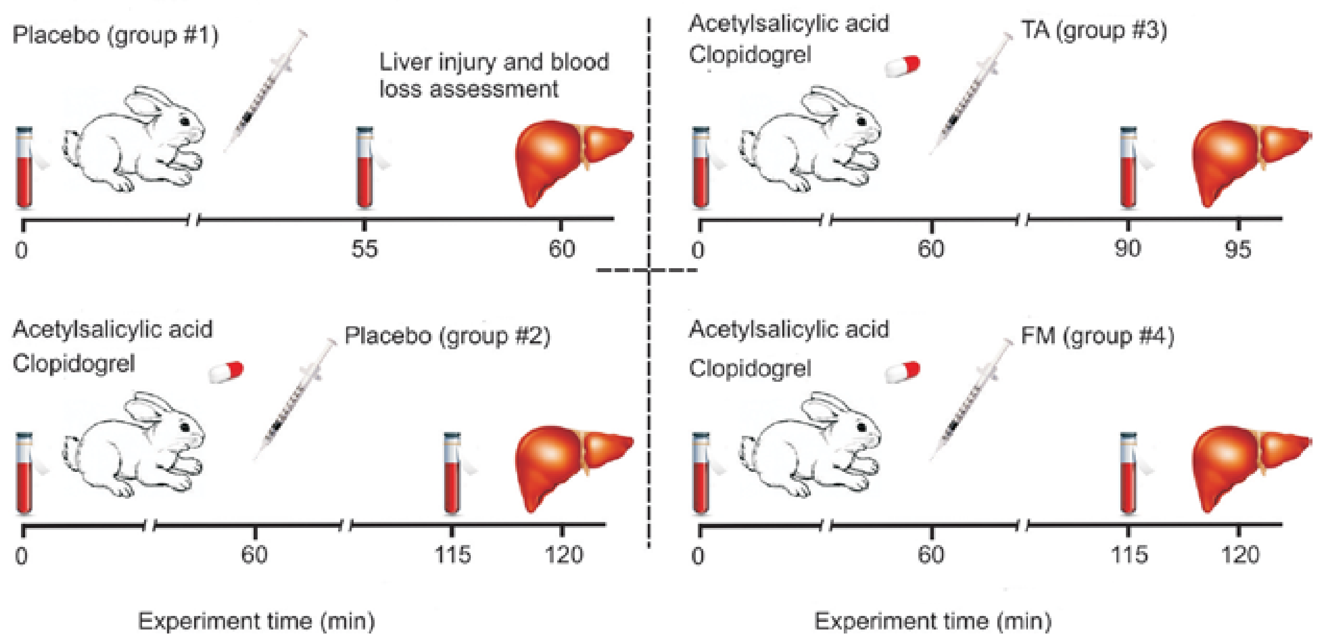

Fig. 1. Design of the study with a controlled liver injury.
Abbreviations and symbols: TXA – tranexamic acid; FM – fibrin monomer;

 – blood sampling to assess hemostasis;  – drug administration

The animals of group 1 (n = 11) were injected into the marginal ear vein with a HMD Cathy I. V. Cannulas (needle catheter Cathy (HMD Company)) with placebo (4.0 M urea solution corresponding to its concentration in the FM solution) in the volume of 0.5 ml. After one hour, a laparotomy was performed and a standard liver injury was inflicted in accordance with the available guidelines [10] under general anesthesia with Telazol (Zoetis, Spain). To suppress platelet aggregation at the beginning of the experiment, the animals of groups 2 to 4 were administered *per os* a mixture of acetylsalicylic acid (Thrombo ACC®, Lannacher Heilmittel GmbH, Austria) at a dose of 2.0 mg/kg and clopidogrel (Plavix®, Sanofi Winthrop Industrie, France) at a dose of 8.0 mg/kg dissolved in water.

An hour after taking these antiplatelets, animals were injected intravenously with solutions of the following drugs: in group 2 (n = 10) – placebo, in group 3 (n = 18) – tranexamic acid (Tranexam®, FSUE Moscow endocrine plant, Russia) at a dose of 15 mg/kg, in group 4 (n = 10) – FM at a dose of 0.25 mg/kg. Used in the experiment FM was obtained by previously registered technology [11]. A standard liver injury was performed under general anesthesia one hour after administration of placebo and FM to the animals of groups 2 and 4, and after 30 minutes, to the animals of group 3. After inflicting an injury, the nature of parenchymal bleeding was evaluated by means of gauze wipes using such criteria as the volume of blood loss as a percentage of the estimated circulating blood volume (% CBV) taking into account animal body weight, and the rate of blood loss per unit of time (mg/s) [10]. To evaluate the hemostasis system, blood was obtained by making an incision of the marginal ear vein (gravity flow) twice before drug administration and liver injury (Fig. 1). Blood was placed into the tubes with the appropriate stabilizers: 0.25 ml was placed into tubes with potassium salt of ethylenediaminetetraacetic acid (AQUISEL® K3E/EDTA 3K, Aquisel S. L. Company, Spain) to count the platelet number blood, and 5.0 ml was placed into polystyrene graduated centrifuge tubes with polyvinylchloride caps containing 0.11 M (3.8 %) sodium citrate solution (blood-stabilizer ratio 9:1) to study other parameters.

Platelet-rich plasma and platelet-poor plasma were obtained according to the common method.

The study of hemocoagulation involved the assessment of the number of platelets in venous blood and their aggregation induced by adenosine diphosphate of disodium salt (ADP), taken at the concentration of 10 μM), activated partial thromboplastin time (APTT) and prothrombin time (PT), as well as fibrinogen concentration and the D-dimer level. The results of APTT and PT assessment were presented in the form of a ratio calculated by the formula: Ratio = $CT_{experiment}/CT_{control}$, where: Ratio is a ratio; $CT_{experiment}$ is coagulation time in experimental plasma (s); $CT_{control}$ is coagulation time in control plasma (s). The platelet number was determined using the hematology analyzer Drew-3 (Drew Scientific Inc., England). Platelet aggregation function was evaluated using the Chrono-Log 490-2D aggregometer (CHRONO-LOG Corporation, USA), the coagulometric parameters were defined with Thrombostat 2coagulation analyzer (Behnk Electronik, Germany) using reagent kits by the company "Technologia Standart" (Russia), the D-dimer level was measured with the analyzer-reflectometer NycoCard Rader II (Axis-Shield PoC AS, Norway) and the test system NycoCard® D-Dimer (Axis-Shield PoC AS).

Distribution of the characteristics was evaluated by Shapiro-Wilk test, the group differences depending on distribution were assessed by Student's t-test, Mann – Whitney U-test, Fisher's exact test, the correlation was evaluated by Spearman's rank correlation coefficient (rS). The differences were considered statistically significant at p J 0.05. The results were processed by MedCalc Version 17.9.7 (license BU556-P12UT-BBS55-YAH5M-UBE51). The data are presented as median (Me), 25th and 75th percentiles Me [Q_{25}÷Q_{75}]).

RESULTS

The study established a high mortality rate of the animals in group 2 (4 out of 10), associated with cardiorespiratory arrest against the background of ongoing bleeding. In contrast, in the other groups no mortality was observed (in groups 1 and 4; p_{1-2} = 0.035; p_{2-4} = 0.035) or it was lower as in group 3 (3 out of 18), p_{2-3} = 0.208).

The mortality of the animals in group 2 was comparable with the severity of blood loss (Fig. 2).

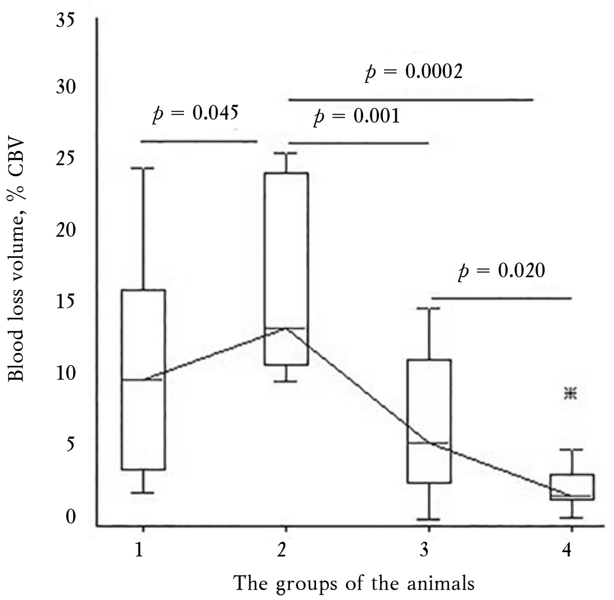

Fig. 2. The parameters of blood loss in the experimental groups: the values are presented as median (*Me*), the horizontal line inside the rectangle; and as the values corresponding to the 2.5th and 97.5th percentiles, the lower and upper vertical bars.

The volume of blood loss in the group of the animals treated with antiplatelets and placebo (group 2) was 1.4 times higher (13.6 [11.3 ÷ 22.0] % of CBV) compared to the same rate in the group of the animals treated with placebo only (group 1) (10.1 [4.1 ÷ 13,5] % CBV). However, the rate of blood loss in the animals in these groups did not differ. These observations indicate the reproduction of hemorrhagic syndrome caused by double antiplatelet therapy in group 2.

It was further found that the volume of blood loss in the groups of the animals pre-treated with antiplatelets after intravenous administration of TXA (group 3) (5.7 [3.1 ÷ 10.6] % CBV) and FM (group 4) (2.0 [1.8 ÷ 3.2] % CBV) was 2.4 times and 6.0 times less, respectively, in comparison with group 2 (placebo). Similar patterns were observed when assessing the rate of blood loss that decreased by 4.8 times after TXA administration (group 3) (6.2 [4.5 ÷ 8.3] mg/s) and by 5.9 times after FM administration (group 4) (5.0 [4.3 ÷ 6.8] mg/s) compared to the placebo group (group 2) (29.9 [11.9 ÷ 34.3] mg/s), table.

Table

Hemostatic characteristics in the experimental groups against the background of antiplatelets, hemostatic agents, and placebo *Me* [Q_{25}÷Q_{75}]

Parameter	Group 1		Group 2		Group 3		Group 4	
	before (1a)	after (1б)	before (2a)	after (2б)	before (3a)	after (3б)	before (4a)	after (4б)
Platelet count, $\times 10^9$/л	477.5 [405.8÷621.5]	480.5 [412.3÷555.0]	430.5 [413.3÷479.8]	475.0 [438.5÷515.3]	429.5 [373.0÷504.8]	408.0 [347.3÷477.5]	475.5 [468.0÷561.0]	460.0 [411.5÷532.8]
		$p_{1a-1б}$ = 0.151		$p_{2a-2б}$ = 0.121		$p_{3a-3б}$ = 0.102		$p_{a-4б}$ = 0.312
ADP-induced aggregation, %	20.5 [19.0÷28.7]	22.0 [19.2÷31.4]	18.4 [17.0÷19.7]	1.3 [0.4÷6.4] $\Delta_{2a-2б}$ -14 раз (times)	20.8 [17.4÷37.6]	6.8 [4.0÷9.8] $\Delta_{3a-3б}$ – 3 раза (times)	19.8 [11.7÷20.8]	1.1 [1.0÷1.9] $\Delta_{4a-4б}$ – 18 раз (times)
		$p_{1a-1б}$ = 0.598		$p_{2a-2б}$ = 0.005		$p_{3a-3б}$ = 0.0003		$p_{4a-4б}$ = 0.00007
APTT, ratio	1.1 [1.0÷1.2]	1.1 [0.9÷1.2]	1.1 [0.9÷1.1]	1.0 [0.9÷1.1]	0.9 [0.8÷1.1]	0.9 [0.8÷1.0]	1.0 [0.9÷1.0]	0.9 [0.9÷1.0]
		$p_{1a-1б}$ = 0.248		$p_{2a-2б}$ = 0.110		$p_{3a-3б}$ = 0.124		$p_{4a-4б}$ = 0.614
PT, ratio	1.1 [0.6÷1.6]	0.9 [0.9÷1.3]	1.1 [1.0÷1.2]	1.1 [1.0÷1.1]	1.0 [0.8÷1.3]	0.9 [0.8÷1.4]	1.1 [1.0÷1.1]	1.0 [1.0÷1.1]
		$p_{1a-1б}$ = 0.476		$p_{2a-2б}$ = 0.645		$p_{3a-3б}$ = 0.458		$p_{4a-4б}$ = 0.251
Fibrinogen, g/l	3.3 [2.8÷4.4]	3.7 [2.8÷4.5]	3.3 [3.0÷3.5]	3.4 [3.2÷3.8]	3.5 [2.9÷3.9]	3.2 [3.0÷3.8]	3.5 [3.2÷4.1]	3.4 [3.0÷4.1]
		$p_{1a-1б}$ = 0.811		$p_{2a-2б}$ = 0.758		$p_{3a-3б}$ = 0.753		$p_{4a-4б}$ = 0.872
D-dimer, нг/мл	100.0 [100.0÷100.0]	100.0 [100.0÷200.0]	100.0 [100.0÷175.0]	100.0 [100.0÷200.0]	300.0 [200.0÷400.0]	1000.0 [525.0÷1350.0] $\Delta_{3a-3б}$ +3.3 times	150.0 [100.0÷275.0]	175.0 [100.0÷300.0]
		$p_{1a-1б}$ = 0.205		$p_{2a-2б}$ = 0.180		$p_{3a-3б}$ ***=0.010***		$p_{4a-4б}$ = 0.463

Notes: p – the achieved level of statistical significance of the differences in the compared values; ADP – adenosine diphosphate; APTT – activated partial thromboplastin time; PT – prothrombin time; Δ – the difference of values.

A positive relationship between the volume and the rate of blood loss was determined in the animals treated with both TXA (group 3) $rS = 0.86$ ($p = 0.002$) and FM (group 4) $rS = 0.79$ ($p = 0.006$), that was absent in placebo groups (1 and 2).

Along with the assessment of blood loss, the rates of ADP-induced platelet aggregation and coagulogram were studied in order to visualize drug-induced thrombocytopathy and to comparatively assess the effects of TXA and FM (Table).

In the animals of groups 2, 3 and 4, inhibition of ADP-induced platelet aggregation function (in %) was reported to be inhibited by 3-18 times against the background of the combined use of antiplatelets, without any change in the platelet number, as well as without shifts in the chronometric values of the coagulogram and fibrinogen concentration.

DISCUSSION

The results obtained in the experiment indicate that the noticeable hemostatic effects of TXA were realized against the background of more than 3-fold (median) increase in the D-dimer plasma level with persistent pharmacologically conditioned thrombocytopathy which is considered to be an evidence of a shift in hemostatic balance towards increased fibrin formation. At the same time, the use of FM in platelet function inhibition was not accompanied by any significant coagulation activation which, however, is in contradiction with the observed systemic hemostatic effects (in terms of volume and rate of blood loss).

As it is known, acetylsalicylic acid irreversibly inhibits platelet cyclooxygenase-1, followed by a decrease in the formation of thromboxane A_2. Clopidogrel, in turn, is a prodrug and turns into its active form as an antagonist of $P2Y_{12}$-platelet receptors through metabolism in the liver [12]. When FM was used, correction of platelet function, reduced under the antiplatelet action, was not reported which currently does not allow to explain the mechanism of noted hemostatic effects associated with FM.

CONCLUSION

Fibrin-monomer (des-AABB-fibrinogen) administered intravenously at a dose of 0.25 mg/kg s able to prevent severe posttraumatic parenchymal bleeding caused by combined use of drugs with different mechanisms of antiplatelet action. The nature of this phenomenon is not quite clear, and more research is required.

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Vdovin V.M. – conception and design, experimental setup, analysis and interpretation of data, critical revision of the manuscript for important intellectual content, final approval of the manuscript for publication. Momot A.P. – conception and design, analysis and interpretation of data, critical revision of the manuscript for important intellectual content, final approval of the manuscript for publication. Orekhov D.A. – experimental setup, analysis and interpretation of data. Tolstokorov I.G. – experimental setup, analysis and interpretation of data. Lycheva N.A. – analysis and interpretation of data. Shevchenko V.O. – experimental setup, analysis and interpretation of data. Shakhmatov I.I. – conception and design, analysis and interpretation of data. Krasyukova V.O. – experimental setup. Fogt E.V. – experimental setup.

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Quality of life with cervical distonia

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ABSTRACT

The aim of the study was to study the physical and psychological components regarding the quality of life of patients with cervical dystonia.

Material and methods. 170 respondents were examined. The main group included 120 patients with cervical dystonia, 50 patients were included in the control group, consisting of patients with cervicalgia of various genesis. The diagnosis of cervical dystonia met uniform criteria for the diagnosis and treatment of dystonia adopted in 2011 by the European Federation of Neurological Societies and the Movement Disorders Society (European Federation of Neurological Societies / Movement Disorders Society, EFNS / MDS). In the control group, the pain syndrome of the cervical spine was caused by a degenerative process and was confirmed by X-ray examination and /or MRI. As part of our research, we determined the quality of life in men and women in both groups using the SF-36 questionnaire with a study of the parameters of physical and psychological well-being.

Results and conclusion. A considerably significant effect of cervical dystonia on the somatic and mental parameters regarding the quality of life in both men and women has been established. Significant decrease in all indicators representing the quality of life in patients with cervical dystonia was revealed compared with respondents without dystonic hyperkinesis. As a chronic disease, cervical dystonia leads to psycho-physiological stress, which significantly impairs the quality of life of patients. Significant gender differences were identified: women from the groups of cervical dystonia and cervicalgia were more often exposed to psychological deprivation and reduced physical activity than men from the same groups.

Key words: cervical dystonia, quality of life, gender differences.

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

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Conformity with the principles of ethics. All participants of the study signed an informed consent. The study was approved by the Ethics Committee at Siberian State Medical University (date of the meeting: November 28, 2016, registration number – 4943).

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Качество жизни при цервикальной дистонии

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РЕЗЮМЕ

Цель. Изучить физические и психологические составляющие качества жизни больных, страдающих цервикальной дистонией.

Материалы и методы. Обследованы 170 респондентов: 120 пациентов с цервикальной дистонией составили основную группу, 50 человек включены в группу контроля – респонденты с цервикалгиями различного генеза. Диагноз цервикальной дистонии установлен клинически, согласно единым критериям по диагностике и лечению дистонии, принятым в 2011 г. Европейской федерацией неврологических обществ и Обществом двигательных расстройств (European Federation of Neurological Societies / Movement Disorders Society, EFNS / MDS). Болевой синдром в шейном отделе позвоночника у группы контроля был вызван дегенеративным процессом и подтвержден рентгенографическим обследованием и (или) магнитно-резонансной томографии. В рамках проводимого нами исследования определялось качество жизни у мужчин и женщин в обеих группах с помощью опросника SF-36 с изучением параметров физического и психологического благополучия.

Результаты и заключение. Установлено достоверно значимое влияние цервикальной дистонии на соматические и психические параметры качества жизни как у мужчин, так и у женщин. Выявлено достоверное снижение всех показателей качества жизни у больных цервикальной дистонией по сравнению с респондентами, не имеющих дистонического гиперкинеза. Цервикальная дистония как хроническое заболевание приводит к психофизиологическому напряжению, что значительно ухудшает качество жизни больных. Выявлены достоверные гендерные внутригрупповые различия: женщины из групп цервикальной дистонии и цервикалгий в большей степени подвержены психологической депривации и снижению физической деятельности, чем мужчины из этих же групп.

Ключевые слова: цервикальная дистония, качество жизни, гендерные различия.

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

Источник финансирования. Авторы заявляют об отсутствии финансирования.

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INTRODUCTION

Diseases that lead to a decrease in the level of general health and social and professional activity are always the focus of attention of researchers and practitioners. Cervical dystonia (CD) belongs to such diseases. It is known that CD is a focal variant of muscular dystonia and is characterized by violent, often painful hyperkinesis of the cervical spine muscles that form an unnatural position of the neck and/or head [1]. The pathogenetic manifestations of dystonic hyperkinesis are still not fully understood. The current leading hypothesis is based on the multifactorial nature of the disease, according to which genetic predisposition is realized under the influence of external triggers [2, 3]. CD can be combined with tremor (fast, rhythmic hyperkinesis) and myoclonus (spasmodic hyperkinesis) [4] and is often combined with pain syndrome [5], insomnia [6] and anxiety-depressive disorder. Pain is the most common comorbid syndrome in CD and occurs in 70% of patients [7]. The disease often starts with pain in the back of the head and cervical spine [8]. Taking into account the specific phenotypic diversity of this hyperkinesis, this concept is used to determine the pattern in which a variant of neck and/or head rotation relative to the body is distinguished [9]. The most common form of CD is torticollis (dystonic turn of the neck to the side). It is known that the onset of the disease occurs in the period of the highest working and social activity between 20 and 60 years. The chronic course of dystonia and the formation of functional deficiency lead to professional and social maladaptation and, consequently, disability of patients [10].

According to the modern paradigm of clinical medicine, the main goal of any disease treatment is to improve the quality of life against reduction or alleviation of clinical symptoms [11]. Since 2004, the President of the Russian Federation has defined the criteria for the development of Russia, which assign both social and economic significance to the quality of life [12]. It is known that the quality of life is both an integral and subjective characteristic that covers the physical and mental condition, and social and professional aspects [13, 14]. In patients with chronic disease, it is especially important to determine the quality of life indicators that allow the patient to analyze his/her condition. This assessment demonstrates not only how the patient tolerates the disease, but also the degree of adaptation to it.

Despite active research into the problem of CD in recent decades, it is noteworthy that small number of studies examine the effect of hyperkinesis on the patient's quality of life. This necessitates research into the quality of life of patients with CD.

The aim of the study was to investigate the physical and psychological characteristics of the quality of life of patients with CD.

MATERIAL AND METHODS

170 people were examined. The main group included 120 patients with CD aged 27 to 82 of which 31 (25.8%) were men and 89 (74.2%) were women. Patients were observed on an outpatient basis at the Novosibirsk Extrapyramidal Disease Center with Botulinum Toxin Therapy. The diagnosis of cervical dystonia met uniform criteria for the diagnosis and treatment of dystonia adopted in 2011 and 2014 [15, 16]: the presence of a dystonic posture, corrective tricks and paradoxical kinesis, the identification of the direct connection between hyperkinesis and static stress, physical and psychological stress, the presence of daily fluctuations. The most common form of CD was torticollis, from which 48.3% of patients (58) suffered. 34.2% of patients (41) were diagnosed with laterocollis, and 17.5% of patients (21) had other forms of CD. Pain syndrome of varying severity was present in 97.5% of patients (117). The combination of CD with tremor was observed in 58.3% (70) of all examined patients, and with myoclonus in 11.6% (14) of patients.

The control group consisted of 50 respondents: 16 (32%) men and 34 (68%) women aged 25 to 82 with cervicalgia due to degenerative changes in the cervical spine confirmed by X-ray and/or MRI. The study of the quality of life was carried out using questionnaire SF-36, in which physical and psychological parameters are considered. Physical health characteristics were determined by 4 scales: 1) physical functioning (PF) – the level of physiological loads; 2) role limitations of the physical state (RLPS) – role functioning dependent on physical status; 3) physical pain (PP) – the severity of pain and its impact on daily activities; 4) general health (GH) – the present somatic condition subjectively assessed by the patient.The mental health component was also evaluated according to four criteria. These criteria were: 1) vitality (V) – a subjective evaluation of the degree of vital activity; 2) social functioning (SF) – the degree of restriction of social activity; 3) role limitation of the emotional state (RLES) – functioning depending on the respondent's temperament; 4) psychological health (PH) – self-esteem characterized by the degree of manifestation of positive and anxiety-depressive experiences. The values of the scales ranged from a low index of the quality of life (0% – 20%) to a high index (81%–100%).

The hypothesis on the concordance of sample distributions to the normal Gauss-Laplace distribution was tested by comparing mean values using the Kolmogorov – Smirnov (K-S) and Lilliefors criteria. The test results show that static distribution of the studied parameters does not correspond to the normal distribution law and, therefore, the values of the measured parameters are given in the *Me* (Q_1;Q_3) format, where *Me* is the median, Q_1 is the lower quartile, and Q_3 is the upper quartile. A comparative analysis was carried out using the Mann-Whitney criterion, the threshold value of the achieved significance level *p* was 0.05. The data were processed using the statistical software package Statistica v.10.0 (StatSoft Inc., USA).

RESULTS AND DISCUSSION

Evaluation of the quality of life criteria revealed significant depression of most performances in patients with CD compared with the control group of patients with cervicalgia.

Analyzing the criteria of the physical functioning scale (Table 1), a significant decrease in the parameters of this performance in men and women of CD group was revealed, compared to patients from the control group (p = 0.0006 and p = 0.0048, respectively). This scale reflects the degree of limitation of physical activity, such as taking the stairs and walking distances, lifting and carrying weights, or self-care at home.

Comparing the results of the scale characterizing the role limitations connected with the physical state (Table), it turned out that role functioning, i.e. daily work or other daily activities, was limited significantly in women with dystonic hyperkinesia compared with women from the cervicalgia group (p < 0.0001). In the meantime, no significant role limitations were identified in men with CD in comparison to men with cervicalgia. It is noteworthy that no significant differences of the results between men and women of the main group were obtained, as well as between men and women of the control group.

According to the patients of the CD group, a significant influence on the quality of life came from pain syndrome while working at home and outside of it (Table).

Table

The quality of life of patients with CD and cervicalgia according to questionnaire SF-36

Scales SF-36	Gender	Control (cervicalgia), n = 16/34		Cervical dystonia, n = 31/89		p
		Me [Q_1;Q_3]	p male – female	Me [Q_1; Q_3]	p male – female	
Physical functioning (PF)	male	95.0 [82.5;95.0]	0.0099	70.0 [50.0;95.0]	0.0042	0.0048
	female	75.0 [50.0;95.0]		50.0 [35.0;65.0]		0.0006
Role limitations associated with physical state (RLPS)	male	50.0 [12.5;87.5]	0.8679	25.0 [0.0;50.0]	0.0903	0.0839
	female	50.0 [25.0;100.0]		0.0 [0.0;25.0]		<0.0001
Physical pain (PP)	male	79.0 [51.0;92.0]	0.2984	41.0 [31.0;61.0]	0.3038	0.0001
	female	72.0 [61.0;84.0]		41.0 [31.0;42.0]		<0.0001
General health (GH)	male	58.5 [55.0;74.5]	0.3824	40.0 [35.0;50.0]	0.1241	0.0009
	female	56.0 [40.0;70.0]		35.0 [30.0;45.0]		<0.0001
Vitality (B)	male	67.5 [52.5;80.0]	0.0055	50.0 [35.0;60.0]	0.0007	0.0131
	female	50.0 [35.0;60.0]		35.0 [25.0;45.0]		0.0019
Social functioning (SF)	male	87.5 [75.0;87.5]	0.0261	62.5 [50.0;75.0]	0.0383	0.0014
	female	75.0 [50.0;75.0]		50.0 [37.5;62.5]		<0.0001
Role limitations associated with emotional state (RLES)	male	66.7 [0.0; 00.0]	0.7081	33.3 [0.0;100.0]	0.1370	0.6214
	female	66.7 [0.0;66.7]		33.3 [0.0;66.7]		0.0357
Mental health (MH)	male	74.0 [58.0; 80.0]	0.0112	60.0 [44.0;68.0]	0.0115	0.0422
	female	56.0 [52.0;68.0]		44.0 [36.0;56.0]		0.0008

Women with CD significantly more often indicated pain as a negative and important factor affecting their lives than women from the control group (p < 0.0001). Similar data were obtained in men from the main group as compared to men from the group of patients with cervicalgia (p = 0.0001). Evaluation of inter-gender differences within each study group (CD and cervicalgia) showed a lack of significant differences in performance.

An analysis of the general health scale revealed that the presence of dystonic hyperkinesis is also an important factor for patients with CD that affects the somatic well-being. The result of a subjective evaluation of their condition by women and men with CD was lower than in respondents with cervicalgia ($p < 0.0001$ and $p = 0.0009$, respectively). It is interesting that no significant gender differences in the main and control groups were found.

While evaluating the performances of scales that reflect the psychological aspect of the quality of life, significant intergroup differences in patients with CD compared to the control group of cervicalgia were revealed.

The vitality scale shows how much the respondent feels alert, tired, or exhausted. Thus, the vitality parameters were the lowest in both women and men with CD compared to women and men from the group of cervicalgia ($p = 0.0019$ and $p = 0.0131$, respectively).

The scale of social functioning shows a low degree of satisfaction of CD patients with their social activity, which includes communication with family members, friends, and colleagues. Thus, the limitation of social contacts was reliably expressed in the group of women and men with CD, compared to the women and men from the control group ($p < 0.0001$ and $p = 0.0014$, respectively).

The influence of the respondents' emotional background on the quality and volume of the work performed was assessed using the scale of role limitation of the emotional state. A decrease in the values to 33.3% (from 100%) in women and men with CD showed that daily activity was significantly reduced and directly depended on their emotional well-being. However, the results obtained are truly significant only in patients with CD compared to women from the group of cervicalgia ($p = 0.0357$). While in men with CD, significant role limitations were not identified compared to men from the control group. Thus, women with CD have a lower emotional background that affects the quality and volume of the routine work. At the same time, no significant differences between the data obtained for women and men in the main and control groups were found.

Mental well-being affecting the quality of life of the patients was evaluated on a mental health scale. Analyzing the performances, deprivation of positive emotions against the background of anxiety-depressive states in both women and men with CD was revealed when compared to women and men from the control group ($p = 0.0008$ and $p = 0.422$, respectively).

Significant differences in the results for men and women in the main and control groups were identified in terms of physical functioning, vitality, social functioning and psychological health. Thus, analysis of physical functioning shows that women with CD ($p = 0.0042$) and women with cervicalgia ($p = 0.0099$) more often believe that their state of health reduces their tolerance to physical loads, as opposed to men from the same groups. The data on the vitality scale also demonstrate that women from both studied groups more often feel more tired and exhausted than men from the same groups. Evaluation of the social functioning criteria revealed low satisfaction with communication with relatives and colleagues in women in the CD and cervicalgia groups, compared to men in the same groups ($p = 0.0383$ and $p = 0.0261$, respectively). Psychological distress caused by the limitation of favorable emotions was significant in patients with CD and cervicalgia compared to male respondents ($p = 0.0115$ and $p = 0.0112$, respectively).

CONCLUSION

1. CD leads to inversions of physical performances of the quality of life in men and women in the form of a significant decrease in the parameters of somatic functioning and the pronounced effect of pain syndrome. Role limitations associated with the physical state in performing daily work were identified only in women with CD.

2. CD also leads to changes in psychological performances of the quality of life in men and women in the form of a decrease in mental health, social and life activity. A significant influence of the emotional state on everyday work in the form of role limitations was found only in female patients with CD. The

3. differences in the degree of decrease in physiological functioning between men and women within both groups with CD and cervicalgia were revealed. Women from these groups have lower tolerance to physical activity in contrast to men.

4. The biggest gender differences in the CD and cervicalgia groups were revealed in the psychological

characteristics of the quality of life, such as vitality, social functioning and mental well-being. These performances are lower in women of both main and control groups (CD and cervicalgia) than in men from the same groups.

5. As a chronic disease, CD leads to psychophysiological stress, significantly reducing the quality of patients' life.

6. Determining the quality of life in patients with CD makes it possible to have a better assessment of the course of the disease in individuals. Besides, it helps to identify the patient's degree of adaptation to functional disorders and analyze the problems connected with the response to the disease, which allows to determine a treatment plan based on a personalized approach.

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Authors contribution

Druzhinina O.A. – conception and design of the study, analysis and interpretation of data. Zhukova N.G. – substantiation of the manuscript, critical revision of the manuscript for important intellectual content, final approval of the manuscript for publication. Sperling L.P. – conception and design of the study.

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Dissimilar tumor cell populations in ascitic fluid of ovarian cancer patients

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ABSTRACT

Ovarian cancer is one of the most aggressive and hard-to-treat cancers. About 75% of ovarian cancer cases are detected at later stages of the disease. Ascitic fluid is promising biological material to get information about the tumor nature in ovarian cancer. Peritoneal dissemination is one of the most unfavorable factors of malignant tumor progression. However, prognostic factors associated with malignant ascites are not well understood.

The aim of the study was to evaluate various tumor cell populations in ascitic fluid of ovarian cancer patients by laser multicolor flow cytometry using a molecular panel of EpCam, CD45, CD44, CD24, CD133, and N-cadherin markers. The prospective study included 16 patients aged 36 to 76 years with newly diagnosed FIGO stage Ic–IV ovarian cancer, who were admitted for treatment to the Cancer Research Institute of Tomsk National Research Medical Center. The study material included EDTA-stabilized ascitic fluid sampled during laparoscopy. Various populations of ascitic tumor cells (with stemness features, with epithelial mesenchymal transition (EMT) features, without stemness and EMT features, with a combination of these features, as well as atypical/hybrid cell populations) were identified by multicolor flow cytometry on a BDFACSCanto apparatus (USA) using fluorochrome-labeled EpCam, CD45, CD44, CD24, CD133, and N-cadherin monoclonal antibodies and the BD FACSDiva software. The study revealed twelve populations of Epcam-positive cells in ascitic fluid of ovarian cancer patients. The cell composition of ascitic fluid in ovarian cancer patients is represented by a heterogeneous population. A large fraction of ascitic tumor cells are atypical/hybrid tumor cells with stemness features as well as Epcam+CD45−CD44+CD24+CD133+/− cancer stem cells, both with and without EMT features.

Key words: ovarian cancer, ascitic tumor cells, cancer stem cells, multicolor flow cytometry, liquid biopsy.

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Различные популяции опухолевых клеток в асцитической жидкости больных раком яичников

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РЕЗЮМЕ

Введение. Рак яичников (РЯ) является одним из самых агрессивных и тяжело поддающихся лечению онкологических заболеваний. Около 75% случаев РЯ выявляется на поздних стадиях заболевания. Асцитическая жидкость является перспективным биологическим материалом для получения информации о характере опухолевого процесса при РЯ. Перитонеальная диссеминация считается одним из наиболее неблагоприятных факторов прогрессирования злокачественных опухолей. Однако прогностические факторы, связанные со злокачественным асцитом, изучены недостаточно.

Целью данного исследования явилась оценка различных популяций опухолевых клеток в асцитической жидкости больных РЯ методом многоцветной проточной лазерной цитометрии на основе молекулярной панели маркеров EpCam, CD45, CD44, CD24, CD133 и N-cadherin. В проспективное исследование включены 16 больных с впервые диагностированным РЯ, стадии Ic–IV по системе FIGO, возраст 36–76 лет, поступившие на лечение в НИИ онкологии Томского НИМЦ. Материалом для исследования служила асцитическая жидкость, стабилизированная ЭДТА, взятая во время лапароскопии. Различные популяции асцитических опухолевых клеток (с признаками стволовости, с признаком ЕМТ (epithelial-mesenchymal transition), без признаков стволовости и ЕМТ, с сочетанием этих признаков, а также атипичные / гибридные популяции клеток определяли методом многоцветной проточной лазерной цитометрии на аппарате BDFACSCanto (США) с помощью меченных различными флуорохромами моноклональных антител к EpCam, CD45, CD44, CD24, CD133 и N-cadherin и программного обеспечения BD FACSDiva. В результате исследования в асцитической жидкости больных РЯ было выявлено 12 популяций Epcam-положительных клеток. Клеточный состав асцитической жидкости больных РЯ представляет собой гетерогенную популяцию. Большую концентрацию асцитических опухолевых клеток представляют собой атипичные / гибридные формы опухолевых клеток с признаком стволовости, а также стволовые опухолевые клетки Epcam+CD45-CD44+CD24+CD133+/- как с признаком ЕМТ, так и без него.

Ключевые слова: рак яичников, асцитические опухолевые клетки, стволовые опухолевые клетки, многоцветная проточная цитометрия, 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 one of the most aggressive and hard-to-treat cancers. More than 225,000 new cases of ovarian cancer are annually detected across the world, and more than 140,000 females die from this disease. Despite success achieved in the diagnosis, about 75% of ovarian cancer cases are detected at later stages of the disease. Treatment of patients with advanced cancer is difficult and not always provides a positive outcome. The five-year survival rate in patients with stage III or stage IV cancer is 23.8% and 11.6%, respectively. The asymptomatic course of the disease results in late diagnosis and a five-year mortality rate of 60%.

Unlike other tumors, ovarian cancer is characterized by a unique metastatic process. The earliest and most common way of metastasis is through 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 promising biological material to get information about the tumor nature. Unlike serum, ascitic fluid is more informative, especially at an early stage of the malignant process. In general, ascites is a multicomponent, dynamic system where all elements combined facilitate the formation of a pro-inflammatory and immunosuppressive environment. Ascites consists of a complex mixture of cell populations and a rich cytokine profile. The variety of cells is related to several factors. Firstly, it is phenotypic plasticity arising from the influence of soluble factors and microenvironment signals from immune and stromal cells. Secondly, the heterogeneity is associated with hydrodynamic forces that significantly change cell morphology [1]. Thirdly, the cause of tumor cells in ascitic fluid is the tumor, in particular, ovarian cancer, that is a heterogeneous cell population itself.

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 rate 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). 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 for cancer stem cells (CSC) [2].

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 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 [3].

Another CTCs marker in ascitic fluid is CD24 that 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 [4].

Morphologically, leucocytes in ascitic fluid resemble circulating tumor cells; therefore, it is advisable to use the differentiation antigen CD45 for affinity binding to leucocytes.

A common EMT feature is reduced expression of epithelial cadherin (E-cadherin) and a concomitant increase 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 signals, and modulates the canonical Wnt pathway, which leads to the formation of an aggressive phenotype [5].

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 cancers [6].

A complete picture of different tumor cell populations in ovarian cancer may enable to predict the disease course, overall and relapse-free survival, and response to chemotherapy. Peritoneal dissemination caused by ascites is one of the most unfavorable factors for progression of malignancies. However, prognostic factors associated with malignant ascites are not well studied. In this regard, investigation of different tumor cell populations in ascitic fluid is a topical issue. The aim of this study was to evaluate tumor cell populations in ascitic fluid of ovarian cancer patients by laser multicolor flow cytometry using a molecular panel of EpCam, CD45, CD44, CD24, CD133, and N-cadherin markers.

MATERIALS AND METHODS

The prospective study included 16 patients aged 36 to 76 years with newly diagnosed FIGO stage Ic–IV ovarian cancer, who were admitted for treatment to Cancer Research Institute of Tomsk National Research Medical Center.

The study material included EDTA-stabilized ascitic fluid sampled during laparoscopy. Different populations of ascitic tumor cells (with stemness features, with epithelial mesenchymal transition (EMT) features, without stemness and EMT features, with a combination of these traits, as well as atypical/hybrid cell populations) were identified by multicolor flow cytometry on a BDFACSCanto apparatus (USA) using a molecular panel of EpCam, CD45, CD44, CD24, CD133, and N-cadherin markers and the BD FACSDiva software.

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 were analyzed on a flow cytometer within an hour. The cell level was expressed as the amount of cells per 1 μL of ascitic fluid.

The obtained data were processed using variation statistics methods. The statistical significance of differences was evaluated by a nonparametric Wilcoxon test for dependent samples with the Bonferroni correction using the Statistica 12.0 statistical software (StatSoft). The data are presented as a median (*Me*) and upper and lower quartiles [Q_1–Q_3]. The differences were considered statistically significant at a significance level of $p < 0.05$.

RESULTS

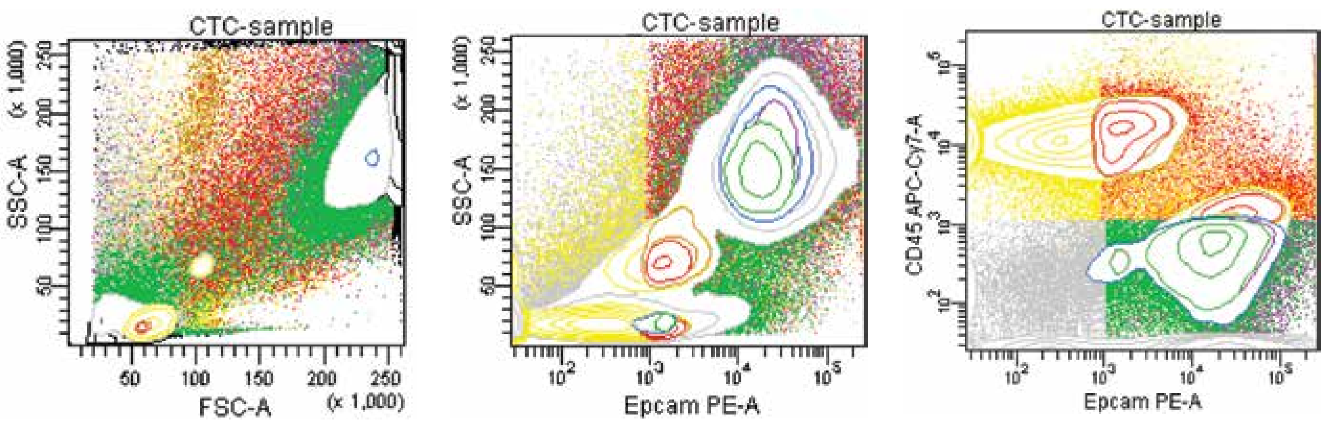
Multicolor flow cytometry of ascitic fluid revealed twelve populations of Epcam-positive cells. These included ascitic tumor cells with stemness features, without EMT features, and with phenotypes Epcam+CD45−CD44+CD24−CD133+Ncadherin−; Epcam+CD45−CD44+CD24−CD133−Ncadherin−; Epcam+CD45−CD44+CD24+CD133+Ncadherin−; ascitic tumor cells without stemness and EMT features: Epcam+CD45−CD44−CD24−CD133−Ncadherin−; ascitic tumor cells without stemness features and with EMT features: Epcam+CD45−CD44−CD24−CD133−Ncadherin+; ascitic tumor cells with stemness features and EMT features: Epcam+CD45−CD44+CD24−CD133+Ncadherin+; Epcam+CD45−CD44+CD24−CD133−Ncadherin+; Epcam+CD45−CD44+CD24+CD133+/−Ncadherin+/−; Epcam+CD45−CD44−CD24+CD133+/−Ncadherin+/−; Epcam+CD45−CD44+CD24+CD133+Ncadherin+; atypical/hybrid cells without stemness features: Epcam+CD45+CD44−CD24−CD133−Ncadh+/−; atypical/hybrid cells with stemness features: Epcam+CD45+CD44+CD24+/−CD133+/−Ncadh+/−.

Figure 1 presents the results of multicolor flow cytometry in assessing different populations of Epcam+ cells in ascitic fluid.

Statistical analysis of the data using a nonparametric Friedman-Kendall test and pairwise Wilcoxon comparison revealed statistically significant differences between levels of these populations. The highest concentration of ascitic tumor cells was observed in atypical/hybrid forms with stemness traits. For example, the level of Epcam+CD45+CD44+CD24+/−CD133+/−Ncadh+/− cells was 240.97 (80.54–383.5) cells/μL, while the levels of ascitic tumor cells without stemness and EMT traits (Epcam+CD45–CD44–CD24–CD133–Ncadh–) and atypical/hybrid tumor cells without stemness traits (Epcam+CD45+CD44–CD24–CD133–Ncadh+/–) amounted to 0.28 (0.11–4.27) cells/μL (p = 0.0009) and 2.07 (0.29–7.16) cells/μL (p = 0.00098), respectively.

The amount of tumor stem cells with positive expression of CD24, both with and without EMT features, (Epcam+CD45–CD44+CD24+CD133+/–Ncadherin+/–) did not significantly differ from the amount of tumor cells without stemness and EMT features (Epcam+CD45–CD44–CD24–CD133–Ncadherin–) and exceeded the level of cells without stemness traits in the EMT state (Epcam+CD45–CD44–CD24–CD133–Ncadherin+) at the trend level (p = 0.073) (Table 1).

In this case, the amount of tumor stem cells with positive expression of CD24 (Epcam+CD45–CD44+CD24+CD133+/–Ncadherin+/–) was significantly higher than that of tumor stem cells with negative expression of CD24, both with and without EMT features (Epcam+CD45–CD44+CD24–CD133+/–Ncadh+ and Epcam+CD45–CD44+CD24–CD133+/–Ncadh–, respectively), and amounted to 2.07 (0.53–11.42) cells/μL vs. 0.29 (0.03–0 72) cells/μL and 0.02 (0.00–0.52) cells/μL, respectively. The level of tumor stem cells with positive expression of CD24 with the Epcam+CD45–CD44–CD24+CD133+/–Ncadherin+/– phenotype amounted to 1.58 (0.22–3.87) cells/μL and was significantly higher than that of tumor stem cells with the Epcam+CD45–CD44+CD24–CD133+/–Ncadherin+ phenotype (Table 1).



a *b* *c*

Fig. 1 Different cell populations in ascitic fluid of an ovary cancer patient, which were identified by multicolor flow cytometry: *a*) ascitic cell populations in forward (FSC) and side (SSC) scattered light modes; *b*) populations of EpCam-positive and EpCam-negative cells in acidic fluid; *c*) populations of CD45+EpCam–, CD45+Epcam+, CD45–Epcam–, and CD45–Epcam+ cells

Table

Levels of different cancer cell populations in ascitic fluid of ovarian cancer patients, *Me* [Q_1–Q_3]

ACC	Phenotype of ascitic cancer cells	Cells / μL
ACC-1	Epcam+CD45-CD44-CD24-CD133-Ncadherin-	0.28 [0.11–4.27]
ACC-2	Epcam+CD45-CD44-CD24-CD133-Ncadherin+	0.09 [0.00–1.22] p_{1-2} = 0.054
ACC-3	Epcam+CD45-CD44+CD24-CD133+/-Ncadherin-	0.02 [0.00–0.52] p_{1-3} = 0.021. p_{2-2} = 0.91
ACC-4	Epcam+CD45-CD44+CD24-CD133+/-Ncadherin+	0.29 [0.03–0.72] p_{1-4} = 0.035. p_{2-4} = 0.27. p_{3-4} = 1.00
ACC-5	Epcam+CD45-CD44+CD24+CD133+/-Ncadherin+	0.34 [0.21–0.53] p_{1-5} = 0.085. p_{2-5} = 0.31. p_{3-5} = 0.77. p_{4-5} = 0.37

Table (continued)

ACC	Phenotype of ascitic cancer cells	Cells / μL
ACC-6	Epcam+CD45-CD44+CD24+CD133+/-Ncadherin-	0.09 [0.00–1.45] $p_{1-6} = 0.21$ $p_{2-6} = 0.76$ $p_{3-6} = 0.91$ $p_{4-6} = 0.76$ $p_{5-6} = 0.88$
ACC-7	Epcam+CD45-CD44-CD24+CD133+/-Ncadherin+	0.09 [0.04–0.78] $p_{1-7} = 0.26$ $p_{2-7} = 0.32$ $p_{3-7} = 0.95$ $p_{4-7} = 0.85$ $p_{5-7} = 0.76$ $p_{6-7} = 0.85$
ACC-8	Epcam+CD45-CD44-CD24+CD133+/-Ncadherin-	0.17 [0.00–0.74] $p_{1-8} = 0.068$ $p_{2-8} = 0.39$ $p_{3-8} = 0.77$ $p_{4-8} = 0.85$ $p_{5-8} = 0.85$ $p_{6-8} = 0.95$ $p_{7-8} = 0.39$
ACC-9	Epcam+CD45-CD44+CD24+CD133+/-Ncadherin+/-	2.07 [0.53–11.42] $p_{1-9} = 0.36$ $p_{2-9} = 0.073$ $p_{3-9} = 0.039$ $p_{4-9} = 0.030$ $p_{5-9} = 0.043$ $p_{6-9} = 0.011$ $p_{7-9} = 0.31$ $p_{8-9} = 0.21$
ACC-10	Epcam+CD45-CD44-CD24+CD133+/-Ncadherin+/-	1.58 [0.22–3.87] $p_{1-10} = 0.36$ $p_{2-10} = 0.086$ $p_{3-10} = 0.084$ $p_{4-10} = 0.047$ $p_{5-10} = 0.514$ $p_{6-10} = 0.374$ $p_{7-10} = 0.027$ $p_{8-10} = 0.017$ $p_{9-10} = 0.27$
Atipical / hybrid cell without stemness features	Epcam+CD45+CD44-CD24-CD133-Ncadherin+/-	2.07 [0.29–7.16] $p_{1-11} = 0.55$ $p_{2-11} = 0.0029$ $p_{3-11} = 0.177$ $p_{4-11} = 0.013$ $p_{5-11} = 0.015$ $p_{6-11} = 0.0506$ $p_{7-11} = 0.011$ $p_{8-11} = 0.011$ $p_{9-11} = 0.68$ $p_{10-11} = 0.506$
Atipical/ hybrid cell with stemness features	Epcam+CD45+CD44+CD24+/-CD133+/-Ncadherin+/-	240.97 [80.54–383.50] $p_{1-12} = 0.0009$ $p_{2-12} = 0.0009$ $p_{3-12} = 0.0009$ $p_{4-12} = 0.0009$ $p_{7-12} = 0.0076$ $p_{8-12} = 0.0076$ $p_{9-12} = 0.0076$ $p_{10-12} = 0.0009$ $p_{11-12} = 0.0009$

Note: ACC- ascitic cancer cells; Wilcoxon Matched Pairs Test. Marked tests are significant at $p < 0.05$

DISCUSSION

Metastatic spread of ovarian cancer occurs mainly due to detachment of cells from the primary tumor and 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.

During metastasis, ovarian cancer cells demonstrate tropism for the omentum. Their interaction leads to the formation of a highly active cancer-associated adipocyte (CAA) phenotype that produces most of the tumor metabolic microenvironment [7]. In addition, adipose tissue is a source of multipotent mesenchymal stem cells (MSCs) capable of self-renewing, differentiating into other pro-oncogenic cells (cancer-associated fibroblasts (CAFs) and CAA), and maintaining the cancer stem cell (CSC) population [8].

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; stromal and immune cell populations, identification and characterization of which may be a useful tool in predicting the disease course and response to chemotherapy.

The largest group of cells in ascitic fluid of ovarian cancer patients has an atypical phenotype and is represented by hybrid cells with stemness features (Epcam+CD45+CD44+CD24+/−CD133+/−Ncadherin+/−). It may be suggested that the formation of hybrid cells promotes carcinomatosis of ovarian cancer and prevents initiation of apoptosis and anoikis of tumor cells in ascites. This suggestion and the identified cells require further research.

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 Мьllerian inhibiting substance (MIS) effectively suppressed its growth [9].

It may be supposed that the presence of tumor cells with the Epcam+CD45−CD44+CD24+CD133+/−Ncadherin+/− phenotype in ascitic fluid is associated with the aggressive course of ovarian cancer. Numerous studies have shown that the Epcam+CD44+CD24+CD133+CD117+ population has increased ability to initiate cancer and/or stimulate metastasis *in vivo* [10]. In a mouse model (NOD/Shi-scid/IL-2Rγ null mice), it was demonstrated that CD24+ and CD133+ cells were 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 connects the CD24 marker to the EMT phenotype. Interestingly, CD24 cells were also capable of initiating tumor growth, albeit to a lesser extent than CD24+. This probably occurs due to the fact that a subset of CD24 cells with stem properties has a lower proliferation rate than CD24+ cells [11].

According to E. Meng et al., ovarian cancer cells with the CD44+/CD24− phenotype have a high potential for intraperitoneal dissemination due to the properties similar to those of CSCs. Compared with other phenotypes, they demonstrate a 60-fold increase in the Matrigel invasion ability of the SKOV3 cell line ($p < 0.001$) and are characterized by an aggressive clinical course of the disease [12].

The presence of CD44 indicates a great potential for the formation of cell spheroids that are known to promote metastasis and chemotherapy resistance. This is confirmed by a study that demonstrated the formation of large hollow spheroids from the CD44high/CD166high population derived from an ovarian cancer line (SKOV3, OV90) in comparison with CD44low/CD166low analogs [13].

Therefore, our findings demonstrate a large heterogeneity of tumor cells in ascitic fluid of ovarian cancer patients. The presence of atypical/hybrid forms of Epcam-positive cells and different populations of tumor cells with stemness features in ascitic fluid is of great interest for further research in the field of personalized medicine.

CONCLUSION

The cell composition of ascitic fluid in ovarian cancer patients is heterogeneous. A large fraction of ascitic tumor cells are represented by atypical/hybrid forms of cells with stemness features and cancer stem cells Epcam+CD45−CD44+CD24+CD133+/−, both with and without EMT. Further investigation of different tumor cell populations in ascitic fluid and their relationship with the clinical course and efficacy of chemotherapy of ovarian cancer patients is very important and opens up great opportunities for practical developments in the field of liquid biopsy.

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Authors contribution

Kaigorodova E.V., Fedulova N.V., Dyakov D.A. – carrying out of research and interpretation of data. Ochirov M.O., Molchanov S.V – diagnostics and treatment of patients with ovarian cancer. Rogachev R.R., Chasovskikh N.Y. – statistical data processing, data retrieval from different bioinformatics resources. Kaigorodova E.V. – conception and design, drafting of the manuscript.

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Eosinophilic cationic protein as a non-invasive marker of the nature of inflammatory response in patients with chronic obstructive pulmonary disease

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ABSRACT

Objective. To estimate the value of measuring plasma eosinophilic cationic protein (ECP) levels in patients with chronic obstructive pulmonary disease (COPD) as a potential biomarker for determining the activity of eosinophilic inflammation. To compare it to determining the number of blood eosinophils and predicting the severity of COPD by determining such clinical characteristics as respiratory function, exacerbation frequency and the BODE index.

Materials and methods. Based on the protocol, 161 patients with COPD participated in the study. They made 2 visits for the collection of anamnestic data and the performance of the main study procedures: respiratory function test, 6-minute step test, dyspnea assessment according to the Medical Research Council Scale questionnaire and sputum and blood analysis in order to determine the level of eosinophils and ECP. The second visit was conducted 12 months after the first to assess the dynamics of the disease. We paid particular attention to the presence of allergies in the case history, the frequency of exacerbations, the number of courses of treatment with antibacterial drugs and inhalants, and systemic glucocorticoids.

Results. The study has demonstrated that high plasma levels of ECP in patients with COPD are associated with a more severe course of disease and the development of more frequent infection-related exacerbations of the disease, which require the administration of inhaled glucocorticoids and antibiotics. We have demonstrated an inverse relationship between the ECP level and forced expiratory volume in 1 second (FEV1). This allows the use of this indicator as a predictor of the severity of COPD in patients.

Conclusion. According to the obtained data, measuring the ECP level of blood plasma can be recommended for use as a clinical marker in the prognosis of COPD and selection of personalized therapy. It is a non-invasive and a relatively easily accomplished research method.

Key words: COPD, eosinophils, eosinophilic cationic protein, phenotype, severity, glucocorticoids.

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

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Эозинофильный катионный белок как неинвазивный маркер характера воспалительного ответа у больных хронической обструктивной болезнью легких

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РЕЗЮМЕ

Цель исследования. Установить ценность измерения уровня эозинофильного катионного протеина в плазме крови у больных хронической обструктивной болезнью легких

(ХОБЛ) как потенциального биомаркера для определения активности эозинофильного воспаления в сравнении с определением количества эозинофилов крови и прогнозирования тяжести ее течения на основании определения таких клинических характеристик, как функция внешнего дыхания, частота обострений и индекс BODE.

Материалы и методы. В исследовании приняли участие больные ХОБЛ (n = 161), для которых предусмотрено два визита, включающих сбор анамнестических данных и выполнение основных процедур исследования (функция внешнего дыхания, 6-минутный шаговый тест, оценка одышки по опроснику Medical Research Council Scale, исследование мокроты и крови с определением уровня эозинофилов и эозинофильного катионного белка). Второй визит проводился через 12 мес после первого для оценки динамики заболевания. Особое внимание уделялось наличию аллергии в анамнезе, частоте обострений, количеству курсов терапии антибактериальными препаратами и приему ингаляционных и системных глюкокортикостероидов.

Результаты. Исследование продемонстрировало, что высокий уровень эозинофильного катионного белка в плазме крови у больных ХОБЛ ассоциирован с более тяжелым течением и развитием более частых инфекционно-зависимых обострений заболевания, требующих назначения ингаляционных глюкокортикостероидов и антибиотиков. Нами была продемонстрирована обратная связь между уровнем эозинофильного катионного белка ECP и ОФВ1, что позволяет использовать данный показатель как предиктор тяжести течения ХОБЛ.

Заключение. Учитывая полученные нами данные, измерение эозинофильного катионного белка плазмы крови, являющееся неинвазивным и относительно легко выполнимым методом исследования, можно рекомендовать использовать в качестве клинического маркера при прогнозе ХОБЛ и персонифицированном подборе терапии.

Ключевые слова: ХОБЛ, эозинофилы, эозинофильный катионный белок, фенотип, степень тяжести, глюкокортикостероиды.

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

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INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a chronic inflammatory disease of the respiratory tract. The development of COPD is associated with the influence of various environmental factors. COPD is a multicomponent disease characterized by a number of pathological changes in the respiratory system, such as mucus hypersecretion, airway obstruction, bronchiolitis, and lung tissue remodeling [1]. According to the published studies, different COPD phenotypes are formed depending on the predominance of certain changes in lung tissue and the rate of their progression.

Recently, scientists have identified more and more new phenotypes of this disease. Thus, the data published in recent years have allowed us to identify a phenotype characterized by bronchial hyperresponsiveness and the presence of eosinophilic inflammation in the respiratory tract [1]. Whereas inflammatory infiltration in the respiratory tract in other COPD types is mainly represented by neutrophils and cytotoxic T-cells. The leading functional criterion for diagnosis of these types is a negative post-bronchodilator test with FEV1/FVC ratio less than 0.7 [1–3]. Scientists have suggested that in the pathogenesis of the COPD phenotype, characterized by the presence of bronchial hyperresponsiveness in the respiratory tract, the T2-dependent mechanism of inflammation prevails. This is more typical for patients with bronchial asthma with eosinophilic inflammation type [2, 3]. Eosinophils are considered to be the key effector cells damaging the mucous membrane of the respiratory tract in this mechanism of the inflammatory process. One of the main proteins that reflect the activity of eosinophilic inflammation is eosinophilic cationic protein (ECP). It is easily detected both in bronchial secretions and in peripheral blood; its level correlates with the severity of asthma and the frequency of exacerbations [4].

M. Saetta and his co-authors have found that the number of tissue eosinophils and the level of ECP increases not only in patients with asthma, but also in some patients with COPD. This increase was most often observed in sputum and bronchial lavage in severe exacerbation phases of the disease. From a pathogenic perspective, the obtained data were explained by the fact that ECP is a powerful chemoattractant for eosinophils, neutrophils and basophils, which are involved in viral and bacterial inflammatory processes in the respiratory tract [5]. A number of recently published studies have discussed that, on the one hand, an increase in ECP in the respiratory tract reflects the degree of eosinophilic inflammation. On the other hand, ECP itself acts as a powerful chemoattractant. It attracts eosinophils, neutrophils and basophils (effector cells involved in viral and bacterial inflammatory processes in the bronchopulmonary area) to the inflammation focus [6].

Today, the global guidelines of the Global Initiative for Chronic Obstructive Lung Disease GOLD and recommendations of the Russian Respiratory Society for the treatment of COPD suggest taking into account the level of eosinophils in the blood of patients who have recurrent exacerbations related to adequate single or dual bronchodilator therapy. This allows the consideration of the advisability of prescribing inhalant glucocorticoids (also called inhaled corticosteroids or ICS) [1]. The degree of inflammation correlates stronger to the blood level of ECP in patients with COPD than to the level of sputum and eosinophils in blood. Thus, it is reasonable to determine its level and use it as a marker of inflammatory activity in the airway for patients with severe COPD and as a predictor of the effectiveness of ICS in patients with eosinophilic phenotype of COPD [7–9].

The aim of the study was to estimate the value of measuring plasma eosinophilic cationic protein (ECP) levels in patients with chronic obstructive pulmonary disease (COPD) as a potential biomarker for determining the activity of eosinophilic inflammation. To compare it to determining the number of blood eosinophils and predicting the severity of COPD by determining such clinical characteristics as respiratory function, exacerbation frequency and the BODE index.

MATERIALS AND METHODS

To achieve this goal, a prospective non-interventional study was planned and conducted. The study involved 161 patients with COPD (average age of 63 [55; 70] years old, smoking index of 40 [25; 60] pack-years). All the patients had a confirmed diagnosis of COPD, according to the GOLD criteria [1], established at least 12 months before inclusion in the study, with the smoking index of more than 10 pack-years.

The study included two visits to COPD patients. During the first visit, all the patients underwent a clinical examination and medical history taking. Exercise tolerance was determined by a 6-minute step test. The degree of dyspnea was assessed by a modified MRC (Medical Research Council Dyspnea Scale) in points. All the patients underwent a study of respiratory function with a bronchodilator test. The study was carried out with the use of the MasterScreen Body equipment (Erich Jaeger, Germany). The obtained data were compared to the proper values, calculated according to the formulas recommended by the European respiratory and American thoracic societies [10]. All the patients underwent a clinical study of blood and induced sputum to determine the number of eosinophils. As a criterion of eosinophilic inflammation outside exacerbation of the disease, the level of eosinophilia ≥300 cells/μl in peripheral blood and ≥3% of eosinophils in induced sputum was used [6, 7, 11]. Also, the level of eosinophilic cationic protein (ECP) in the peripheral blood was assessed for all the patients. The level of ECP > 24 ng/ml in peripheral blood was used as a criterion for increasing ECP outside exacerbation period [4]. The second visit was carried out 12 months after the first one to assess the dynamics of the disease.

The patients were stratified by the level of eosinophils and ECP in peripheral blood during the remission period of the disease. Stratification was based on the clinical, functional, and laboratory criteria obtained during the examination. The formed groups were comparable in age and smoking history. The data are presented in Tables 1 and 2.

Table 1

Characteristics of COPD patients with different levels of eosinophils in peripheral blood during remission, n = 161, Me [Q_{25}; Q_{75}]

Level of eosinophils in peripheral blood	Average age, years	Smoking history
≥ 300 cells/mcl	62 [54; 69]	40 [24.5; 53.3]
< 300 cells/mcl	64 [56; 72]	45 [40.0; 52.2]

Table 2

Characteristics of COPD patients with different levels of eosinophilic cationic protein in peripheral blood during remission of the disease, n = 161, Me [Q_{25}; Q_{75}]

Level of ECP in peripheral blood	Average age, years	Smoking history
> 24 ng/ml	63 [53; 72]	42 [26.0; 54.5]
≤ 24 ng/ml	65 [55; 70]	44 [38.5; 52.0]

The average age in the COPD group with elevated levels of eosinophils was 62 [54; 69.25], the smoking index was 40 [24.5; 53.25]. The average age in the COPD group with normal indicators of eosinophils was 64 [56; 72], the smoking index was 45 [40; 52.5].

Data processing. Statistica software package for Windows 10.0 was used for statistical processing of anamnesis data, clinical, functional and laboratory parameters. The χ^2 criterion was used for comparing the frequencies of qualitative features. The Mann – Whitney U-test was used to estimate the difference of means in pairwise unrelated samples. Qualitative data are presented in the form of absolute or relative (%) frequencies, and quantitative data are presented in the form of Mediana [Q_{25}; Q_{75}]. The difference of values at $p < 0.05$ was considered statistically significant. The Spearman coefficient was used in the correlation analysis. The correlation strength was estimated as follows: strong: ± 0.7 to ± 1; medium: ± 0.3 to ± 0.699; weak: 0 to ± 0.299.

Regression analysis was performed to identify the relationship between the level of eosinophilic cationic protein and clinical and functional features of COPD. The level of eosinophilic cationic protein was used as a dependent variable, and clinical and functional parameters were integrated as independent variables. The p values are given after the Benjamini-Hochberg adjustment. The threshold level of significance is less than 0.05.

RESULTS

In the course of the examination of COPD patients with different eosinophils and ECP levels in peripheral blood during remission of the disease, a comparative analysis of the main clinical and functional indicators of severity and phenotypic features of the disease was performed.

No significant differences have been found when comparing the clinical and functional parameters of COPD patients with elevated and normal levels of eosinophils in peripheral blood and induced sputum.

We analyzed the obtained data based on published studies that showed a significant increase in the level of eosinophils in COPD patients during exacerbation of the disease [12, 13]. It should be noted that there were patients with a transient increase in the level of eosinophils in periods of exacerbation of COPD in the group of patients with normal eosinophils level in peripheral blood during remission of the disease. During further analysis of the data from the medical history archive, we noticed that an increase in eosinophils in the peripheral blood with a level of ≥300 cells/μl was detected not at each exacerbation in the same patient. This did not allow us to identify the group with a transient increase in eosinophils in peripheral blood correctly and to conduct a comparative analysis.

We used the determination of the level of eosinophilic cationic protein in peripheral blood as a marker of the activity of eosinophilic inflammation. The average level of eosinophilic cationic protein in patients in the study was 15 [9; 23] mg/l. The comparative analysis revealed significant levels of difference in the content of ECP between groups of patients with the eosinophils level in peripheral blood ≥300 cells/μl and < 300 cells/μl (p = 0.029). However, the correlation analysis showed significant positive correlations of the average strength of the ECP level only with the frequency of exacerbation of COPD (p = 0.035, r = +0.06).

No significant differences were found when comparing the clinical and functional parameters of COPD patients with elevated and normal levels of eosinophils in induced sputum. Therefore, at the next stage of the study, COPD patients were divided into 2 groups depending on the presence of increased ECP in peripheral blood.

To assess the differences between the groups, a comparative assessment of clinical and functional parameters was performed in the groups of patients with COPD with elevated ECP levels and the group with its normal values. Table 3 presents the clinical and functional characteristics of these groups of patients.

Table 3

Comparative characteristics of clinical and functional parameters of COPD patients with different levels of eosinophilic cationic protein in peripheral blood, *Me* [Q_{25}; Q_{75}]

Parameters	COPD patients, n = 161		p
	with elevated levels of eosinophilic cationic protein (>24 ng/ml) n = 38	with normal levels of eosinophilic cationic protein (≤24 ng/ml) n = 123	
Exacerbations within 12 months, n	3.00 [2.00; 3.75]	1 [1; 2]	0.024
ICS +LABA+LAAC therapy (12 months), n (% of patients)	14 [36; 84]	14 [11; 38]	–
ICS (12 months), n (% of patients)	20 [52; 63]	24 [19; 51]	–
Course ABT frequency (12 months), n	1.5 [1.0; 3.0]	1 [0; 1]	0.042
Smoking index, pack/years	46.50 [26.75; 75.00]	40.0 [25.5; 60.0]	0.34
BODE index, points	3 [2; 4]	1 [0; 2]	0.031
mMRC, points	3 [2; 4]	1 [0.5; 2.0]	0.046
6-minute step test, meters	450.0 [302.5; 637.5]	540 [372; 670]	0.23
Sputum production, points	1.5 [1.0; 2.0]	1 [1; 2]	0.83
Pneumonias within 12 months, %	50	22	–

Table 3 (continued)

Parameters	COPD patients, n = 161		p
	with elevated levels of eosinophilic cationic protein (>24 ng/ml) n = 38	with normal levels of eosinophilic cationic protein (≤24 ng/ml) n = 123	
FEV1, % of the norm (after a bronchodilator test)	69.0 [53.75; 75.00]	67.0 [57.0; 76.5]	0.34
Level of eosinophils in blood (outside exacerbation), 10^9/l	0.35 [0.34; 0.41]	0.17 [0.11; 0.25]	0.026

Note. ICS – inhaled corticosteroids; ABT – antibacterial therapy; LAAC – long-acting anticholinergics; LABA – long-acting beta-agonists; FEV1 – forced expiratory volume in 1 second (here and in Table 4).

A comparative analysis of clinical and functional parameters of patients with different levels of eosinophilic cationic protein in blood plasma has revealed that patients with high content of ECP received courses of antibacterial therapy significantly more often. They were characterized by significantly more severe course of the disease, a higher frequency of exacerbations, more significant dyspnea and a higher value of the BODE index. Patients with high plasma ECP tended to have a higher risk of developing pneumonia (50% of patients in this group had a history of pneumonia). They also tended to need a higher volume of basic treatment (36% received dual bronchodilator therapy in combination with ICS). At the same time, these indicators did not differ statistically significantly between groups of patients stratified by the ECP level.

Regression analysis was performed to identify the relationship between the level of ECP and clinical and functional features of COPD. The significance of the ECP level for the severity of COPD and its connection with the inflammatory genesis of the disease exacerbation was proved by the method of multiple linear regression. The content of eosinophilic cationic protein was used as a dependent variable, and other clinical and functional factors were integrated as independent variables. The data obtained are presented in Table 4.

Table 4

Eosinophilic cationic protein level and associated clinical and functional parameters in COPD patients

Parameter	SLC	SLC std. dev.	p	adj. p
Sputum production, points	5.385	2.022	0.009	0.02
Exacerbations within 12 months	2.765	0.42	0.006	0.01
Level of eosinophils in blood (outside exacerbation), $\times 10^9$/л	8.64	3.232	0.008	0.022
Number of ABT courses within 12 months	2.808	0.441	0.002	0.03
ICS (12 months), n (% of patients)	5.538	1.428	0.0002	0.0007
LABA±LAAC (12 months), n (% of patients)	3.573	1.375	0.01	0.03
ICS +LABA+LAAC therapy (12 months), n (% of patients)	6.169	1.687	0.0003	0.001
FEV1, % of the norm (after a bronchodilator test)	4.782	1.421	0.003	0.02

* SLC – slope of line coefficient adj.p.
* p – value after the Benjamini – Hochberg adjustment.

The presented table shows that clinical and functional parameters in COPD patients with higher ECP levels in peripheral blood were characterized by a greater frequency of exacerbation of the disease, higher sputum production, more frequent courses of antibacterial therapy. The regression analysis has revealed a connection between the increase in ECP and the increase in the volume of treatment. COPD patients with elevated levels of eosinophilic cationic protein in peripheral blood were significantly more likely to receive continuous triple inhaled therapy, which includes inhaled long-acting beta-2-agonist and muscarinic antagonist in combination with ICS. This indicates a more severe course of the disease. It is noteworthy that maintenance therapy of the patients in this group included inhaled corticosteroids, the need for the prescription of which was associated with the level of ECP and was reliable (p = 0.0002; adj. p = 0.0007).

DISCUSSION

The performed work confirms that the high level of eosinophilic cationic protein in blood plasma in patients with COPD is associated with a more severe course of COPD and the development of more frequent infection-dependent exacerbations of the disease requiring the prescription of inhaled corticosteroids and antibiotics. We have demonstrated an inverse relationship between the level of ECP and FEV1, which allows us to use this indicator as a predictor of the severity of COPD. A small number in the sample of patients with extremely severe course of the disease (GOLD3-4) can explain the absence in our study of the relationship between the level of eosinophils in induced sputum and peripheral blood and the clinical and functional features of COPD in remission (identified in other studies) [14–16]. Our study will be continued in order to confirm the findings that, compared to the level of blood and sputum eosinophils, EPC is a more accurate marker of the nature of inflammation in COPD, a prognostic criterion for the severity of the disease and one of the leading criteria for the necessity to prescribe glucocorticoids to COPD patients.

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Authors contribution

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Macrophages as homeostatic regulators in the ischemically damaged myocardium after use of allogenic biomaterial

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ABSTRACT

Macrophages as the effector cells play a key role in initiating the inflammatory process and predetermine the manifestation of the postinfarction cardiosclerosis. The population of these cells is heterogenous and is mainly represented by M1 and M2 phenotypes. Alloplant biomaterial (ABM) is resorbed by the macrophages which became the regulators of the cellular interaction in tissues.

The aim of the investigation was to reveal the peculiarities of the postinfarction healing of the myocardium following the ABM insertion and to assess the population change in the dynamics of macrophages and c-kit+ cells.

Materials and methods. The experimental investigations were carried out on 100 male Wistar's rats weighing 0.18–0.25 kg. All the animals had coronary occlusion by way of ligating the arteries. In the experimental group, the ABM (12 mg) suspension was intramyocardially administered simultaneously with the vessel stricture formation. The harvesting of hearts was carried out at 3, 7, 14, 30, 45 days.

Results. In the experimental group the course of the inflammatory process was characterized by the onset of the early proliferative stage, whereas in the control group colliquative necrosis was developing. It was caused by different degrees of the macrophage reaction expression. The number of CD68+ cells in the rat reactive zone of the control group was bigger than in the experimental one. In the experimental group the ABM-induced macrophages of mesenchyme origin were revealed and c-kit+ cells were considerably more in number than in the control one. After 45 days, the scar area index in the experimental group was significantly less than in the control group.

Conclusion. ABM had a histoprotective effect under the conditions of the acute myocardial ischemia due to the inhibition of macrophage migration and induction of cellular cardiomyogenesis.

Key words: alloplant biomaterial, scar area, myocardium.

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Макрофаги как регуляторы гомеостаза миокарда после его ишемического повреждения в условиях применения аллогенного биоматериала

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РЕЗЮМЕ

Ведение. Макрофаги как клетки-эффекторы играют ключевую роль в инициации воспалительного процесса, предопределяют выраженность постинфарктного кардиосклероза. Популяция этих клеток является гетерогенной и представлена преимущественно фенотипами М1 и М2. Аллогенный биоматериал аллоплант (БМА) резорбируется макрофагами, продукты резорбции влияют на их способность регулировать клеточные взаимодействия.

Цель. Раскрыть особенности постинфарктного заживления миокарда после введения БМА. Оценить динамику изменения численности макрофагов и c-kit$^+$-клеток.

Материалы и методы. Экспериментальные исследования были проведены на 100 самцах крыс линии Вистар массой 0,18–0,25 кг. Всем животным была проведена коронароокклюзия верхней трети левой нисходящей коронарной артерии. В опытной группе сразу после стенозирования артерии в ее бассейн интрамиокардиально вводили суспензию, содержащую 12 мг БМА. Использовали гистологические, электронно-микроскопические, иммуногистохимические (CD 68, c-kit, Timp-2), морфометрические и статистические методы исследования. Забор сердец проводили через 3, 7, 14, 30, 45 сут.

Результаты. В опытной группе течение воспалительного процесса характеризовалось наступлением ранней пролиферативной стадии, в то время как в контрольной развивался колликвационный некроз. Группы характеризовались различной степенью выраженности макрофагальной реакции. Число CD68$^+$-клеток в реактивной зоне в контрольной группе было больше, чем у опытной группы. Напротив, в опытной группе выявлены БМА-индуцированные макрофаги мезенхимного происхождения, а численность c-kit$^+$-клеток была значительно больше, чем в контроле. Спустя 45 сут индекс площади рубца в опытной группе был статистически значимо меньше, чем в контроле.

Заключение. БМА в условиях острого ишемического поражения миокарда оказывал гистопротекторный эффект за счет ингибирования миграции макрофагов и индукции клеточного кардиомиогенеза.

Ключевые слова: биоматериал аллоплант, площадь рубца, миокард.

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

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INTRODUCTION

In the course of experiments using alloplant biomaterial (ABM) it was established that the key histion cells during the regeneration of fibrous connective and skeletal muscle tissue are macrophages with M1 phenotype. Their number significantly exceeds the amount of these cells in the control groups in which the defect infliction was not treated with the biomaterial in question [1, 2]. It was shown that the use of ABM had a positive effect upon the cardiac muscle condition and improved its structure following ischemic damage [3]. The ABM biodegrates into the tissue and its resorption products are the chemoattractant of the stem progenitor cells which induce the regeneration process [4, 5]. There are conflicting views on the negative role of M1 macrophages in the healing process of the ischemic damaged myocardium as key cells promoting cardiomyocyte damage, inflammation manifestation and fibrosis progression. Consequently, the study of macrophage involvement in inflammatory and degenerative processes developing in the cardiac muscle, following coronary occlusion experiments and when administering the ABM, appears relevant.

The aim of the study was to understand the effect of ABM on the post-infarction myocardium healing process and evaluate the dynamics behind the changing number of macrophages and c-kit+ cells.

MATERIALS AND METHODS

Experiments involving ABM were carried out on 100 male Wistar rats weighing 0.18–0.25 kg. All the animals were divided into two groups. The myocardium infarction modeling in the control group (n = 50) was performed as follows: all the animals under general anesthesia (intramuscular injection of Zoletil) underwent left-sided thoracotomy with further ligation in the upper third interventricular branch of the coronary artery (*r. interventricularis paraconalis a. coronarii sin.*). 12 mg of ABM suspended in physiological solution was administered into the cardiac muscle, in its pool zone, of the rats in the experimental group (n = 50) immediately after the coronary artery ligation. The ABM dose was chosen arbitrarily. The rats in both groups were euthanized after the experiment by lethal insufflation of ether vapors after 3, 7, 14, 30, 45 days. Ten rats were taken for each point of the study.

The studies were conducted according to the Rules of good laboratory practice of the Russian Federation in line with legislation adopted from the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasburg,1986). The studies were also carried out in accordance with the approved written protocol on standard operating procedures of a researcher as well as official laboratory guidelines on animal treatment and alternative models in biomedical studies [7].

Alloplant® biomaterial was developed in the Federal State Government-Funded Institution "Russian Eye and Plastic Surgery Centre" under the Ministry of Health of the Russian Federation, in the city of Ufa. This biomaterial is produced according to technical specifications 42-2-537-87; it is certified and was approved for clinical use by the order of the USSR Ministry of Health No. 87 901-87 dated 22.07.1987.

Histological study. The allogeneic biomaterial in this study was made from rat tendons and enlarged to a size of 50–80 mcm. The Ethics Committee approved the study protocol No. 31 dated 12.10.2015. For the histological investigation the hearts were fixed with 10% solution of neutral formalin, then dehydrated with increasing concentrations of alcohol and embedded in paraffin as per the generally accepted method. The sections were prepared with the use of LEICA RM 2145 microtome (Germany) and stained using the Mallory's staining technique.

Immunohistochemical study. The 4 mcm-thick paraffin sections were stained by Leica Microsystems Bond TM immunohistostainer (Germany). CD 68 and Timp-2 diluted in the proportion of 1 : 300 (Santa Cruz Biotechnology, USA) were used as primary antibodies. Single and double immunolabeling of cells for the given antibodies was carried out. An indirect Leica Bond (Novocastra TM , Germany) streptavidin-biotin detection system was used for unmasking. Assessment of reaction specificity when staining the sections was determined without primary antibodies. The positively stained cells were calculated in 20 fields of view of each specimen (n = 6) when magnified by X400. The investigation and visualization of the specimens was conducted with the use of the Leica DMD 108 (Germany) light microscope equipped with specialized software to manage settings and capture images.

Electron microscopic study. The myocardium pieces 1–2 mmi in size fixed by 2.5% glutaraldehyde solution were used for the electron microscopic study. The solution was prepared on the cacodylate buffer (pH 7.2–7.4) with further post-fixation by 1% OsO_4 solution on the same buffer. The material was dehydrated in increasing concentrations of alcohol and embedded into Epon-812 according to the generally accepted method. EM UC7 (Leica, Germany) ultramicrotome was used to prepare semithin sections which were stained by toluidine blue solution based on 2.5% anhydrous sodium solution. Areas were chosen on the specimens for the electron microscopic studies. The ultrathin sections were contrasted by 2% water solution of uranyl acetate and of lead citrate according to Reynolds. They were studied by JEM-1011(Jeol Ltd.; Japan) transmission microscope.

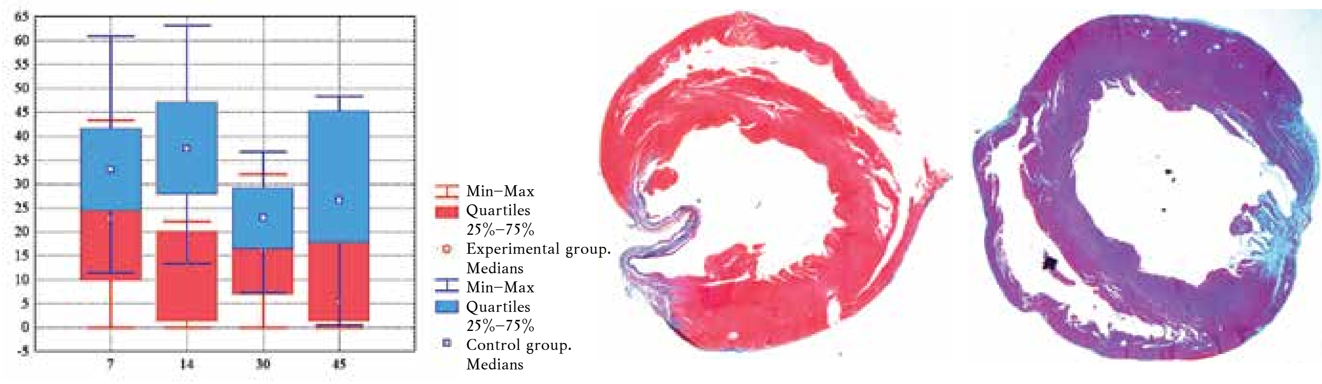
STATISTICS

Each heart was cut into five sections to determine the size of the postinfarction scar. The scar area index (SAI) was measured in the specimens of the heart cross-sections using ITEM software in the following way: the ratio between the scar area and left ventricle wall area was multiplied by 100%. The analysis of SAI values was performed using non-parametric methods, namely, the univariate Kruskal – Wallis analysis of variance and comparison of uncorrelated data by Mann – Whitney method [8].

RESULTS

The difference in healing between the ischemic damaged myocardium in the control group and that in the experimental ones was significant. The SAI data in the experimental group insignificantly depended upon the follow-up periods ($\chi^2 = 5.7$, $p > 0.12$). However, the values of this parameter tended to reduce gradually. The distribution medians by the 7th day totaled 22.7%, (0%, 43.3%) and dropped significantly to 13.4% (0%, 22.2%). They decreased on the 14th day, whereas on the 30th and 45th days they reached 16% (0%, 32.1%) and 5.2% (0%, 33.8%) ($p = 0.14$ and $p = 0.02$, respectively). The difference between the 14th, 30th and 45th days turned out to be statistically insignificant ($p = 0.23 \div p = 0.75$).

In the control group, the dependence of SAI on follow-up time was also statistically insignificant ($\chi^2 = 6.3$, $p = 0.10$). The differences in the SAI level from the initial one (day 7) were statistically significant only on the 30th day ($p = 0.01$). Figure 1 shows that during the whole period of observation in the control group there were no cases of zero SAI values. Comparison of both experimental groups at different time periods of the study showed that at implantation of ABM at all follow-up periods, SAI was statistically significantly less than in the control ($p = 0.01 \div p < 0.0001$).



a *b* *c*

Fig. 1. Index in the experimental and control groups (*a*): on the abscissa axis – observation time (days). The ordinate of the index area of the scar (%): *b* – cross-section of the myocardium after 45 days in the control group, × 40; *c* – myocardial cross-section after 45 days in the experimental group, ×40. Mallory's staining

Macrophage cells are of great importance in fibrous progression and scar manifestation [6]. The number of CD68 macrophages in the control group within the reactive zone of the ischemically damaged cardiac muscle exceeded the values of the experimental group almost throughout the entire experiment. In the control and experimental groups, the recurring rise and subsequent fall of the cell number was, on the whole, highly significant ($\chi^2 = 76.3$, $p << 0.0001$ and $\chi^2 = 45.2$, $p << 0.0001$, respectively). The number of CD68$^+$ cells in the control group was statistically significantly greater than the number in the experimental group during the follow-up period from the

3th to 14th day (p = 0.003 and less). The remodeling attenuation process of myocardium and scar formation took place over a period from the 30th to 45th day. This caused a decrease in the number of macrophages in both groups (p = 0.12) on the 45th day and transformation from the exudative-proliferative phase of inflammation into the recovery stage (Fig. 2).

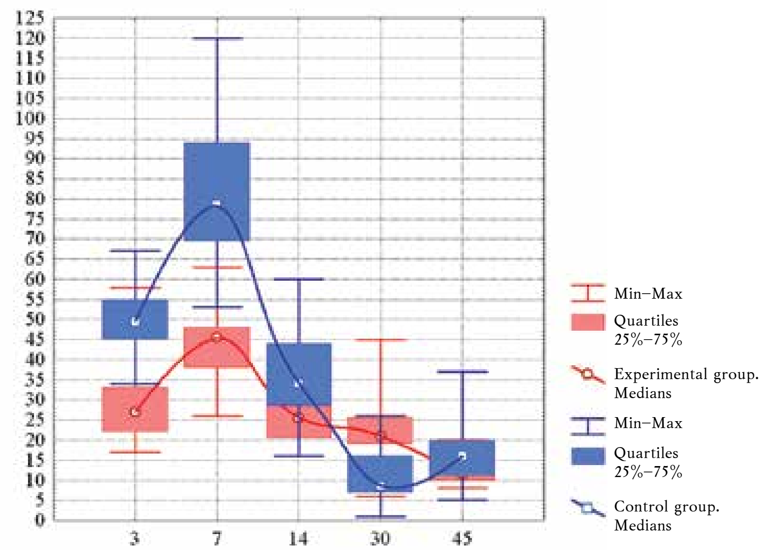
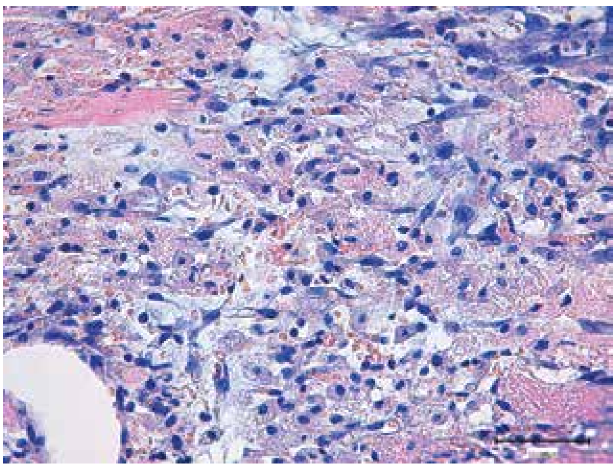


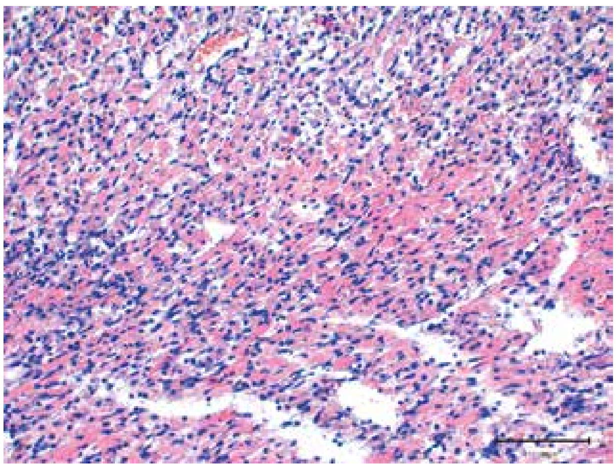
Fig. 2. The number of CD68+ macrophages in rat myocardium in experimental and control groups. On the x-axis is "days". On the ordinate axis – the number of cells

Through evaluation of the dynamics of pathomorphological changes, it was revealed that the initial stage of inflammation (day 3) was characterized by the early onset of the proliferative phase and formation of the granulation tissue in the perifocal area of the ischemically damaged myocardium. This is where thin collagen fibres, mesenchymal and macrophagal and fibroblastic infiltration were observed (Fig. 3, *a*).

In the control group, a wide cell shaft consisting of macrophages, lymphocytes, and neutrophils, was formed in place of the decaying cardiomyocytes. In this study, C-kit $^{+}$ cells in both groups were determined mainly in the peri-infarction and perivascular zones. Despite the autogenous origin of C-kit^{+} stem cells and absence of antigenicity factors they were subjected to phagocytosis by macrophages (Fig. 4, *a*).



a



b

Fig. 3. Morphological changes in the myocardium after 3 days: *a* – formation of granulation tissue in the perifocal zone, infiltration by macrophages, mesenchymal cells, fibroblasts in rat myocardium 3 days after coronary occlusion and ABM administration, *b* – macrophage-lymphocytic cell wall in the zone of necrotically changed cardiomyocytes 3 days after coronary occlusion. Stained with Hematoxylin and Eosin

Numerous macrophages phagocyting undifferentiated cells on the electron microscopic level were also recorded. Fragments of cytoplasm and pyknotic nuclei were detected in phagocytic vacuoles, and macrophage cells showed signs of activation. The nuclei were oval-shaped and contained large amounts of euchromatin; numerous large mitochondria with a darkened matrix and parallel oriented lamellar crystal were observed in the wide cytoplasma rim. The cytolemma formed deep invaginations. Golgi apparatus was well developed with piled up elongated flat cisterns and uncoupled vesicles (Fig. 4B).

When determining free C-kit+ cells, which were not subjected to macrophagal resorption, it was revealed that their number in the experimental group had surpassed statistically significantly the control group during the follow-up period (P< 0,001) (Fig. 5).

a *b*

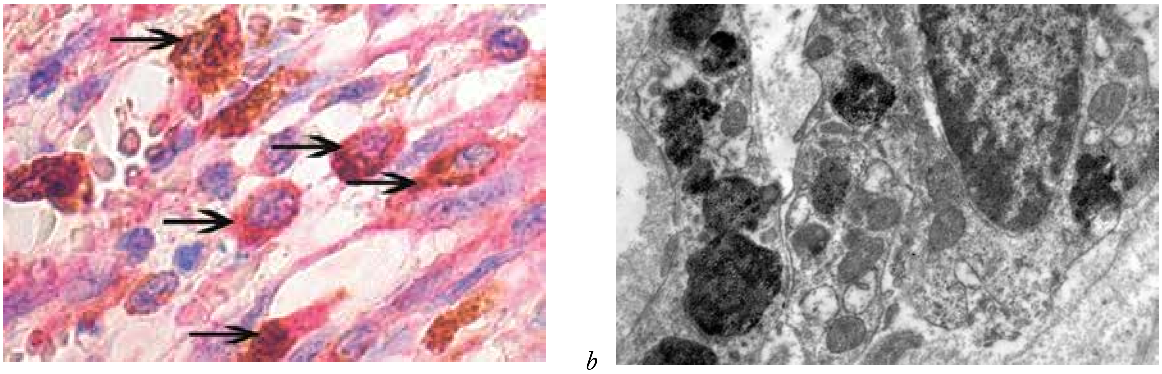


Fig. 4. Phagocytic macrophages: *a* – CD68+/c-kit+ 7 days after coronary occlusion. CD68 (brown), c-kit (red). Chromogen granules are detected in the cytoplasm of macrophages. Double indirect immunoperoxidase method for antigen detection with Hematoxylin staining, ×600; *b* – Phagocytic macrophage with vacuoles of cellular detritus 7 days after coronary occlusion, ×10.000. Electronograms

Hale positive macrophages expressing Timp-2 were revealed in the zone of implantation in the subepicardial space (Fig. 6).

DISCUSSION

Numerous factors, one of which being macrophage reaction induced by ABM, contributed more favorably to myocardial infarction healing in the experimental group. It has already been proven that the products of ABM biodegradation turn into chemoattractants of monocytes and macrophages during the connective tissue healing followed by the inflammatory and destructive process and after inflicting damage [2]. Macrophage cells displayed regeneration efficiency as a result of full-fledged phagocytosis and regula-

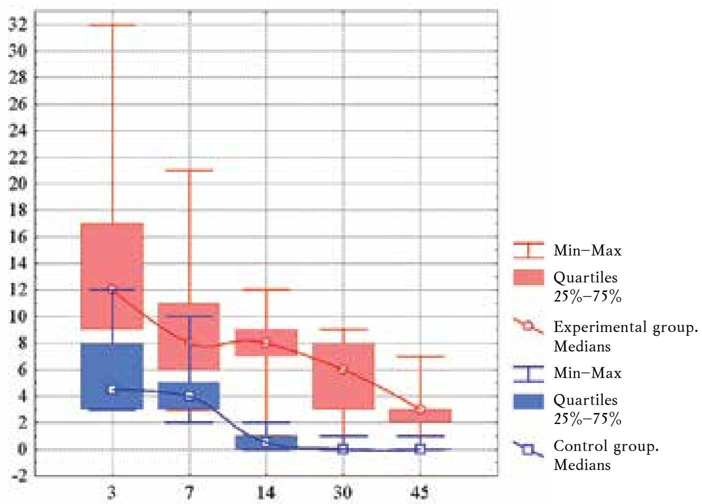


Fig. 5. The number of free c-kit+ cells in rat myocardium in the main and control groups, on the x-axis is "days", on the ordinate axis – the number of cells

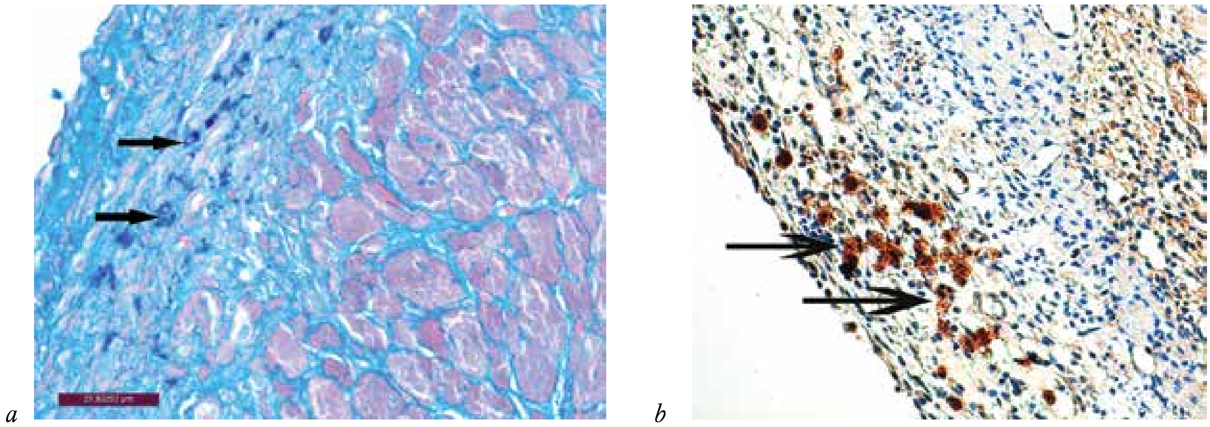


Fig. 6. Macrophages in subepicardial space after AMB administration after 7 days: *a* – GAG-positive macrophages (↑). Hale staining; *b* – Timp-2+ macrophages (↑). Indirect immunoperoxidase method for detection of Timp-2 with additional Hematoxylin staining, ×400

tion of the proliferative phase of the inflammation. They inhibited the fibroblastic activity by M1 stimulation of macrophages and prolongation of cytoxic phase [9, 10]. We got the opposite result in this study in case of acute myocardial infarction. Within 3 days, the ABM particles were resorbed and were not detected in the tissue. One can assume that after the biomaterial resorption, the ABM-induced macrophages became the regulators of intercellular interactions and stimulated the onset of the early proliferative inflammation phase activating fibroblastic cells.

In the control group the ischemically damaged cardiomyocytes initiated a succession of inflammatory cell reactions which resulted in increased inflammation, expansion of the damaged zone and scar manifestation. This observation was confirmed by the data of other researchers who had illustrated that peak levels of the corresponding family of proinflammatory (CD14+) macrophages and/or monocytes negatively correlated with the restoration of the left ventricle function following the acute myocardial infarction [11]. Dysregulated infiltration leads to the extension of myocardial infarction, expansion of the left ventricle and cardiac insufficiency. Monocytosis increases and extends stages of alteration and exudation due to the spectrum expression of inflammatory monokines (TNFa, IL1, IL6 etc) which, in turn, induce the exudative stress spreading over the nearby cardiomyocytes, thus expanding the necrosis zone. As a result of inflammation, the left ventricle remodeling is increased in case of the ischemic damages to the myocardium [12].

Macrophages are the polymorphic cellular population, the phenotype of which is determined by the microenvironment signals. In the experimental group after the use of ABM, the products of its biodegradation create a certain microenvironment, together with an anti-fibrogenic effect [9], which induces TIMP-2 expression by random cells. This phenomenon helps to decrease inflammation when acute ischemia occurs [13]. The modulation approach of macrophages changes according to their environment. It was revealed that phenotypes and functions of macrophages are formed by the corresponding organ microenvironment [14]. The transplantation of differentiated peritoneal macrophages into the pulmonary medium, for example, induced the transcriptional landscape reprogramming of those cells and their acquisition of new specific tissue functions [11, 15]. Thus, in case of acute ischemic myocardial damage, ABM has an anti-inflammatory effect and is a factor in the switching of the phenotype of macrophages from M1 to M2. Conversely, the destructive cardiomyocytes in the control group provoked a number of inflammatory reactions, due to the pronounced expression of metalloproteinases MMP-9 [13]. It is known that the regenerative process participants in the myocardium are not only effector fibroblast cells, but also cardiomyogenic progenitor cells. It is assumed that stem cell niches as well as epicardial cells, hematopoietic stromal cells etc. can be the source of stem cells [16,17]. The differentiation direction of progenitor stem cells is often unpredictable. This is explained by high probability of teratoma formation [18]. ABM stimulated migration of poorly differentiated C-kit+ cells. Phagocytosis by macrophages of C-kit+ cells is probably connected with the genetically programmed mechanism of anti-tumorigenicity. In spite of this fact, the level of progenitor cells in the experimental group remained high enough which contributed to a more wholesome regeneration of myocardium and inhibition of scar tissue development.

Macrophages populate heterogenous cells.

Macrophages of mesenchymal origin, otherwise known as "matrix-forming", have also been identified during previous experiments with ABM [1, 19, 20]. They featured Vimentin+ /Hale+ / CD68+ /PCNA phenotype and secreted glycosaminoglycanes (GAG); this phenomenon being typical of fibroblast cells. Macrophages appeared to be of the mesenchymal origin. It was revealed in the study that they had expressed Timp-2 tissue inhibitor of metalloproteinase. Presumably these cells play a structural and informative role for cellular cooperation and create homeostasis in the inflammation focus. Their presence is connected with synthesis of the hydrocarbon component. Timp-2 has a histoprotective effect due to the anti-inflammatory mechanism in the myocardium [21] which can set in motion the early proliferative phase of inflammation and homeostasis regulation. The discovery of macrophages of the given phenotype is consistent with the observation that the adult human heart contains macrophages of embryonal origin capable of tissue restoration. It is worth noting that though these families are present in the resting adult heart, the tissue-resident macrophages are lost after injury of heart in adults and are substituted by inflammatory monocytic macrophages of the medullary origin [14]. Thus, ABM had a histoprotective effect in the case of acute ischemic myocardium. Differences in the number, composition and function of microphages contribute to varying models of restoration and remodeling of the left ventricle observed in the given experiment.

CONCLUSION

Coronary artery stenosis alongside with ABM use allows to reduce the myocardium scar area by 2.74 times.

The ABM use decreases myocardium infiltration by macrophage cells.

During the myocardium restoration after the ischemic damage, macrophages are capable of actively phagocytizing autogenic stem cells.

There exists a population of GAG-positive macrophages in the ABM implantation zone.

ABM usage ensures a substantial prevalence of C-kit+ cells compared with the control group.

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Authors contribution

Lebedeva A.I. – collection and processing of material, carrying out of morphological studies, drafting of the article; Afanasiev S.A. – analysis and interpretation of data; final approval of the manuscript for publication; Muslimov S.A., Popov S.V. – conception and design of the study; Gareev E.M. – statistical processing of the obtained data; Kondratieva D.S. – carrying out of the main stages of the experiment.

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Gender and age related features of metabolically healthy obesity phenotype prevalence

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ABSTRACT

Aim. The study objective was to assess the age and gender characteristics of the metabolically healthy obesity phenotype (MHO) prevalence, taking into account various classifications.

Materials and methods. The materials used are the cross-sectional studies of the population cohort (Health, Alcohol and Psychosocial factors in Eastern Europe (HAPIEE) project, Novosibirsk), with the total of 3,197 people, among them 857 men (26.8%) and 2,340 women (73.2%), with BMI ≥30 kg/m². The MHO is defined according to different classifications: 1. IDF (International Diabetes Federation, 2005) – Waist circumference (WC) ≥ 94 cm in men and ≥80 cm in women and one or none of the components of metabolic syndrome (MS); 2. NCEP ATP III (the National Cholesterol Education Program Adult Treatment Panel III, 2001) in the presence of 2 and / or less components of the metabolic syndrome and 3. RSC (The Royal Society of Chemistry) - the index of waist circumference / hip circumference (WC / HC) ≤0.9 in men and ≤0.85 in women.

Results. According to IDF the frequency of MHO in the group was 23.2%; NCEP ATP III – 41.8; RSC criteria – 27.1%. The frequency of MHO was higher in women than in men, and it significantly decreased with the age in women population. In all classifications, increased average blood pressure (BP) level, with normal average values of the level of triglycerides (TG) and high-density lipoprotein (HDL) is typical for persons with MHO. The surveyed according to the RSC criteria people with MHO demonstrate higher frequency levels of all cardio metabolic risk factors than those surveyed with the use of other criteria of MHO.

Conclusion. The frequency of MHO varies depending on the used classification. In women, the frequency of MHO is reliably higher than in men. With the age, a significant reduction of the frequency of MHO in women is manifested. The frequency of arterial hypertension and abdominal obesity, the level of fasting blood glucose and LDL (low density lipoprotein), hypertriglyceridemia is higher in persons with MHO according to the criteria RSC.

Key words: obesity, prevalence, sex differences, metabolically healthy obesity phenotype.

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

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Conformity with the principles of ethics. All patients signed an informed consent to participate in the study. The study was approved by the local Ethics Committee at IIPM (Protocol No. 1 of 03.14.2002).

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Половозрастные особенности распространенности метаболически здорового фенотипа ожирения

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РЕЗЮМЕ

Цель. Изучить половозрастные особенности метаболически здорового фенотипа ожирения (МЗФО).

Материалы и методы. Использованы материалы кросс-секционного исследования популяционной когорты (проект HAPIEE, г. Новосибирск) (*n* = 3 197 человек, среди них 857 (26,8%) мужчин и 2 340 (73,2%) женщин, с индексом массы тела (ИМТ) ≥30 кг/м²). МЗФО определен в соответствии с различными классификациями: 1) IDF (2005) – окружность талии (ОТ) ≥94 см у мужчин и ≥80 см у женщин и любой компонент метаболического синдрома (МС) по IDF или без него; 2) NCEP ATP III (2001) при наличии 2 и (или) менее компонентов МС; 3) критерии РКО (2017) – индекс окружность талии/окружность бедер (ОТ/ОБ) ≤0,9 у мужчин и ОТ/ОБ ≤ 0,85 у женщин.

Результаты. Среди лиц с ожирением частота МЗФО по критериям IDF – 23,2%; NCEP ATP III – 41,8%; РКО – 27,1%. Частота МЗФО выше у женщин, чем у мужчин, и она значимо снижается с возрастом в женской популяции. Для лиц с МЗФО по всем классификациям характерны повышенное среднее значение артериального давления при нормальных средних значениях уровня триглицеридов и холестерина липопротеидов высокой плотности. Обследованные с МЗФО по критериям РКО демонстрируют более высокие показатели частоты всех изучаемых кардиометаболических факторов риска, чем при использовании других критериев МЗФО.

Выводы. Частота МЗФО варьирует в зависимости от используемой классификации. У женщин частота МЗФО достоверно выше, чем у мужчин. С возрастом отмечается значимое снижение частоты МЗФО у женщин. Частота артериальной гипертонии, абдоминального ожирения, уровень глюкозы крови натощак, холестерина липопротеидов низкой плотности и гипертриглицеридемия выше у лиц с наличием МЗФО по критериям РКО.

Ключевые слова: ожирение, распространенность, половые различия, метаболически здоровый фенотип ожирения

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

Источник финансирования. Работа выполнена в рамках бюджетной темы НИИТПМ – филиал ИЦиГ СО РАН ГЗ № 0324-2018-0001, № АААА-А17-117112850280-2, проект HAPIEE частично поддержан грантами Wellcome Trust, UK064947/Z/01/Z; 081081/Z/06/Z; National Institute of Aging, USA (1R01 AG23522).

Соответствие принципам этики. Все пациенты подписали информированное согласие на участие в исследовании. Исследование одобрено локальным этическим комитетом НИИТПМ (протокол № 1 от 14.03.2002).

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INTRODUCTION

The modern obesity epidemy is one of the most serious public health problems of our century. Usually, obesity is accompanied by an unfavorable metabolic profile, such as carbohydrate metabolism disorders, altered lipid profile, increased blood pressure (BP), systemic inflammation, altered liver enzymes, etc. [1]. The cluster of changes caused by obesity is also known as metabolic syndrome (MS).

However, recent evidence suggests that obesity does not always lead to adverse metabolic effects and it is increasingly recognized not as not being a homogeneous condition [2]. Approximately 10–30% of obese people are metabolically healthy despite excessive body fat accumulation. This phenomenon is referred to in modern literature as a metabolically healthy obesity phenotype (MHO) [3]. However, the main obstacle to understanding the epidemiology of MHO and its long-term perspective is the contradictory definition in various studies [4–6]. For example, some studies show that the prevalence of MHO varies depending on the definition used. This circumstance contributes to the discrepancy between this phenotype and the health consequences. Rey-Lopez and coauthors conducted a systematic review of the prevalence of MHO and they reported that the frequency of this phenotype varies from 6% to 75%. They also suggested that prevalence may vary depending on several socio-demographic factors, such as gender, age, and ethnicity. The authors then stratified the analysis by gender and age, and found that the prevalence of MHO was higher in women and younger individuals [7].

Thus, it is important to understand that researchers may introduce overweight and/or obesity and/or different MS criteria into this concept. Thus, participants with the absence of metabolic changes, or with the presence of one/two components of metabolic syndrome (MS), depending on the definitions of the latter, may fall under the definition of metabolically healthy individuals [6].

Despite different study designs and population differences, the variability in the frequency of MHO reported in both comparative studies and meta-analyses underscores the need for larger representative population studies and the need for a global consensus on a standard definition of MHO.

The aim was to assess the age and gender characteristics of MHO prevalence, taking into account various classifications.

MATERIALS AND METHODS

The survey of a representative sample of Novosibirsk residents was conducted in 2003-2005, within the framework of the international project HAPIEE (Health, Alcohol and Psychosocial factors In Eastern Europe), which is a prospective cohort study designed to study the impact of classical and non-traditional risk factors, as well as social and psychosocial factors on cardiovascular and other non-communicable diseases in Eastern Europe and the CIS countries [8]. The analysis included persons with a body mass index (BMI) ≥ 30 kg / m2: There were 3,197 people, 857 (26.8%) males and 2,340 (73.2%) females. In the initial survey the following data was analyzed: age, anthropometry, systolic blood pressure (SBP), diastolic blood pressure (DBP), total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), fasting plasma glucose (BG), table 1.

Table 1

Main characteristics of the studied sample of 45–69 years, $M \pm \sigma$

Parameters	Male, n = 857	Female, n = 2.40	Both genders, n = 3.197	$p_{м/ж}$
SPB (mmHg)	151.6 ± 23.3	151 ± 26.5	151.2 ± 25.7	0.573
DBP (mmHg)	96.5 ± 13.1	94.6 ± 13.1	95.1 ± 13.1	<0.001
BMI (kg/m^2)	33.1 ± 3.0	34.9 ± 4.2	34.4 ± 4.0	<0.001
BG (mmol/l)	6.8 ± 2.3	6.3 ± 1.8	6.4 ± 2.0	<0.001
TC (mmol/l)	6.2 ± 1.1	6.6 ± 1.3	6.5 ± 1.2	<0.001
LDL-C (mmol/l)	4.0 ± 1.0	4.3 ± 1.1	4.2 ± 1.1	<0.001
HDL-C (mmol/l)	1.4 ±0.3	1.5 ± 0.3	1.4 ± 0.3	<0.001
TG (mmol/l)	1.9 ± 1.0	1.8 ± 0.9	1.8 ± 0.9	<0.001

BP was mesasured three times with an interval of two minutes in the right hand in a sitting position after a 5-minute rest using an automatic tonometer Omron M5-I (Japan). The average value of three measurements was recorded. We found out the awareness of the screening participants about the presence of previously elevated blood pressure and about taking antihypertensive drugs during the last two weeks. Persons with previously diagnosed arterial hypertension (AH), but with normotonia on screening, in cases of taking drugs that reduce blood pressure, were also considered as patients with AH.

Standing height was measured without outer clothing and shoes with a standard height meter. Body weight was determined without clothing and shoes with a doctor's scales that passed metrological control. The measurement accuracy was 0.1 kg. Body mass index (BMI) was calculated by the formula: BMI (kg/m^2) = weight (kg) / height (m)^2 (WHO (World Health Organization), 1997).

Blood for biochemical studies was taken by venipuncture using vacutainers in fasting state, after 12-hour fasting. The content of TG, HDL-C and glucose was determined by enzymatic methods using an automatic biochemical analyzer "KoneLab 300". Conversion of fasting serum glucose into blood plasma values was carried out according to the formula proposed by experts of the European Association for the study of diabetes in 2007: plasma glucose concentration (mmol/l) = −0.137 + 1.047 × serum glucose concentration (mmol / l).

Three variants of criteria were used to single out the metabolically healthy phenotype of obesity: presence of BMI ≥30 kg/m^2 and

1. (IDF, 2005) from ≥ 94 cm in men and ≥80 cm in women and in the presence or absence of one of the following MS components: TG≥1.7 mmol/l or prior treatment (hyperTG); HDL-C – <1.0 mmol / l in men and <1.3 mmol / l in women or prior treatment (hypo-HDL); Blood pressure ≥130/85 mmHg or previous antihypertensive therapy (AH); Fasting plasma glucose ≥5.6 mmol / l or presence of D2.

2. (NCEP ATP III, 2001) presence of one or 2 of the following MS components: FROM >102 cm in men and >88 cm in women; TG ≥1.7 mmol/l; HDL-C <1.0 mmol / l in men and <1.3 mmol / l in women; Blood pressure≥130/85 mmHg; Blood plasma glucose ≥ 6.1 mmol / l (BG) or prior treatment.

3. Project (RSC, 2017) index of waist circumference / hip circumference (WC/HC) ≤0.9 in men and WC/HC ≤0.85 in women.

Statistical analysis was carried out using the statistical software package SPSS (Statistical Product and Service Solutions) 13.0 for Windows (1 Sep. 2004). The level of statistical significance of the differences was assessed by the Student's criterion (t) in the presence of two groups. The distribution of features obeyed the normal distribution (Kolmogorov – Smirnov criterion was used to assess the normality of the distribution), in the case of distribution other than normal, for analysis using parametric criteria, the transformation of indicators using natural logarithm was carried out. The data obtained are presented in the tables and the text as absolute and relative values (n, %), as well as ($M \pm \sigma$), where M is the arithmetic mean; σ is the standard deviation. Differences were considered as statistically significant at $p < 0.05$; $p \leq 0.01$-very significant; $p \leq 0.001$-highly significant.

RESULTS

The sample of obese persons (BMI ≥30 kg/m^2) was 3,197 people: 857 males (26.8%) and 2,340 females (73.2%). The frequency of the metabolically healthy obesity phenotype varies significantly depending on the criteria used, as shown in Fig. 1.

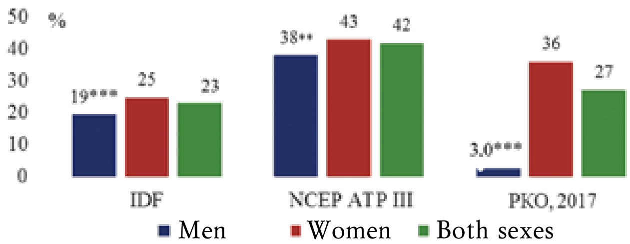

Fig. 1. The prevalence of the metabolically healthy obesity phenotype by different classifications. ** $p < 0.01$, *** $p < 0.001$ statistical significance between men and women

The frequency of MHO according to IDF criteria, 2005 was 23% (n = 743 people), NCEP ATP III, 2001 – 41.8% (n = 1,338 people), RSC, 2017 – 27% (n = 867 people), $p < 0.001$. At the same time, according to the criteria of the RSC, an interesting peculiarity was obtained in men. The frequency of MHO in them is 3%, which indicates a high prevalence of abdominal obesity.

According to the data obtained MHO is more common in women than in men, Fig. 1. Since age and gender are important factors in the development of MHO, we estimated the frequency of MHO in different age groups, Figure 2–4. In women, the highest frequency of MHO was determined in the age range of 45–49 – 34.1% (IDF, 2005), 54.0% (NCEP ATP III), 52.9% (RSC, 2017), $p < 0.001$, is significantly less common in women at the age of over 55, compared with the age of 45–49. In men, no statistical significance of differences in the frequency of MHO in all age groups was obtained ($p > 0.05$), Fig. 2–4.

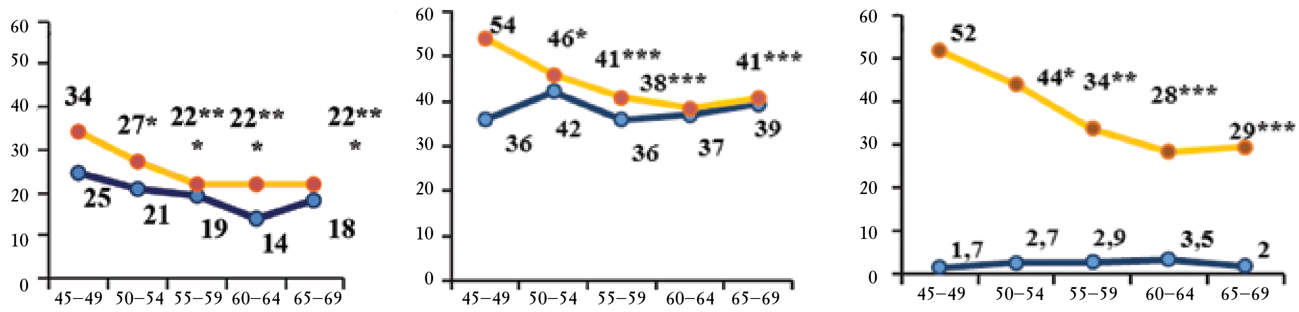

Fig. 2. Age characteristics and gender characteristics of the prevalence of MHO: * $p < 0.05$, *** $p < 0.001$, the statistical significance of the differences in age ranges compared with age 45–49

Thus, our results indicate a higher incidence of MHO in women than in men, as well as a decrease in the incidence of MHO in women over the age of 55.

The analysis of the main components was carried out in persons with MHO according to different classifications where it was found that the average values of SPB, DBP presented in Table 2 are higher than recommended by modern recommendations for the diagnosis and treatment of hypertension [15]. The analysis of lipid spectrum components showed normal values of TG and HDL-C levels in all analyzed classifications, in contrast to the levels of TG and LDL-C, which exceed the reference values for the general population, in persons with low cardiovascular risk [16].

Table 2

The main characteristics of the components in persons with MH, $M \pm \sigma$

Parameters	IDF $n = 743$	NCEP ATP III $n = 1.338$	RSC $n = 867$
SPB (mmHg)	142.9 ± 25.5	145.4 ± 26.1	146.7 ± 25.3
DBP (mmHg)	90.8 ± 13.0	92.0 ± 13.5	92.5 ± 12.5
BMI (kg/m²)	33.7 ± 3.6	33.7 ± 3.6	34.1 ± 4.1
WC (cm)	101.0 ± 9.6	101.3 ± 9.6	96.1 ± 7.9
BG (mmol/l)	5.3 ± 0.7	5.5 ± 0.7	5.8 ± 1.2
TC(mmol/l)	6.0 ± 1.0	6.1 ± 1.1	6.4 ± 1.2
LDL-C (mmol/l)	3.9 ± 0.9	4.0 ± 1.0	4.2 ± 1.1
HDL-C (mmol/l)	1.5 ± 0.2	1.5 ± 0.2	1.5 ±0.3
TG(mmol/l)	1.1 ± 0.3	1.2 ± 0.3	1.5 ± 0.8

The highest rates of cardiometabolic risk factors were determined in individuals with MHO according to the criteria proposed by the RSC 2017, despite lower mean values of WC.

The analysis of the frequency of risk factors in individuals with MHO showed a high prevalence of abdominal obesity (AO) in both men and women. At the same time, a comparative analysis of gender characteristics revealed that AO is more common in women than in men: NCEP ATP III – 90% and 71%, respectively, $p < 0.001$; IDF – 99% and 97 %, respectively, $p < 0.001$;

RSC – 99% and 86%, respectively, $p < 0.001$, Figure 3.

The frequency of AH in the NCEP ATP III group in men and women is the same, $p < 0.01$. We found a higher prevalence of AH in men (91%) than in women (84%) according to the criteria of the MHO RSC, $p < 0.001$. In the MHO group, IDF AH is more often determined in women than in men: 70% and 67%, respectively, $p < 0.05$.

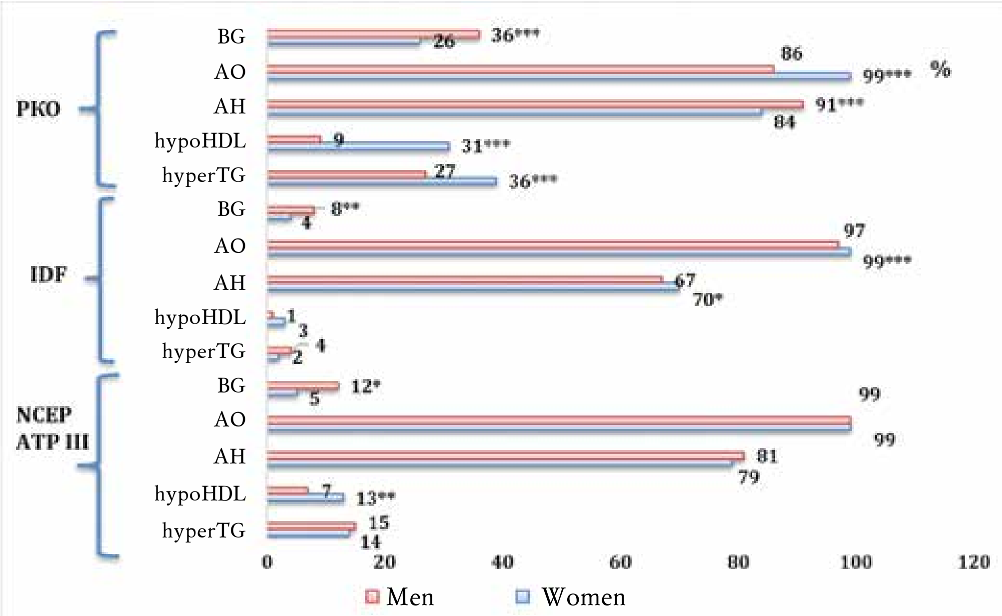

Fig. 3. Frequency of MS components in individuals with MHO according to IDF and NCEP ATP: III criteria
* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ statistical significance of differences between men and women within the same classification of MHO

Carbohydrate metabolism disorders have a low incidence in individuals with MHO, according to NCEP ATP III and IDF criteria. However, according to the RSC criteria, the prevalence of hyperglycemia in men was 36%, in women – 26%, $p < 0.001$. Various lipid spectrum disorders such as hyperTG and hypoHDL have a low incidence in MHO according to IDF and NCEP ATP III criteria and no statistical significance of differences between men and women was obtained, $p < 0.05$. However, according to the RSC criteria, the prevalence of hyperTG and hypoHDL in women is quite high: 39% and 31%, respectively, while in men the frequency of hyperTG is lower (27%), and hypoHDL is 9%, $p < 0.001$.

Based on the above, a high frequency of such components of the metabolic syndrome as AO and AH in all studied classifications was revealed in persons with MHO. At the same time, in accordance with the criteria of RSC, MHO demonstrates higher rates of all cardiometabolic risk factors.

DISCUSSION

Recently, the lack of a standard approach to the use of the same criteria and limit values sets in order to determine metabolic disorders has been identified as the main source of the high variability in the prevalence of MHO obesity, which was reported earlier [3].

Both around the world and in the Russian Federation, experts are searching for the criteria of the MHO. The Russian cardiological society published a draft of recommendations relating to obesity in 2017. This paper actively discusses the feasibility of isolating a group of patients with a metabolically healthy obesity phenotype. The authors propose to allocate this phenotype of obesity in each category of BMI on the basis of the index WC/HC [9]. This may be due to the fact that a number of data suggests that body fat distribution is a strong metabolic and cardiovascular risk factor [10-11]. The HUNT-II study suggested that indicators of abdominal obesity, such as the WC/HC index, may serve as better predictors of coronary heart disease than BMI [12]. A similar data was obtained in the Australian national representative cross-sectional study (AusDiab), among 11,247 thousand participants aged ≥25, it was revealed that those with a larger WC and a small HC had the highest prevalence of undiagnosed diabetes. A similar pattern was shown for the prevalence of undiagnosed hypertension and undiagnosed dyslipidemia [13].

In our study, the analysis of the prevalence revealed that the frequency of MHO according to the criteria of IDF 2005 is 23%, according to NCEP ATP III is 41.8%. In women, the frequency of MHO is determined significantly more often than in men. However, the results of the BioSHaRE researchers, who analyzed data from several epidemiological studies using the same standard criteria, also demonstrate a significant diversity in the prevalence of MHO in Europe. The highest percentage of MHO in men was found in CHRIS and KORA studies, and in women in NCDS, LifeLines, KORA and CHRIS, while the lowest prevalence was found in Finnish cohorts and in HUNT2 [14]. According to Ostrovskaya E. V., in the analysis of 389 case records of patients at the age of 18–60 with obesity, the frequency of MHO, taking into account the criteria of IDF 2005, was 38.6%. It is possible that this difference in frequency is connected with a younger average age of the participants of the study [15]. The group of domestic authors (O. Rotar et al.) studied the prevalence of MHO in 13 regions of Russia (Volgograd, Vologda, Voronezh, Vladivostok, Ivanovo, Kemerovo, Krasnoyarsk, Orenburg, Tomsk, Tyumen, St. Petersburg and the Republic of North Ossetia-Alania) with the participation of 1,600 people aged 25–65. The maximum prevalence of MHO, taking into account the criteria of the IDF MS in 2005, was noted in Tyumen at 52.2%, the minimum in Voronezh at 25.7% with a total prevalence of 41% and no significant gender differences [16]. In another domestic study of scientists from St. Petersburg, the prevalence of MHO was significantly lower at only 8.7% [17]. The lower rates were predetermined by the fact that in this work the combination of the minimum number of manifestations of metabolic syndrome together with normal tissue sensitivity to insulin was assigned to the criteria of MHO.

According to the data we have obtained, gender differences in the frequency of MHO in different age groups were revealed. Thus, women over 55 have a significant decrease in the frequency of MHO, as distinct from men. The obtained data can be explained by the fact that at this age women go through menopause. According to the literature, menopausal women show a greater incidence of MS and an increase in the prevalence of MS components: BP, AO, hyper-LDL-C, hyper-HDL-C, hyper-TG, high levels of glucose [18].

In men, there is a slight decrease in the frequency of MHO in older age groups, perhaps this is connected with the average life expectancy, which in men is 66.5 years in Russia for 2016. This age is an order of magnitude less than that of women: 77.1 years [19].

According to the data we have obtained, the average values of SBP, DBP presented in Table 2 are higher than the levels indicated by modern recommendations for the diagnosis and treatment of hypertension, but the average values of TG and HDL-C the level are in the reference range [20].

It is also worth noting that MHO is a transitional state [6], in which components of the metabolic syndrome may join over time. According to our findings on the frequency of cardiometabolic risk factors, persons with MHO, according to the criteria of RSC, have the highest frequency of all risk factors in both men and women, Figure 3. Traditionally, it is believed that the WC/HC index should reflect the presence of AO, but our data shows a fairly high frequency of AO, despite the normal values of the WC/HC index against MHO. And in the general population of Novosibirsk aged 45–69, the most common components among people with MS, according to the criteria of NCEP-ATP III 2001 in the urban Siberian population aged 45–69, are hypertension (95 %) and abdominal obesity (85%) [21].

Thus, data on variability in the prevalence of MHO, as well as higher prevalence at a younger age, correspond to world sources [24, 25]. This situation makes the future prospects of this condition unclear. So a unified classification of the metabolically healthy phenotype of obesity is needed, in order to determine such outcomes as myocardial infarction, cerebrovascular accident, type 2 diabetes, etc. And it remains unclear at what stage it is necessary to carry out medical intervention to the change of the lifestyle, in order to obtain further benefits for the health of the patient, which requires further comprehensive study of this problem.

CONCLUSIONS

The frequency of MHO varies depending on the classification used: IDF, 2005 – 23%; RSC, 2017 – 27%; NCEP ATP III – 41.8%.

In the female sample, the frequency of MHO is statistically significantly higher than in men in all classifications.

In women older than 55, there is a statistically significant decrease in the frequency of MHO.

Women, according to the RSC criteria, show higher rates of all cardiometabolic risk factors than when using other MHO criteria.

Persons with MHO are characterized by increased mean values of SBP, DBP, with normal mean values of TG and HDL-C.

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Authors contribution

Mustafina S. V. – conception and design; justification of the manuscript and critical revision of the manuscript for important intellectual content; final approval of the manuscript for publication. Vinter D.A. – conception and design; analysis and interpretation of data. Shcherbakova L. V. – conception and design; justification of the manuscript and critical revision of the manuscript for important intellectual content. Malyutina S. K. – conception and design. Ragino Yu. I. – conception and design. Rymar O.D. – conception and design; justification of the manuscript and critical revision of the manuscript for important intellectual content; final approval of the manuscript for publication.

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Association of rs10507391 polymorphism with the development of acute cerebrovascular accident in patients with cardiovascular pathology

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ABSTRACT

The aim of the study was to investigatey the association of single-nucleotide polymorphism (SNP) rs10507391 (A>T) with the acute cerebrovascular accident (CVA) development in patients of the East Siberian population with cardiovascular pathology and its risk factors.

Material and methods. The study involved 260 patients with acute CVA (age [57.0; 51.0–62.0]) and 272 patients of the control group (age [55.0; 51.0–62.0]). Among the patients who had acute CVA there were 157 men and 103 women. The control group included 170 men and 102 women. The examination of the experimental group included: collection of complaints, anamnesis, clinical examination, computed tomography of the brain, electrocardiography, echocardioscopy, ultrasound duplex scanning of extracranial brachiocephalic arteries, daily blood pressure and heart rate monitoring, and analysis of the blood coagulation system. In patients of the experimental group, the following cardiovascular pathology and risk factors were present: arterial hypertension, paroxysmal supraventricular tachycardias, dyslipidemia, atherosclerosis of the brachiocephalic arteries, and disorders of the hemostatic system. The control group was surveyed in the framework of the international "HAPIEE" project. Molecular genetic research was performed by real-time PCR. Statistical processing of the material was carried out using the following software: Statistica for Windows 7.0, Excel and SPSS 22.

Results. When studying the association of SNP rs10507391 (A>T) with the acute CVA development in all the analyzed groups and subgroups of patients, a link was established between the rare TT genotype and the T allele and an increased risk of acute CVA.

Conclusion. TT genotype and T allele of the SNP rs10507391 (A>T) increase the risk of acute CVA in patients regardless of previous cardiovascular pathology and its risk factors, including patients with arterial hypertension, supraventricular tachyarrhythmias, atherosclerosis of brachiocephalic arteries, impaired lipid metabolism and hemostasis system.

Key words: acute CVA, supraventricular tachycardia, arterial hypertension, dyslipidemia, atherosclerosis, hemostasis, rs10507391.

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

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Conformity with the principles of ethics. All study participants signed a written informed consent. The study was approved by the local Ethics Committee at KrasSMU n.a. prof. V.F. Voyno-Yasenetsky (Protocol No. 29 of 18.01.2011).

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Ассоциация полиморфизма rs10507391 с развитием острого нарушения мозгового кровообращения у пациентов с сердечно-сосудистой патологией

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РЕЗЮМЕ

Цель. Изучить ассоциацию однонуклеотидного полиморфизма (ОНП) rs10507391 (A>T) с развитием острого нарушения мозгового кровообращения (ОНМК) у пациентов с сердечно-сосудистой патологией и факторами риска ее развития, являющихся представителями восточносибирской популяции.

Материалы и методы. В исследовании приняли участие 260 пациентов с ОНМК, (возраст [57,0; 51,0–62,0]) и 272 пациента контрольной группы (возраст [55,0; 51,0–62,0]). Среди пациентов, перенесших ОНМК, – 157 мужчин и 103 женщины. Контрольная группа – 170 мужчин и 102 женщины. Обследование основной группы включало: сбор жалоб, анамнеза, клинический осмотр, компьютерную томографию головного мозга, электрокардиографию, эхокардиоскопию, ультразвуковое дуплексное сканирование экстракраниальных брахиоцефальных артерий, суточное мониторирование артериального давления и сердечного ритма, анализ свертывающей системы крови. У пациентов основной группы присутствовала следующая сердечно-сосудистая патология и факторы риска: артериальная гипертензия, пароксизмальные наджелудочковые тахикардии, дислипидемия, атеросклероз брахиоцефальных артерий, нарушения системы гемостаза. Контрольная группа обследована в рамках международного проекта HAPIEE. Молекулярно-генетическое исследование проводили методом полимеразной цепной реакции в реальном времени. Статистическая обработка материала проводилась с применением набора прикладных программ Statistica for Windows 7.0, Excel и SPSS 22.

Результаты. При изучении ассоциации ОНП rs10507391 (A>T) с развитием ОНМК во всех анализируемых группах и подгруппах пациентов установлена связь между редким генотипом TT и аллелем T и повышенным риском ОНМК.

Заключение. Генотип TT и аллель T ОНП rs10507391 (A>T) повышают риск развития острого нарушения мозгового кровообращения у пациентов вне зависимости от предшествующей сердечно-сосудистой патологии и факторов риска, в том числе у пациентов с артериальной гипертензией, наджелудочковыми тахиаритмиями, атеросклерозом брахиоцефальных артерий, нарушением липидного обмена и системы гемостаза.

Ключевые слова: ОНМК, наджелудочковая тахикардия, артериальная гипертензия, дислипидемия, атеросклероз, гемостаз, rs10507391.

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

Источник финансирования. Работа поддержана грантом президента Российской Федерации № МД-58887.2018.7.

Соответствие принципам этики. Все участники исследования подписали письменное информированное согласие. Исследование одобрено этическим комитетом КрасГМУ (протокол № 29 от 18.01.2011).

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INTRODUCTION

The single-nucleotide polymorphism (SNP) rs10507391 (A> T) is located on chromosome 13, position 30737959, at the locus of the ALOX5AP gene. The gene encodes a protein necessary for the synthesis of leukotrienes, which are metabolites of arachidonic acid and are involved in various types of inflammatory reactions. ALOX5AP genotypes are associated with a risk of developing such cardiovascular diseases as myocardial infarction (MI) and ischemic stroke [1].

The relationship between SNP rs10507391 of the ALOX5AP gene and five other polymorphisms (ALOX5 rs12762303 and rs12264801, LTA4H rs2072512, rs2540487, rs2540477) and myocardial infarction and risk factors for its development (dyslipidemia, alcohol consumption, smoking) were studied in the Chinese population. 401 patients with history of myocardial infarction (MI) and 409 people in the control group were genotyped according to these polymorphisms. SNP rs10507391 was significantly associated with lipid levels in patients with MI (p <0.006–0.008) [2].

The associations of ALOX5AP gene polymorphisms with subclinical atherosclerosis in families of type 2 diabetes (T2D) patients were studied by K.P. Burdon et al. (2010, USA). The study involved 998 patients, of whom 828 suffered from T2D and 170 did not have diabetes. As a result of the study, associations of the ALOX5AP gene single-nucleotide polymorphisms (rs9506352 and rs4769060) with subclinical atherosclerosis of the coronary and carotid arteries were revealed. Regarding the rs10507391 polymorphism, no statistically significant results were obtained [3].

A number of studies have confirmed the association of ALOX5AP gene polymorphisms with stroke among the Chinese population.

In the Chinese population, the relationship between ALOX5AP-SG13S114A/T (rs10507391), COX-2-765G/C and COX-1-50C/T polymorphisms and acute CVA was evaluated. The study included 411 patients with acute CVA and the same number of people in the control group. None of the genes showed significant associations with acute CVA in the isolated analysis. However, in carriers of the AA rs10507391 genotype of the ALOX5AP gene and the CC genotype of the COX-2-765CC polymorphism, the risk of acute CVA increased by 2.84 times (95% confidence interval (CI) 1.344–6.543). The obtained results confirm the polygenic etiology of acute CVA [4].

The effect of gene interactions on the risk of acute CVA in the Chinese population was confirmed by L.F. Chi et al. (2014). In 292 patients with acute CVA and 259 healthy people, the authors conducted a study of 8 SNPs in five candidate genes. Multivariate analysis showed a pronounced interaction of the ALOX5AP gene rs10507391 and the CYP3A5 gene rs776746 (p = 0.0107). This interaction was associated with an increased risk of acute CVA in patients (taking into account age, hypertension and diabetes; odds ratio (OR) = 1.804; 95% CI 1.180–2.759, p = 0.006) [5].

Associations of six ALOX5A gene polymorphisms with acute CVAs were studied in the northeastern Chinese population. Genotyping by polymorphisms of the studied genes was carried out by real-time polymerase chain reaction (PCR) and DNA sequencing. The results showed that only the G allele of rs9579646 polymorphism was significantly associated with an increased risk of acute CVA. There were no statistically significant differences in the genotype frequencies of rs9551963, rs9315050, rs4769874, rs10507391 and rs4147064 polymorphisms in the experimental and control groups. However, association analysis of rs9579646 and rs10507391 polymorphisms showed that the increased risk of acute CVA was significantly associated with the GT and GA haplotype [6].

The association of ALOX5AP gene polymorphisms with stroke has been studied in a population of East China. By PCR, 507 patients with stroke and 510 healthy individuals were genotyped. A haplotype-based analysis of the rs10507391 and rs12429692 associations showed that the reduced risk of stroke was significantly linked to the AA haplotype (OR = 0.66; 95% CI 0.46–0.95) [7].

Single-nucleotide polymorphisms of the ALOX5AP gene were studied in individuals of the Taiwanese population with history of a atherothrombotic stroke. The results of the study showed that the rare allele combination of three polymorphisms – rs4293222, rs10507391 and rs12429692 – reduces the risk of atherothrombotic stroke by 44% (OR = 0.56; 95% CI 0.37–0.84, p = 0.005) [8].

A study of SNP rs10507391 as a genetic marker of stroke risk was conducted in the Icelandic population. The study involved 639 patients with acute CVA and 736 patients in the control group. Twenty-two SNPs of the ALOX5AP gene were analyzed. The most statistically significant association with acute CVA was demonstrated by SNP rs10507391 (OR = 1.24; 95% CI 1.04 to 1.55; p = 0.017). Moreover, in the male subgroup with acute CVA, associations were more pronounced than in the female subgroup [9].

Thus, most studies confirm the association of the ALOX5AP gene with acute CVA. However, the independent effect of SNP rs10507391 on the risk of stroke is proved only in the European population, which makes this polymorphism attractive for further studies in various populations.

The aim of the research was to study the association of SNP rs556621 (G>T) with the acute CVA development in patients with cardiovascular disease and risk factors for its development. The patients are representatives of the East Siberian population.

MATERIALS AND METHODS

The study included 260 patients with acute CVA (experimental group) and 272 patients in the control group. The study was carried out in accordance with Good Clinical Practice standards and the principles of the Declaration of Helsinki. Prior to inclusion in the study, a written informed consent was received from all participants. The age of patients in the experimental group ranged from 32 to 69 years [57.0; 51.0–62.0], the age of the control group – from 37 to 68 years [55.0; 51.0–62.0]. Among the patients who had an acute CVA there were 157 men (age [56.5; 51.0–62.0]) and 103 women (age [57.0; 51.0–62.0]). The control group included 170 men (age [55.0; 51.0–62.0]) and 102 women (age [55.0; 51.0–62.0]).

Patients of the main group were at inpatient treatment and under examination at the Krasnoyarsk Interdistrict Clinical Hospital No, 20 n. a. I.S. Berzon, Krasnoyarsk. Examination of individuals of the main group included: collection of complaints, anamnesis, clinical examination, computed tomography of the brain, electrocardiography, echocardioscopy, ultrasound duplex scanning of extracranial brachiocephalic arteries, daily monitoring of blood pressure and heart rhythm, and analysis of the blood coagulation system. Clinical and instrumental examination of patients from the experimental group was aimed at verifying the diagnosis and detecting the concomitant cardiovascular pathology and risk factors for the development of acute CVA. 199 patients (123 men and 76 women) of the main group had an ischemic stroke, 51 patients (28 men and 23 women) were diagnosed with hemorrhagic stroke, and 10 patients (6 men and 4 women) showed a mixed type of acute CVA.

Of 260 patients, 19 (13 men and 6 women) had a repeated stroke. None of the examined patients had clinical, anamnestic and instrumental data indicating the presence of coronary heart disease. Arterial hypertension (AH) (249 people, of which 153 men and 96 women) was the most common cardiovascular pathology preceding acute CVA. Heart rhythm disturbances of the paroxysmal supraventricular tachycardia type, including atrial fibrillation, were detected in 31 patients (20 men and 11 women). The following risk factors for stroke in the study group of patients were observed: dyslipidemia (159 patients, of which 95 men and 64 women), atherosclerosis of the brachiocephalic arteries (BCA) (160 patients, of which 94 men and 66 women), hemostatic disorders leading to hypercoagulability (90 patients, of which 53 men and 37 women), 28 patients (19 men and 9 women) had aggravated hereditary history of acute CVA.

The control group is represented by a population sample of the Novosibirsk city inhabitants examined in the framework of the international HAPIEE project [3]. The examination of the control group included: questionnaires (social economic conditions of life, chronic diseases, level of physical activity, mental health), anthropometry (height, body weight, waist, hips), a survey on smoking and alcohol consumption (frequency and typical dose), blood pressure measurement, lipid profile evaluation, a survey for the detection of exertional angina (Rose), resting ECG with 12 leads, and a study of respiratory and cognitive functions. In the control group, AH occurred in 177 patients, of which 98 were men and 79 were women. There were no other cardiovascular diseases and risk factors for their development at the time of the examination in the control group.

Molecular and genetic research of individuals from the experimental and control groups was carried out by real-time PCR at the Research Institute for Treatment and Preventive Medicine of the Siberian Branch of the Russian Academy of Medical Sciences.

Statistical processing of the material was carried out using the following software: Statistica for Windows 7.0, Excel and SPSS 22.

When performing a statistical analysis of the obtained material, standard operating procedures for conducting statistical exercises were used, while the methods of statistical processing were used in accordance with the nature of the accounting features and the number of comparison groups. Fisher's exact test was used when the desired frequencies were less than 5. The relative risk of disease probability for a particular allele or genotype was calculated as the odds ratio (OR). The value of the critical significance level (p) when testing statistical hypotheses was designated 0.05 [10–12].

Correspondence of the distribution of the observed frequencies in the studied genotypes theoretically expected by the Hardy – Weinberg principle was checked using the χ^2 criterion. The counting was carried out using a calculator for statistics in case-control studies on the Gen Expert website (Russia, http://www.oege.org/software/hwe-mr-calc.shtml).

RESULTS

The frequency distribution of SNP rs10507391 genotypes and alleles (A>T) among the patients with acute CVA and in control group is presented in Table 1. A statistically significant number predominance of the rare TT genotype and T allele carriers among the patients with acute CVA was compared with the control group. There was also a statistically significant decrease in the number of the AA genotype and A allele carriers in the group of patients who had acute CVA, compared with the control group. Differences in the frequencies of the heterozygous AT genotype in the compared groups were not statistically significant.

When analyzing the frequency distribution of SNP rs10507391 genotypes and alleles (A>T) in the subgroup of men with acute CVA and in the male control group, results similar to the experimental group were obtained. The TT genotype was statistically significantly more common among men with acute CVA (50.6 ± 7.9%) than among men in the control group (11.2 ± 4.74%; p = 0.000; OR 8.1; 95% CI 4.60–14.45). The frequency of the AA genotype in the subgroup of men with acute CVA was significantly lower (7.1 ± 4.07%) than in the control group (46.5 ± 7.5%; p = 0.000; OR 11.2; 95% CI 5.71–22.22). The frequencies of the AT genotype were comparable in the subgroup of men with acute CVA (42.2 ± 7.8%) and among men in the control group (42.4 ± 7.43%; p = 0.98). The frequency of the A allele in the subgroup of men with acute CVA was 28.2 ± 5.03%, and in the control group of men it was 67.6 ± 4.97%. T allele in the subgroup of men with acute CVA was found with a frequency of 71.8 ± 5.03%, and with a frequency of 32.4 ± 4.97% in the control group. The differences in alleles were also statistically significant (p = 0.000; OR 5.34; 95% CI 3.79–7.46).

When analyzing the frequency distribution of genotypes and alleles of SNP rs10507391 (A>T) in the subgroup of women with acute CVA and in the female control group, results were obtained that are similar to the results in the experimental group and in the subgroup of men with acute CVA. The frequency of the AA genotype among women with acute CVA was 6.8 ± 4.86%, among women in the control group it was 56.9 ± 9.1% (p = 0.000; OR 18.18; 95% CI 7.63–43.48). The AT genotype in the subgroup of women with acute CVA was found with a frequency of 34.0 ± 9.15%, and in the control group of women with a frequency of 35.3 ± 9.27% (p = 0.84). The TT genotype was identified in 59.2 ± 9.49% of women with acute CVA and in 7.8 ± 5% of women in the control group (p = 0.000; OR 17.06; 95% CI 7.50–38.82). In the subgroup of women with acute CVA, 23.8 ± 5.81% of patients were carriers of the A allele, 76.2 ± 5.81% of patients were carriers of the T allele. In the control group, 74.5 ± 5.98% of women were carriers of the A allele, 25.5 ± 5.98% of women were carriers of the T allele (p = 0.000; OR 9.34; 95% CI 5.98–14.70).

Table

Frequency distribution of SNP rs10507391 genotypes and alleles (A>T) among patients with acute CVA and in control group

Genotypes and alleles	Patients with acute CVA (n = 257)			Control group (n = 272)			p
	total	%	m	total	%	m	
Genotypes							
AA	18	7.0	3.12	137	50.4	5.94	p = 0.000
AT	100	38.9	5.96	108	39.7	5.81	p = 0.85
TT	139	54.1	6.09	27	9.9	3.55	p = 0.000
Alleles							
A	136	26.5	3.81	382	70.2	3.84	p = 0.000
T	378	73.5	3.81	162	29.8	3.84	
OR A/T; 95% CI	6.53; 5.00–8.55						
Total alleles							
AA	18	7.0	3.12	137	50.4	5.94	p = 0.000
AT + TT	239	93.0	3.12	135	49.6	5.94	
OR; 95% CI	13.5; 7.87–23.25						
TT	139	54.1	6.09	27	9.9	3.55	p = 0.000
AA + AT	118	45.9	6.09	245	90.1	3.55	
OR; 95% CI	10.7; 6.70–17.05						

Note. OR – odds ratio; 95% CI – 95th confidence interval, p – significance level when comparing the distribution of genotypes with indices of the control group.

The frequencies of the SNP rs10507391 (A>T) genotypes and alleles in the subgroups of patients who suffered from acute CVA and with various cardiovascular pathologies and risk factors were analyzed. The analysis results of the frequencies and alleles distribution in all subgroups corresponded to the distribution in the experimental group of patients with acute CVA.

In the subgroup of patients with arterial hypertension (AH) and history of acute CVA, the genotypes were distributed as follows: the AA genotype – 7.3 ± 3.25%, the AT genotype – 39.0 ± 6.10%, the TT genotype – 53.7 ± 6.23%. In the control group of patients without hypertension and acute CVA, the AA genotype was detected in 46.3 ± 10.03% of patients, the AT genotype in 44.2 ± 9.99%, and the TT genotype in 9.5 ± 5.89% of patients. Among patients with AH and acute CVA, compared with the control group, a statistically significant predominance of the number of TT genotype carriers (p = 0.000; OR 11.06; 95% CI 5.33–22.98) and a statistically significant decrease in the number of AA genotype carriers were found (p = 0.000; OR 10.87; 95% CI 5.85–20.41). Differences in the AT genotype were statistically insignificant (p = 0.38) (Fig. 1). The A allele frequency in the subgroup of patients with AH and acute CVA was 26.8 ± 3.92%, in the control group it was 68.4 ± 6.61%. The T allele in the subgroup of patients with AH and acute CVA occurred with a frequency of 73.2 ± 3.92%, and with a frequency of 31.6 ± 6.61% in the control group. The differences were statistically significant (p = 0,000; OR 5.92; 95% CI 4.09–8.55).

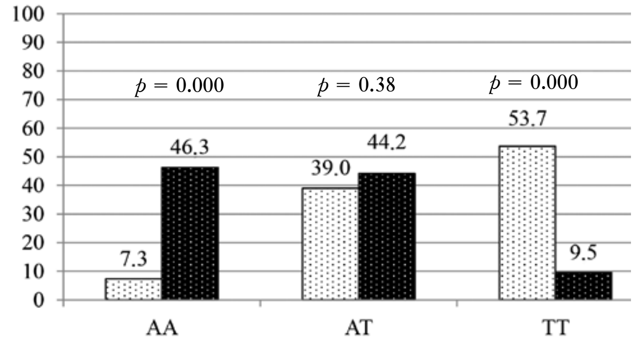

Fig. 1. Frequency distribution of SNP rs10507391 genotypes and alleles (A>T) among patients with AH and history of acute CVA and people in the control group without AH and acute CVA

In the subgroup of patients with cardiac rhythm disturbances (CRD) and history of acute CVA, the frequency of AA genotype was 3.3 ± 6.42%, the history of the AT genotype was 30.0 ± 16.4%, and that of the TT genotype was 66.6 ± 16.87%. The frequencies of SNP rs10507391 genotypes and alleles (A>T) in the control group are presented in Table. Among patients with CRD and acute CVA, compared with the control group, a statistically significant predominance of the number of TT genotype carriers (p = 0.000; OR 18.15; 95% CI 7.70–42.75) and a statistically significant decrease in the number of the AA genotype carriers were established (p = 0.000; OR 29.41; 95% CI 3.95–200.0). Differences in the AT genotype were not statistically significant (p = 0.30). A statistically significant predominance of the number of T allele carriers and a decrease in the number of A allele carriers in the subgroup of patients with CRD and acute CVA were compared with numbers in the control group (p = 0.000; OR 11.11; 95% CI 5.32–20.83) (Fig. 2).

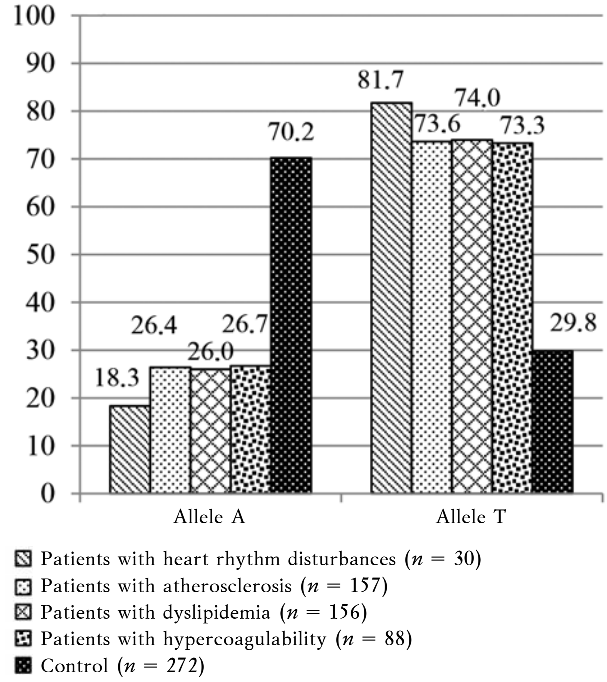

Fig. 2. Frequency distribution of SNP rs10507391 alleles (A>T) among the patients with cardiovascular pathology, its risk factors and history of acute CVA and individuals in the control group

In the subgroup of BCA atherosclerosis patients with history of acute CVA, the frequencies of genotypes and alleles were distributed as follows: AA genotype – 5.7 ± 3.64%, AT genotype – 41.4 ± 7.70%, TT genotype – 52.9 ± 7.81%, A allele – 26.4 ± 4.88%, T allele – 73.6 ± 4.88%. The frequencies of SNP rs10507391 genotypes and alleles (A>T) in the control group are presented in Table 1. In the subgroup of patients with BCA atherosclerosis and acute CVA, compared with the control group, the TT genotype was statistically significantly more likely to be found (p = 0.000; OR 10.18; 95% CI 6.13–16.88) and the AA genotype was statistically significantly less common (p = 0.000; OR 16.67; 95% CI 8.19–34.48). The frequencies of the AT genotype were comparable in the compared groups (p = 0.73). The T allele significantly prevailed among patients with atherosclerosis of BCA and acute CVA in comparison with the control group (p = 0.000; OR 6.58; 95% CI 4.81–8.93) (see Fig. 2).

In the subgroup of patients with dyslipidemia and history of acute CVA, the frequencies of genotypes and alleles were distributed as follows: AA genotype – 5.1 ± 3.46%, AT genotype – 41.7 ± 7.74%, TT genotype – 53.2 ± 7.83%, A allele – 26.0 ± 4.86%, T allele – 74.0 ± 4.86%. The frequencies of SNP rs10507391 genotypes and alleles (A>T) in the control group are presented in Table 1. In the subgroup of patients with CRD and acute CVA, compared with the control group, the TT genotype was statistically significantly more frequent (p = 0.000; OR 10.32; 95% CI 6.21-17.13) and the AA genotype was statistically significantly less common (p = 0.000; OR 18.87; 95% CI 8.85–40.0). The frequencies of the AT genotype were comparable in the compared groups (p = 0.69). The T allele significantly prevailed among patients with dyslipidemia and acute CVA compared with the control group (p = 0.000; OR 6.571; 95% CI 4.93–9.17) (see Fig. 2).

In the subgroup of patients with impaired hemostasis system and history of acute CVA, the AA genotype was 6.8 ± 5.27%, the AT genotype – 39.8 ± 10.23%, and the TT genotype – 53.4 ± 10.42%. The frequencies of SNP rs10507391 (A> T) genotypes and alleles in the control group are presented in Table. A statistically significant predominance of the number of TT genotype carriers (p = 0.000; OR 10.40; 95% CI 5.84–18.53) and a statistically significant decrease in the number of AA genotype carriers were found (p = 0.000; OR 13.89; 95% CI 5.85–33.33) in the subgroup of patients with hypercoagulation and acute CVA, compared with the control group. There were no significant differences when comparing the frequencies of the AT genotype (p = 0.99). The T allele significantly prevailed among patients with hypercoagulation and acute CVA as opposed to individuals in the control group (p = 0.000; OR 6.45; 95% CI 4.42–9.43) (see Fig. 2).

DISCUSSION

When studying the association of SNP rs10507391 (A>T) with the development of acute CVA in all analyzed groups and subgroups of patients, a connection was established between the rare TT genotype and the T allele and the increased risk of acute CVA. The results are consistent with published data and are determined by the mechanism by which the action of polymorphism is realized: participation in most types of inflammatory reactions. The uniqueness of this study is the confirmation of the SNP rs10507391 TT genotype (A>T) role as an independent predictor of acute CVA in individuals of the East Siberian population. Previously it was established only among patients of European origin [9].

CONCLUSION

TT genotype and T allele of the SNP rs10507391 (A>T) increase the risk of developing acute cerebrovascular accident in patients regardless of previous cardiovascular pathology and its risk factors, including patients with arterial hypertension, supraventricular tachyarrhythmias, atherosclerosis of brachiocephalic arteries, impaired lipid metabolism and hemostatic system.

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Experimental estimation of the effects of exogenous carbon monoxide on blood cells

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ABSTRACT

The aim of the study was to investigate the effect of the carbon monoxide (CO) donor on the Ca^{2+} -activated potassium permeability of the erythrocyte membrane and platelet aggregation ability.

Materials and methods. Healthy volunteers (n = 27) and patients with chronic coronary heart disease (CHD) (n = 32) of both sexes were examined. The material of the study was packed red blood cells and platelet-rich plasma obtained from patient's venous blood. The change of Ca^{2+} -dependent potassium conductivity of the erythrocyte membrane was evaluated by potentiometric method, and the platelet aggregation was studied by turbidimetric method. Carbon monoxide releasing molecule-2 (CORM-2) was used as a CO donor. The amplitude of A23187- and redox-induced hyperpolarization response (HR) of erythrocytes, and the rate and degree of platelet aggregation were estimated.

Results. It was shown that the addition of CORM-2 (10 and 100 μM) in the erythrocyte suspension caused a dose-dependent decrease in the amplitude of A23187- and redox-dependent HR in healthy donors, as well as in patients with chronic CHD. The maximum decrease was observed in the presence of 100 μM CORM-2. The effect of CORM-2 at concentrations of 10 and 100 μM on collagen-induced platelet aggregation led to a decrease in the degree and rate of aggregation in healthy donors. The maximum effect was shown at 100 μM of CO donor. However, such an unambiguous effect of CORM-2 on the aggregation parameters in patients with CHD was not observed.

Conclusion. The results suggest that CO has a significant effect on the ion transport function of the erythrocyte membrane and platelet aggregation activity of both healthy donors and patients with CHD.

Key words: carbon monoxide, red blood cells, ion transport systems, platelets, aggregation.

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Conformity with the principles of ethics. All patients signed an informed consent to participate in the study. The study was approved by the Ethics Committee at SSMU (Protocol No. 4340 of 30.11.2015).

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Экспериментальная оценка влияния экзогенного монооксида углерода на клетки крови

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РЕЗЮМЕ

Цель исследования – изучить влияние донора монооксида углерода (СО) на Ca^{2+}-зависимую калиевую проницаемость мембраны эритроцитов и агрегационную способность тромбоцитов.

Материалы и методы. Обследованы здоровые добровольцы (n = 27) и пациенты с хронической ишемической болезнью сердца (ИБС) (n = 32) обоего пола. Материалом исследования являлись упакованные эритроциты и обогащенная тромбоцитами плазма, полученные из венозной крови. Потенциометрическим методом изучали изменение Ca^{2+}-зависимой калиевой проводимости мембраны эритроцитов, турбидиметрическим методом – агрегационную активность тромбоцитов при действии донора СО (CORM-2). Оценивали величину A23187- и редокс-индуцированного гиперполяризационного ответа (ГО) эритроцитов, скорость и степень агрегации тромбоцитов.

Результаты. В присутствии 10 и 100 мкМ CORM-2 амплитуда A23187- и редокс-зависимого ГО здоровых доноров, как и пациентов с хронической формой ИБС дозозависимо уменьшалась, причем максимальное снижение отмечено в присутствии 100 мкМ донора СО. Воздействие CORM-2 в концентрациях 10 и 100 мкМ на коллаген-индуцированную агрегацию тромбоцитов приводило к снижению степени и скорости агрегации у здоровых доноров, достигая максимального эффекта при 100 мкМ донора СО. Однако столь однозначного влияния CORM-2 на параметры агрегации у пациентов с ИБС не наблюдалось.

Заключение. Полученные результаты указывают, что СО оказывает существенное влияние на ион-транспортную функцию мембраны эритроцитов и агрегационную активность тромбоцитов как здоровых доноров, так и пациентов с ИБС.

Ключевые слова: монооксид углерода, эритроциты, ион-транспортные системы, тромбоциты, агрегация.

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INTRODUCTION

Carbon monoxide (CO), nitric oxide (NO) and hydrogen sulfide (H2S) are a new class of gas regulatory molecules [1, 2]. The formation of CO occurs during the degradation of the hemoprotein in the heme molecule (hemoglobin, myoglobin, catalase, etc.), which is catalyzed by the heme oxygenase (HO) enzyme, that has inducible (HO-1) and constitutive (HO-2) isoforms [3].

Currently, CO is considered as an important mediator in the cardiovascular system that regulates vascular tone and has anti-inflammatory, anti-apoptotic, and antiproliferative effects [4]. There is evidence that CO modulates surface architectonics and red blood cell energy metabolism [5]. At the same time, changes in the structural and functional status of red blood cells can be an indicator of the degree of damage to membranes during various pathological processes in the body.

Abnormalities in the rheological properties of blood are of great importance among the factors determining hemodynamic disturbances in patients with coronary heart disease (CHD) [6, 7]. It has been shown that in patients with CHD, there is a change in the phospholipid composition of the erythrocyte membrane due to the increased incorporation of cholesterol, as well as possible phosphatidylserine externalization [8].

Structural disorganization and changes in the biomechanical properties of the erythrocyte membrane cause disruption in its ion transport function [9, 10], in which Gardos channels – Ca^{2+}-activated potassium channels (K_{Ca}-channels) – are significant. An increase in the activity of these channels causes eryptosis and also reduces the erythrocyte deformability. [2, 11]. A special place in the pathogenesis of CHD is given to the increased platelet aggregation and related to it relevant antiplatelet therapy. The literature data indicate that CO donors can produce an antiplatelet effect [12].

Thus, the aim of this work was to study the effect of CO on the ionic permeability of the erythrocyte membrane and platelet aggregation.

MATERIAL AND METHODS

The study included 32 patients (20 men and 12 women) aged 40 to 65 years, with a clinically verified chronic form of CHD, functional class II–III. Of these, 21 (65.6%) patients had myocardial infarction in past medical history. The patients had 5 (2; 8) years' experience of CHD. Arterial hypertension (AH) was diagnosed in 14 (43.7%) of the examined individuals. Clinical and laboratory studies were conducted before the start of conservative treatment for chronic CHD. The comparison group consisted of 27 healthy volunteers (16 men and 11 women) aged 38 to 62 years who did not have cardiovascular, endocrine or genetic diseases in the medical history. The characteristics of patient groups are given in Table 1. The work complied with the ethical standards developed in accordance with the Declaration of Helsinki (as amended in 2000) and the Guideline for good clinical practice. Each individual in this study signed a written informed consent.

Venous blood taken from elbow vein of fasted patients in the morning in test tubes of the BD Vacutainer® type with an anticoagulant served as material for the study. Hematological (XN-1000 analyzer, Sysmex, Japan), biochemical (Konelab 60i analyzer, Thermo Scientific, USA) and hemostasiological tests (coagulation analyzer ACL TOP 700, Instrumentation Laboratory Company, USA) were performed.

The erythrocyte suspension was obtained by centrifugation (5 min, 1,000 g, 4 °C) of whole blood (heparin, 17 IU / ml). Plasma and white blood cells were removed, and then the red blood cells were washed twice with 150 mM NaCl containing PBS (5 mM, pH 7,4) under the same centrifugation conditions. The erythrocyte sediment was washed with iso-osmotic medium (320 mOsm/L) containing 150 mM NaCl, 10 mM glucose, 1 mM KCl, and 1 mM $MgCl_2$. The red blood cells were transferred to ice and stored for no more than 12 hours. For the study, packed red blood cells were diluted in 1:5 ratio in their incubation medium. To obtain platelet-rich plasma, blood with sodium citrate (blood: citrate ratio – 9:1) was centrifuged for 7 min at 800 g.

The study of Ca^{2+}-activated potassium permeability of the erythrocyte membrane was conducted by the potentiometric method. The erythrocyte hyperpolarization response (HR) was estimated in response to the addition of 0.5 μM Ca^{2+}-ionophore A23187 or the ascorbate (10 mM) – phenazine methosulfate (PMS, 0,1 mM) system. The quazi stationary pH_i level was determined during cell hemolysis in the presence of detergent Triton X-100 (0.2%).

The aggregation ability of platelets was studied by the turbidimetric method with the use of a laser analyzer (220 LA "NPF Biola", Russia). Platelet aggregation was caused by collagen at a final concentration of 2 mg/ml. The degree and rate of aggregation were estimated from the curve of the average aggregate size.

Statistical analysis of the data was performed in SPSS Statistics 17.0 using Mann – Whitney U test and Chi-square test. Data are presented as median (*Me*), interquartile range (Q_1; Q_3), and *n* (%). $p < 0.05$ was considered statistically significant.

Table 1

Clinical and laboratory characteristics of examined individuals

Parameter	Groups		*p*
	Healthy donors, $n = 27$	Patients with chronic CHD, $n = 32$	
Age, years, *Me* (Q_1; Q_3)	53 (42.5; 58)	56 (53.5; 62)	0.272
Body Mass Index, kg/m^2, *Me* (Q_1; Q_3)	24 (23; 25)	30 (28; 32)	0.028
Smoking, *n* (%)	9 (33.3)	13 (40.6)	0.041
Red blood cells, 10^{12}/л, *Me* (Q_1; Q_3)	4.6 (4.4; 4.8)	4.5 (4.3; 4.9)	0.361
Hemoglobin, г/л, *Me* (Q_1; Q_3)	147 (135; 155)	144 (131; 154)	0.118
White blood cells, 10^9/л, *Me* (Q_1; Q_3)	6.7 (5.1; 8.2)	7 (5.5; 8.4)	0.16
Platelets, 10^9/l *Me* (Q_1; Q_3)	240 (217; 264)	232 (205; 255)	0.121
INR, rel. units, *Me* (Q_1; Q_3)	1.1 (1.07; 1.14)	1 (0.95; 1.1)	0.224
aPTT, sec, *Me* (Q_1; Q_3)	30.7 (27;34.6)	28.9 (26.8;33)	0.183
Fibrinogen, g/l, *Me* (Q_1; Q_3)	3.1 (2.7; 5)	2.8 (2.5; 4.8)	0.11
Cholesterol, mmol/l, *Me* (Q_1; Q_3)	4.2 (3.6; 5)	5.5 (4.9.6.4)	0.018
Triglycerides, mmol/l, *Me* (Q_1; Q_3)	1.1 (0.6; 1.5)	2.3 (1.6; 2.7)	0.015

RESULTS

An increase in the cytosolic Ca^{2+} concentration in red blood cells, induced by the Ca^{2+}-ionophore A23187, as well as the effect of the ascorbate-PMS redox system lead to the development of a hyperpolarization response (HR). Change in the HR amplitude characterizes the conductivity of the K_{Ca}-channels of the erythrocyte membrane [11, 13].

To study the role of CO in the regulation mechanisms of erythrocyte Gardos channels, its donor, tricarbonyldichlororuthenium(II) dimer (CORM-2) - ruthenium carbonyl, was used. Despite the release of CO, which binds to hemoglobin of erythrocytes with the formation of carboxyhemoglobin (COHb), it is noted that the COHb content is less than 5%, and the effective concentration of CO is in the range of those observed in vivo [1].

It was found that under the effect of 10 and 100 μM CORM-2, the amplitudes of A23187- and redox-dependent HR of healthy donors and patients with chronic CHD decreased dose-dependently. The maximum decrease in HR was observed in the presence of 100 μM CO donor. Under the action of 10 μM CORM-2, the amplitude of Ca^{2+}-ionophore induced HR in patients' erythrocytes decreased more than that of healthy donors. For the ascorbate–PMS-caused HR this dependence was detected only in the presence of 100 μM CO donor (Table 2).

Platelet aggregation was caused by collagen, which interacts with glycoprotein VI (GPVI) and integrin $\alpha_2\beta_1$ of platelets, and, thus, triggers a complex cascade of processes, including activation of phospholipase C and phospholipase A_2, protein kinases C (PKC), MAP kinases (MAPKs), an increase in cytosolic Ca^{2+} level, thromboxane A_2 synthesis and platelet granule secretion [14, 15].

The effect of CORM-2 in concentrations of 10 and 100 μM on the collagen-caused processes in platelets led to a dose-dependent decrease in the degree and rate of aggregation in healthy donors. The maximum decrease in platelet aggregation was observed with the effect of 100 μM CO donor. However, there was no unambiguous effect of CORM-2 on the aggregation parameters in patients with coronary artery disease, although the initial values did not differ between CHD patients and healthy volunteers (Table 3).

Table 2

The effect of CORM-2 on the hyperpolarization response of erythrocytes of healthy donors and patients with chronic CHD, *Me* (Q_1; Q_3)

Parameter Group	Healthy donors, n = 27		Patients with chronic CHD, n = 32	
	Amplitude of the hyperpolarization response (HR), mV			
	A23187-induced	Redox-induced	A23187-induced	Redox-induced
Control	−25.4 (−26.3; −23.2)	−48.6 (−50.1; −47.5)	−34.7 (−37.1; −31.5) p_3 = 0.02	−49.5 (−53.7; −45.5)
+CORM-2 (10 μM)	−18.3 (−21.1; −16.9) p_1 = 0.004	−38.8 (−43.4; −34.2) p_1 = 0.003	−25.2 (−28.5; −21.4) p_1 < 0.001; p_3 = 0.004	−35.6 (−38.9; −31.2) p_1 < 0.001
+CORM-2 (100 μM)	−10.2 (−12.5; −8.4) p_1 < 0.001; p_2 = 0.01	−28.3 (−31.4; −22.7) p_1 < 0.001; p_2 = 0.003	−17.7 (−22; −13.5) p_1 < 0.001; p_2 = 0.011; p_3 = 0.02	−20.6 (−25.5; −17) p_1 < 0.001; p_2 = 0.004; p_3 = 0.005

Note. The level of statistical significance of differences in comparison with control for given HR (p_1); CORM-2 (10 μM) for given HR (p_2); the same parameter for healthy donors (p_3).

Table 3

The effect of CORM-2 on the collagen-induced platelet aggregation of healthy donors and patients with chronic CHD, *Me* (Q_1; Q_3)

Parameter Group	Healthy donors, n = 27		Patients with chronic CHD, n = 32	
	Degree of aggregation, rel. units	Rate of aggregation, rel. units/min	Degree of aggregation, rel. units	Rate of aggregation, rel. units/min
Control	10.2 (8.5; 13.2)	32.5 (29.2; 37.1)	11.8 (9.7; 13)	31.7 (26.5; 36.8)
+CORM-2 (10 μM)	5.4 (4.1; 8.2) p_1 = 0.001	22.1 (18.6; 24.5) p_1 < 0.001	10.6 (8.8; 12.1) p_3 = 0.003	22.8 (20.3; 24.4) p_1 < 0.001
+CORM-2 (100 μM)	2.3 (1.8; 3.6) p_1 < 0.001; p_2 = 0.003	10.4 (8.8; 14.3) p_1 < 0.001; p_2 < 0.001	6.1 (2.4; 5.5) p_1 = 0.008; p_2 = 0.001; p_3 = 0.015	15.6 (−21.5; −17) p_1 < 0.001; p_2 = 0.001; p_3 = 0.01

Note. The level of statistical significance of differences in comparison with control for given parameter (p_1), CORM-2 (10 μM) for given parameter (p_2); the same parameter for healthy donors (p_3).

DISCUSSION

During the past three decades, electrophysiological studies revealed that human red blood cell membrane includes a large variety of ion transporting systems that are involved in the homeostasis of cationic and, to a lesser extent, anionic cell conductivity [10]. It is known that activation of Gardos channels contributes to the release of potassium ions to the outside, causes a shift in the membrane potential towards hyperpolarization and creates a driving force for the displacement of chlorine from red blood cells. The release of cations and anions is accompanied by a loss of water, which leads to dehydration and cell shrinkage [2].

A decrease in the HR amplitude in response to the action of various concentrations of the CO donor indicates a decrease in the Ca2+-activated potassium conductivity of the membrane and leakage of potassium ions from the cell. This, probably, happens due to the interaction of CO with the channel proteins or its regulatory protein kinases [16]. At the same time, the more significant effect of CO in patients with chronic CHD, unlike healthy donors, can be associated not only with structural membrane restructuring and increased lipid peroxidation [9], but also with its antioxidant properties and increased levels of reduced glutathione (GSH) in blood cells [17]. It was found that the electron-donor system, ascorbate–PMS, promotes the formation of redox agents, which influence the Gardos channels of the erythrocyte membrane through oxidation or reduction of SH groups [18].

Platelets are small anucleate cell fragments that circulate in blood playing a crucial role in managing vascular integrity and regulating hemostasis. Nevertheless, the functional reaction of platelets can be changed either by increasing pro-aggregation stimuli or by reducing the number of antiaggregation substances. These factors contribute to increased platelet aggregation and often occur in cardiovascular diseases. It is known that CHD is associated with a systemic imbalance in hemostasis caused by the presence of a hypercoagulable state and a decrease in fibrinolysis [19]. The proportion of large platelets which are metabolically and enzymatically more active increases in patients with chronic CHD [20]. The number of platelets incapable of expressing P-selectin and having a significantly greater tendency to form microaggregates in a citrate anticoagulant increases at the same time. [21]. Larger platelets contain more prothrombotic material, with increased thromboxane A_2 and B_2 per unit volume and glycoprotein IIb–IIIa receptor expression.

In our study, it was shown that a CO donor reduced the degree and rate of collagen-induced aggregation in healthy donors and patients with CHD. A higher concentration of CO was required for the latter. Our data are consistent with the results of Chlopicki S. and colleagues [12], confirming the anti-aggregation effect of exogenous CO donors.

Also, in the presence of a guanylate cyclase inhibitor (ODQ), the decrease in collagen-induced aggregation caused by CORM-3 was not blocked, but increased. This fact indicates additional effector targets of CO in platelets. It is noted that CO, not being a powerful inhibitor of platelet activation, acquires this property when there is a lack of other antiplatelet agents (NO and prostacyclin) [12]. It is known that antiplatelet therapy, usually with aspirin, may not be effective, because there are other important ways of platelet activation that are not affected by cyclooxygenase block [22]. In this regard, CO donors appear to have high potential as antiplatelet agents.

CONCLUSION

We have found that CO has a significant effect on the ion transport function of the erythrocyte membrane and platelet aggregation in both healthy donors and patients with CHD. The CO-dependent decrease in the amplitude of Ca^{2+}-and redox-induced HR can have a positive effect in the mechanisms of regulation of erythrocyte deformability. The CO influenced decrease in platelet aggregation creates the basis for the development of methods for optimizing antiplatelet therapy in patients with CHD, in which this gasomediator participates.

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Authors contribution

Petrova I.V., Gusakova S.V. – critical revision for important intellectual content, approval of the manuscript for publication. Birulina J.G, Trubacheva O.A. – conception and design, interpretation and analysis of data, drafting of the manuscript. Nosarev A.V., Shnaider O.L. – manuscript substantiation. Belyaeva S.N. – experimental procedure. Vasilev V.N., Suhanova G.A. – conception and design.

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Decision rule for stratification of patients with chronic heart failure of functional class II and III

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ABSTRACT

The aim of the study was focused on the development of a decision rule for classifying patients as functional class (FC) II or III of chronic heart failure (CHF) by discriminant analysis with inflammatory markers.

Materials and methods. The study included CHF patients (n = 61) of both sexes. According to symptom severity, they were assigned to FC II (n = 20) and III (n = 41). In addition to conventional clinical and biochemical parameters to evaluate a patient's state, parameters characterizing inflammation (IL-6, soluble IL-6 receptor, sgp130) were used. Statistically significant differences were revealed with the use of Mann–Whitney U test, Student's t-test, Pearson's χ^2 test and Fisher's exact test. Discriminant analysis was employed to formulate the decision rule. Receiver Operating Characteristic (ROC) analysis was used to evaluate the quality of the developed diagnostic test. The results were considered statistically significant at $p < 0.05$.

Results. Discriminant analysis included significantly different variables (age, brain natriuretic peptide, sgp130, CHF etiology, ischemic heart disease) and additional clinically important variables (diastolic and systolic arterial blood pressure (BP), IL-6). The decision rule for assigning patients to different CHF FC was developed. The optimum cut-off value was found with the use of the ROC curve with a sensitivity of 75.6% and specificity of 85%.

Conclusion. The decision rule for assigning CHF patients to FC II or III was developed using discriminant analysis. In addition to conventional clinical parameters, the model included the ones reflecting inflammatory processes (IL-6 and sgp130). ROC analysis revealed high quality of the model.

Key words: chronic heart failure, interleukin 6, sgp130, decision rule, discriminant analysis.

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Решающее правило для стратификации больных хронической сердечной недостаточностью II и III функционального класса

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РЕЗЮМЕ

Цель. Разработать решающее правило отнесения пациентов ко II и III функциональным классам (ФК) хронической сердечной недостаточности (ХСН) методом дискриминантного анализа с включением маркеров воспаления.

Материалы и методы. Исследование включало 61 пациента обоего пола с ХСН. В зависимости от выраженности симптомов ХСН пациенты были отнесены ко II (n = 20) и III (n = 41) ФК. В работе помимо общепринятых клинических и биохимических показателей, оценивающих состояние пациентов, дополнительно исследовались параметры, отражающие течение воспалительного процесса (IL-6, растворимый рецептор IL-6, sgp130*)*. Для выявления статистически значимо различающихся переменных в группах использовали U-критерий Манна – Уитни, t-критерий Стьюдента, χ^2 Пирсона, точный критерий Фишера. Для построения решающего правила применялся метод дискриминантного анализа. Для оценки качества разработанного диагностического теста использовали ROC-анализ. Статистически значимыми считались результаты при уровне значимости $p < 0{,}05$.

Результаты. В дискриминантный анализ были включены выявленные при сравнении групп II и III ФК ХСН значимо различающиеся переменные – возраст, мозговой натрийуретический пептид, sgp130, этиология ХСН, ишемическая болезнь сердца, а также дополнительные переменные, имеющие существенное значение в клинике (артериальное давление (АД) систолическое, АД диастолическое, IL-6). На основании данных показателей было построено решающее правило для отнесения пациентов к различным функциональным классам ХСН. При оценке качества полученного решающего правила было найдено оптимальное значение точки отсечения с использованием ROC-кривой, которой соответствует чувствительность – 75,6%, специфичность – 85%.

Заключение. С помощью метода дискриминантного анализа разработано решающее правило разделения больных на II и III ФК ХСН. Наряду с общепринятыми клиническими показателями ХСН в модель включены новые параметры, отражающие степень воспалительного процесса (IL-6 и sgp130). ROC-анализ выявил очень хорошее качество полученной модели.

Ключевые слова: хроническая сердечная недостаточность, интерлейкин 6, sgp130, решающее правило, дискриминантный анализ.

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

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INTRODUCTION

Chronic heart failure (CHF) remains a current problem of cardiology, being an unfavorable, progressing cardiovascular disease all over the world. Epidemiological studies demonstrate a constant increase in CHF incidence in Western Europe and the USA, particularly in the aging population [1–3]. Epidemiological studies performed in the Russian Federation indicate a similar increase in CHF incidence [4]. The number of patients with more severe forms of the disease increases considerably. Therefore, identification of the early stages of CHF is an important strategy in the treatment of patients with CHF. Effectiveness of treatment is strongly associated with the choice of a therapeutic scheme based on evaluation of the disease symptoms. Severity of CHF symptoms is characterized according to NYHA functional classification [1]. However, evaluation of the CHF severity and identifying patients' functional class often cause difficulties in medical practice. Decision rules have been widely used for assigning patients to the appropriate functional class. Greater number of clinical parameters reflecting patient's condition allows for better stratification and minimizes errors in classification according to the functional class (FC). In addition to clinical parameters, biochemical markers that reflect organic damage to the heart and hemodynamic load increase have been used in CHF diagnostics [5]. However, the indicators of inflammatory processes that intensify with CHF progression have not received sufficient attention.

The aim of the study was to develop the decision rule that includes inflammatory markers (IL-6 and sgp130) for assigning CHF patients to FC II or III.

MATERIALS AND METHODS

Parameters characterizing the state of 61 patients aged 31–83 years suffering from functional class II–III chronic heart failure [New York Heart Association (NYHA)] with decreased left ventricular ejection fraction are given in Table 1.

Table 1

Baseline characteristics of patients

Parameter	II FC (n = 20)	III FC (n = 41)	p
Age, y/o, Me (Q_1–Q_3)	50.5 (42.75–58.25)	62 (53–67)	0.003
Men / Women, abs. (%)	19/1 (95/5)	35/6 (85/15)	0.409
Etiology of CHF (IHD, HT, DCM), abs. (%)	5/6/9 (25/30/45)	24/13/4 (58.5/31.7/9.8)	0.004
LVEF, (%), Me (Q_1–Q_3)	27.5 (25–31.75)	30 (23.5–35)	0.595
BP systolic, mmHg, Me (Q_1–Q_3)	120 (105.25–140)	120 (110–140)	0.871
BP diastolic, mmHg, Me (Q_1–Q_3)	80 (70–88.75)	80 (70–85)	0.374
Heart rate, beats / min, Me (Q_1–Q_3)	70 (68.50–92.75)	85 (70–96)	0.725
Degree of mitral regurgitation (1, 2, 3), abs. (%)	4/11/5 (20/55/25)	5/20/16 (12.2/48.8/39)	0.490
Congestion Ro, abs. (%)	15 (75)	36 (87.8)	0.205
Hypertension, abs. (%)	12 (60)	34 (83)	0.064
IHD, abs. (%)	5 (25)	25 (61)	0.008
NT-proBNP (pg/ml), Me (Q_1–Q_3)	395.5 (224.5–825)	793 (408.5–1746.5)	0.009
Creatinine (μmol/l), Me (Q_1–Q_3)	88 (74–130)	101 (72.5–112.25)	0.432
IL-6, pg/ml, Me (Q_1–Q_3)	3.48 (2.34–6.66)	3.95 (2.57–8.35)	0.249
pIL-6P, pg/ml, Me (Q_1–Q_3)	36.25 (32.46–3.26)	40.48 (35.22–48.77)	0.167
Sgp130, pg/ml, Me (Q_1–Q_3)	333 (309.5–359.5)	415 (355–469)	0.001

Note. Data are presented as median (25th percentile–75th percentile) or absolute number (%). LVEF – left ventricular ejection fraction; Congestion Ro – congestion in the lungs according to X-ray; IHD – ischemic heart disease; HT – hypertension; DCM – dilated cardiomyopathy; NT-proBNP – N-terminal pro-brain natriuretic peptide; IL-6 – Interleukin 6; sIL-6R – soluble Interleukin-6 receptor.

Diagnostics and FC assignment were performed according to up-to-date national recommendations. In order to meet the research purpose, CHF etiology was defined as a major disease that, according to the researchers' opinion and clinical examination, was a main cause of the heart failure symptom complex. Patients with an acute cardiovascular event within preceding 30-day period, acute and chronic inflammatory diseases requiring specific anti-inflammatory therapy and potentially influencing test parameters, pronounced renal and/or hepatic functional disorders, malignant tumors, or with other chronic diseases of internal organs, or with left ventricular outflow tract obstruction were excluded from the study. All patients who participated in the study signed an informed consent.

Statistical analysis was done with IBMSPSS Statistics 23. Statistically significant differences between quantitative variables for two independent samples with distribution other than normal were revealed with Mann – Whitney *U*-test. Pearson's χ^2 test and Fischer's exact tests were used to reveal statistically significant differences between qualitative variables in two groups. Discriminant analysis was employed to establish the decision rule for assigning patients to the appropriate FC. The quality of diagnostic test was evaluated by ROC analysis. The results were regarded as statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

In order to develop the decision rule, test variables were compared and the following statistically significant variables in patients with CHF of FC II and III were revealed: age, N-terminal pro-brain natriuretic peptide (NT-proBNP), sgp130, etiology of CHF and IHD (Table 1). Clinically important parameters (systolic and diastolic BP, IL-6) were also included in the discriminant analysis.

CHF classification according to FC (NYHA) is based on the severity of clinical manifestations. BP parameters are used in clinical practice to choose pharmacotherapy and control its effectiveness. In addition, BP deviations represent the risk of decompensation of a patient's condition [5]. Therefore, such variables as diastolic and systolic BP were included in the discriminant analysis.

IL-6 is another variable that was selected as a clinically important one. IL-6 is currently regarded as a major cytokine participating in immune response and inflammatory reaction. High level of IL-6 correlates with more severe CHF and high mortality [6–9]. Pro-inflammatory effects of IL-6 are realized via trans-signaling pathway which is inhibited by plasma sgp130. It was reported that increase and decrease in sgp130 are related to the severity of inflammation [10–12]. This parameter was also included in the development of the decision rule.

The following linear discriminant functions (LDF) were obtained:

$$Z_1 = 0{,}907*X_1+27{,}645*X_2+33{,}546*X_3-0{,}001*X_4+ +0{,}102*X_5+0{,}285*X_6+0{,}464*X_7+0{,}002*X_8- -115{,}749 \quad (1.1)$$

Для группы больных, относящихся ко II ФК ХСН ЛДФ:

$$Z_2=0{,}828*X_1+28{,}609*X_2+33{,}643*X_3- -0{,}001*X_6+0{,}088*X_5+0{,}245*X_6+ +0{,}558*X_7-0{,}011*X_8-109{,}853 \quad (1.2)$$

for patients with FC II CHF, where X_1 – age; X_2 – CHF etiology; X_3 – IHD; X_4 – *NT*-proBNP; X_5 – sgp130; X_6 – systolic BP; X_7 – diastolic BP; X_8 – IL-6. Z_1 is the first discriminant function and Z_2 is the second discriminant function.

The quality of the discriminant function was assessed with the help of the learning sample and cross-validation (Tables 2, 3). The first method is based on inclusion of each patient from the learning sample in the decision rule. Percentages of correct and incorrect classifications were calculated. Cross-validation implies that each patient is alternately excluded from the sample. An excluded patient was classified with the help of the obtained decision rule and returned to the learning sample.

Table 2

Coefficients of linear discriminant functions (LDF) for patients with CHF of FC II and III

Variable		FC	
Used in LDF	Explanation	II	III
X_1	Age	0.828	0.907
X_2	Etiology of CHF	28.609	27.645
X_3	IHD	33.643	33.546
X_4	NT-proBNP	0.001	0.001
X_5	sgp130	0.088	0.102
X_6	BP systolic	0.245	0.285
X_7	BP diastolic	0.558	0.464

Table 2 (continued)

Variable		FC	
Used in LDF	Explanation	II	III
X_8	IL-6	0.011	0.002
C	Constant	−109.853	−115.749

Note. LDF – linear discriminant functions; CHF – chronic heart failure; IHD – ischemic heart disease; NT-proBNP – N-terminal pro-brain natriuretic peptide; BP – blood pressure; IL-6 – interleukin 6.

Table 3

Classification results for the learning sample method

Parameter	FC	Predicted Group Membership		Total
		II	III	
Count	II	15	5	20
	III	9	32	41
Count, %	II	75.0	25.0	100
	III	22.0	78.0	100

Note. 77% of original grouped cases were classified correctly; FC – functional class.

Table 4

Cross-validated classification results

Parameter	FC	Predicted Group Membership		Total
		II	III	
Count	II	14	6	20
	III	11	30	41
Count, %	II	70.0	30.0	100
	III	26.8	73.2	100

Note. 72.1% of cross-validated grouped cases were classified correctly; FC – functional class.

The decision rule was based on the following principle: predictor variables of a patient were inserted into functions Z_1 and Z_2 (1.1 and 1.2). The calculated Z_1 and Z_2 values were compared and if $Z_1 \geq Z_2$, the patient was assigned to FC III, if $Z_1 < Z_2$, the patient was assigned to FC II.

It should be noted that sensitivity and specificity were used to evaluate reliability of the diagnostic test.

Since this study had no objective of classifying patients as sick or healthy, patients with CHF FC III were regarded as sick and those with FC II as healthy. Thus, when using the learning sample, sensitivity of the given model was Se = (32/(32+9))*100% = 78% and specificity Sp = (15/(15+5))*100% = 75%. When employing cross-validation, sensitivity was Se = (30/(30+11))*100% = 73.2% ad specificity was Sp = (14/(14+6))*100% = 70%.

The ROC-curve reflecting the correlation between correct and incorrect assignments (Fig.1) was constructed to evaluate the quality of the model [13]. A new variable $Z = Z_1 - Z_2$ was created for this purpose.

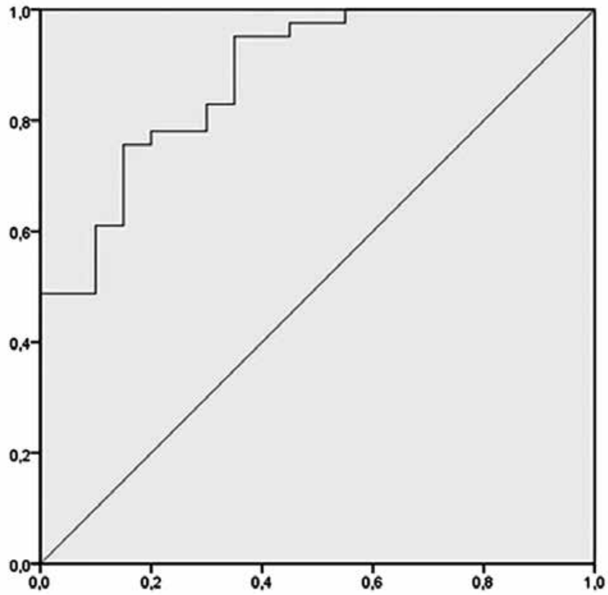

Fig. 1. ROC-curve to evaluate the quality of the developed model for assigning CHF patients to II or III FC

An ideal model is a model with 100% sensitivity and specificity. Since this is difficult to achieve in reality, a cut-off value is used, i.e., the most adequate point to cut off one diagnosed group from the other.

The maximum sensitivity and specificity were chosen to determine the cut-off point. Cut-off = 0.07438 corresponds to sensitivity 0.756 (75.6%) and specificity 0.85 (85%). Thus, if $Z \geq 0.07438$, the patient should be assigned to FC III, $Z < 0.07438$, the patient is assigned FC II.

In addition, the quality of a model can be evaluated by the square under a ROC-curve (AUC): the greater the square, the higher the model quality. In this study, AUC = 0.879 ± 0.045, which indicates excellent quality (0.8–0.9) of the model [13].

CONCLUSION

A comparison of CHF patients of FC II and III was performed so as to develop the decision rule for the assignment of patients to the appropriate FC. The following statistically significant variables were revealed: age, NT-proBNP, sgp130, CHF etiology and IHD. In addition to these parameters, clinically important parameters (systolic and diastolic BP, IL-6) were included in the decision rule. The obtained results allowed for the development of the decision rule for assigning CHF patients to FC II or III. Evaluation of the model by the square under the ROC-curve demonstrated excellent quality (0.8–0.9) of the model. Additional parameters reflecting severity of the inflammatory process (IL-6 and agp130) were used along with conventional clinical parameters.

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Authors contribution

Samoilova E.V. – conception and design, analysis and interpretation of data, critical revision for important intellectual content. Fatova M.A. – analysis and interpretation of data, statistical data processing, graphical representation of data. Mindzaev D.R. – selection and examination of patients, database creation, statistical data processing. Zhitareva I.V. – conception and design, critical revision for important intellectual content. Nasonova S.N. – selection and examination of patients, treatment of patients. Zhirov I.V. – substantiation of the manuscript, critical revision for important intellectual content. Tereschenko S.N. – substantiation of the manuscript, critical revision for important intellectual content. Korotaeva A.A. – substantiation of the manuscript, critical revision for important intellectual content.

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Functional analysis and signaling pathway enrichment analysis of genes associated with Alzheimer's disease and Parkinson's disease

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ABSTRACT

We identified significant functions of susceptibility-genes and performed an analysis of pathway enrichment for Alzheimer's disease, Parkinson's disease and for both of them. Genes were extracted from a Catalog of Published Genome-Wide Association Studies (GWAS). We uploaded genes into Cytoscape version 3.2.1. ClueGO plugin was used for functional and pathway enrichment analysis of genes based on the hypergeometric test. Two databases, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway and REACTOME, were selected for analysis.

The identified susceptibility genes are involved in the synthesis regulation and accumulation of toxic proteins, β-amyloid and α-synuclein, and lead to apoptosis of neurons. We have defined 14 shared functions: collagen catabolic process, cellular response to retinoic acid, regulation of calcium-mediated signaling, negative regulation of cell projection organization, negative regulation of neuron projection development, glial cell activation, microglial cell activation, macrophage activation, regulation of cholesterol metabolism, clathrin-mediated endocytosis, regulation of protein oligomerization, regulation of dendritic spine development, kinesin binding and clathrin binding. Also, we have defined 3 shared signaling pathways: trans-Golgi Network Vesicle Budding, Clathrin derived vesicle budding, Intestinal immune network for IgA production. These pathways contain genes susceptible to Alzheimer's disease and Parkinson's disease. The results suggest the metabolic, neuronal and immunological factors participate in the development of Parkinson's disease and Alzheimer's disease.

Key words: Parkinson's disease, Alzheimer's disease, GWAS, ClueGO Cytoscape, functional annotation of genes.

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Функциональная аннотация и анализ обогащения сигнальных путей генов, ассоциированных с болезнью Альцгеймера и болезнью Паркинсона

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РЕЗЮМЕ

Цель исследования. Охарактеризовать in silico функции генов предрасположенности и провести анализ обогащения сигнальных путей при болезни Паркинсона и болезни Альцгеймера.

Материалы и методы. Гены, ассоциированные с болезнью Паркинсона и болезнью Альцгеймера, были получены на основе анализа информации из каталога GWAS (каталог ассоциаций однонуклеотидных полиморфизмов с заболеваниями). Оценка принадлежности генов к биологическому процессу, молекулярным функциям, к иммунной системе в терминах генной онтологии осуществлялась с помощью алгоритма, реализованного в плагине ClueGO Cytoscape version 3.2.1. Анализ обогащения путей был выполнен при помощи плагина ClueGO Cytoscape с использованием KEGG и REACTOME и с применением гипергеометрического теста.

Результаты. Выявленные гены предрасположенности к болезни Паркинсона и болезни Альцгеймера участвуют в регуляции синтеза и накопления токсичных белков β-амилоида и α-синуклеина, приводя к апоптозу нейронов. Установлено наличие 14 общих функций (процесс катаболизма коллагена, клеточный ответ на ретиноевую кислоту, регуляция кальций-опосредованного сигналинга, негативная регуляция защиты клеточной организации, негативная регуляция развития нейронов, активация глиальных клеток, активация микроглиальных клеток, активация макрофагов, регуляция метаболизма холестерина, клатрин-зависимый эндоцитоз, регуляция олигомеризации белка, регуляция развития дендритного отростка, связывание кинезина, связывание клатрина) и три общих сигнальных пути (везикуло-опосредованный транспорт, клатрин-производное почкование везикул, обеспечение продукции иммуноглобулина А), в которые вовлечены гены предрасположенности к болезни Альцгеймера и болезни Паркинсона.

Заключение. Полученные результаты свидетельствуют об участии метаболических (гены *MMP12, COL13A1, APOE, DGKQ*); нейрональных (гены *CLU MAPT, SNCA, STAP1, RNF6 GAK, INPP5F, MAP4K4*) и иммунологических факторов (гены *LA-DQB1, HLA-DRA, AICDA*) в механизмах развития болезни Паркинсона и болезни Альцгеймера.

Ключевые слова: болезнь Паркинсона, болезнь Альцгеймера, GWAS, ClueGO Cytoscape, гены предрасположенности.

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

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INTRODUCTION

Neurological diseases are in the top three most common diseases in the world. Parkinson's diseases (PD) comes second next to Alzheimer's disease (AD), which affects 1-2% of people over 65 years old [1].

In the recent years, progress in understanding biochemical and molecular mechanisms of these diseases has been made. Nevertheless, issues of their co-occurrence need to be studied further. A significant role here is played by genetic predisposition to the disease and environmental factors.

Patients with Alzheimer's disease and Parkinson's disease often obtain overlapping clinical presentations and brain neuropathology. β-amyloid and α-synuclein accumulation happens in substantia nigra of the human brain in PD, and in hippocampus in AD. It makes the brain unable to produce sufficient amount of dopamine [2,3].

Methods that include identification of genes responsible not only for particular disorders, but also for susceptibility to various diseases, are used in research of molecular mechanisms of neurological diseases. This susceptibility can be expressed by changes in genes, included in the most important biological processes, molecular functions and signaling and metabolic pathways. Occurring disorders of cellular homeostasis lead to the activation of pathological process mechanisms [4].

The search for genes functions and biological pathways that allow researchers to characterize general molecular genetic mechanisms is of specific interest in studying the co-occurrence of diseases. Existing bioinformatics tools allow for characterizing the participation of genes in the development of pathological process and evaluating their role in regulating intracellular signaling and cellular homeostasis [5]. Such tools include the Gene Ontology initiative.

Aim. In silico characterization of susceptibility genes functions and signaling pathways enrichment analysis for Parkinsons's disease and Alzheimer's disease.

MATERIALS AND METHODS

Susceptibility to Parkinson's and Alzheimer's diseases genes were obtained with Catalog of Published Gemone-Wide Association Studies (GWAS). It is a supervised resource that contains results of genome wide search of disease-associated single nucleotide polymorphism [6]. A ClueGO Cytoscape version 3.2. plugin was used to evaluate genes involvement in biological process, molecular functions and immune system in genetic ontology terms [7].

2 is the minimum number of groups required to form functional groups. Hypergeometric test with $p < 0.05$ and K 0.4 was used to evaluate functional links between genes [8].

Pathways enrichment analysis was conducted with ClueGO Cytoscape plugin and KEGG and REACTOME databases. Hypergeometric test with $p < 0.05$ was also used for it. Specifiicity level of 60% was chosen, meaning that enriched pathway must include more than 60% of susceptibility genes of a disease in order to be ranked with this disease. Additionally anriched pathways were clustered into groups with similar biological significance and content of susceptibility genes.

RESULTS AND DISCUSSION

A list of susceptibility genes was formed for each disease. For Parkinson's disease it consists of 119 following genes: *CLRN3, CTC1, CCDC82, TMC3-AS1, TMC3, COL13A1, LRRK2, SPPL2C, MAPT, MCCC1, FAM47E-STBD1, TMEM175, SREBF1, SLC41A1, RIT2, WNT3, MGC57346-CRHR1, ASS1P14, HLA-DRB1, GBA, SYT10, GAK, GCH1, MCCC1, RAB29, NUCKS1, RAD1P1, FAM47E, BST1, RIT2, CCDC62, OR5AZ1P, SH3GL2, SYT17, NDUFAF2, CA8, HSD17B1P1, OR5BD1P, WNT9A, COL5A2, IGSF11, RN7SL383P, RPA2P1, MDGA2, UNC13B, HLA-DRA, GAK, RAB25, ACMSD, LAMTOR2, MCCC1, TMEM175, BST1, MGC57346-CRHR1, CCDC62, MGC57346, DLG2, STAP1, RPL9P21, SEMA5A, EIF2AP4, BST1, SLC2A13, PLEKHM1, SNCA, PAX7, BRINP1, DGKQ, TAS1R2, LHFPL2, TRPS1, KLHDC1, TPM1, DSG3, PRRG4, ATF6, QSER1, AAK1, AGAP1, SPTSSB, PINK1, ZNF165, GBF1, CAB39L, RPL13AP3, MED13, ITGA2B, POLRMTP1, HMGN2P18, RAB29, NUCKS1, SIPA1L2, VDAC2P4, KTN1, MCCC1, TMEM175, BST1, KRTCAP2, HLA-DQB1, MTCO3P1, GPNMB, INPP5F, DLG2, CCDC62, GCH1, GPHN, TMEM229B, BCKDK, RIT2, TMPRSS9, DDRGK1, ITPKB, MAP4K4, SCN2A, SPPL2B, IP6K2, ITIH1, CAMK2D, NDUFAF2.*

For Alzheimer's disease – of 57 genes: *DSTNP5, WDR1, SLC2A9, PCDH7, RNF6, ATP8A2P3, DCHS2, SEPT5, TST, TEX33, RANP7, SALL4P5, CLDN18, HSPA8P9, STK32B, ZCCHC10, EN2, CIR1P1, TOMM40, PINK1, MTATP6P30, PVRL2, EXOC4, PARVB, PHF14, RPL26P32, ST18, AICDA, RPL5P26, MS4A2, SLC24A4, BCAM, CHRNA2, ZFP3, ZNF232, ACE, FBXL7, IGSF23,MS4A3, YWHAZP9, IGSF23, CEACAM19, BLOC1S3, EXOC3L2, BCL3, PPP1R37, APOC1, APOE, CLU, MMP3,GBA, PICALM, LRRK2, RELN, DISC1, TREM2, PAX2.*

An analysis of these lists revealed that products of *SNCA*, *MAPT* and *PINK1* genes when interacting with each other can contribute to the co-occurrence of Parkinson's disease and Alzheimer's disease. In particular, amyloid beta (Aβ), changes of which underlie proteopathy of Alzheimer's disease, stimulates aggregation of alpha-synuclein (αS), protein encoded by *SNCA* gene. *MAPT* gene promotes oligomers and alpha-synuclein fibrils production. Alpa-synuclein expression pattern influences manifestation of Parkinson's disease. Thus, *MAPT*-mediated interaction between Aβ and α-synuclein can concern patients with Alzheimer's disease and Parkinson's disease [9].

PINK1 gene encodes mitochondrial protein kinase. PINK1 mutations lead to mitochondrial dysfunction, thus, causing early-onset Alzheimer's disease [10]. It was revealed that *PINK1* in AD is associated with classic senile plaques, vascular amyloid deposition and reactive astrocytes, related to typical lesions of Alzheimer's [11].

Study of AD susceptibility gene functions allowed to create two main groups. The first group of genes (*GPNMB, MAP4K4, NDUFAF2, SREBF1)* is responsible for processes of negative regulation of insulin secretion, such as decrease in speed, frequency and the degree of insulin secretion from secretory granules (GO:0090278; GO:0046676). Genes of the second group (*GAK, LRRK2, SH3GL2, SNCA, SYT10, SYT17, UNC13B*) influence transport and secretion of neurotransmitters (GO:0099504, GO:0051588).

The study revealed that genes associated with AD were joined in one main group (ABCA7, APOE, CLU, PICALM). This group is in charge of production, regulation and metabolism of Alzheimer's amyloid precursor protein. Alzheimer's amyloid precursor protein is the main component of amyloid plaques in AD (GO:1900221, GO:0042987, GO:1902993).

Functional analysis discovered 14 shared gene functions, which characterize biological processes and molecular functions in the diseases (table 1).

Table 1

Functions associated with AD and PD

Marker	Genes from the functional group	
	Alzheimer's disease	Parkinson's disease
Collagen catabolic process [GO:0030574]	*MMP12, MMP3*	*COL13A1, COL5A2, CTSB*
Cellular response to retinoic acid) [GO: 0071300]	*PAX2*	*BRINP1, LTK, SREBF1, WNT3, WNT9A*
Regulation of calcium-mediated signaling [GO: 0050848]	*TREM2*	*BST1, CAMK2D, LRRK2, RIT2*
Negative regulation of cell projection organization [GO: 0031345]	*APOE, RNF6*	*GAK, INPP5F, MAP4K4, RAB29, RIT2, SEMA5A, STAP1, WNT3*
Negative regulation of neuron projection development [GO: 0010977]	*APOE, RNF6*	*GAK, INPP5F, MAP4K4, RAB29, RIT2, SEMA5A, WNT3*
Glial cell activation [GO: 0061900]	*CLU*	*MAPT, SNCA, STAP1*
Microglial cell activation [GO: 001774]	*CLU*	*MAPT, SNCA, STAP1*
Macrophage activation [GO: 0042116]	*CLU, MAPT*	*MAPT, SNCA, STAP1*
Regulation of cholesterol metabolic process [GO: 0090181]	*APOE*	*DGKQ, FDFT1, SREBF1*
Clathrin-dependent endocytosis [GO: 0072583]	*PICALM*	*GAK, INPP5F, SH3GL2, SNCA*
Regulation of protein oligomerization [GO: 0032459]	*APOE, CLU, GBA, MMP3*	*GBA*
Regulation of dendritic spine development [GO: 0060998]	*APOE, DISC1, LRRK2, RELN*	*LRRK2*
Kinesin binding [GO: 0019894]	*DISC1*	*KTN1, RAB29, SNCA*
Clathrin binding [GO: 0030276]	*PICALM*	*LRRK2, SYT10, SYT17*

The results of the functional analysis demonstrate that shared functions of AD and PD susceptibility genes are connected with the influence on neurons and nervous system functions. For example, microglial activation is a distinctive feature of neuroinflammation, typical for both, Alzheimer's disease and Parkinson's disease. Changes in the regulation of neuronal development can increase or decrease their sensitivity and, thus, lead to disorder in cell response to irritants [12].

Studied gene products enforce processes of collagen disorganization in extracellular matrix. This promotes neuronal dysfunction [13, 14]. Change in cellular response to retinoic acid can disturb cell proliferation and differentiation functions and cause changes cell state and activity. In particular, retinoic acid has neuroprotective effect on dopaminergic neurons in Parkinson's disease [15].

Influence on calcium-mediated signaling induces change in calcium concentration in endoplasmic reticulum. High Ca^{2+} concentration may lead to synaptic deficit and contribute to amyloid plaque accumulation in Alzheimer's disease [16].

The following analysis of signaling pathways enrichment and metabolic pathways has shown that PD susceptibility genes are specifically involved in pathways R-HSA:202165, R-HSA:202291, R-HSA:202427 and R-HSA:2130378. These pathways are engaged into transfer of histocompatibility complex (MHC) class II and activation of Lck (lymphocyte kinase) which phosphorylates immunoreceptor tyrosine-based inhibitory motif (ITIM) in T-cell receptor (TCR) family. TCR is responsible for specifically bind antigen recognition and triggers cellular response [17].

Gene associated with AD specifically enrich pathways concerned with regulation of cholesterol metabolism. Cholesterol takes part in regulation of β-amyloid products (KEGG:04979; R-HSA:174824; R-HSA:8963898) [18].

The analysis of pathways enrichment identified significant pathways shared by AD and PD ($p < 0.05$). These pathways are responsible for the following processes (table 2):

Vesicle-mediated transport (R-HSA:199992). It provides directed movement of substances from Golgi complex to other cell parts, including organelles and plasma membrane.

Clathrin-derived vesicle budding (R-HSA:421837). It is a transport vesicle formation under the effect of clathrin and adaptor proteins on the Golgi membrane.

Immunoglobulin A (IgA) production with intestinal immune network (KEGG:04672). It is a production of a large number of non-inflammatory antibodies for IgA.

Table 2

Enriched pathways specific for Parkinson's disease and Alzheimer's disease

Marker	Genes included in biological pathway	
	Parkinson's disease	Alzheimer's disease
Vesicle-mediated transport	*GAK, GBF1, SH3GL2*	*BLOC1S3*
Clathrin-derived vesicle budding	*GAK, GBF1, SH3GL2*	*BLOC1S3*
Immunoglobulin A (IgA) production with intestinal immune network	HLA-DQB1, HLA-DRA, HLA-DRB1	AICDA

The conducted study of signaling and metabolic pathways demonstrate that genes associated with PD and AD specifically enrich pathways, which contribute to synthesis and accumulation of toxic proteins. These toxic proteins are β-amyloid and α-synuclein which lead to neuronal apoptosis. Studied genes also influence products of non-inflammatory antibodies to immunoglobulin A (IgA), thus, providing active protection from microorganisms and toxins.

CONCLUSION

Three components influencing development mechanisms of Parkinson's disease and Alzheimer's disease can formulated based on the analysis of AD and PD susceptibility genes participation in molecular functions and signaling and metabolic pathways:

Metabolic component including collagen catabolism, cholesterol metabolism, clathrin-mediated endocytosis (MMP12, COL13A1, APOE, DGKQ)

Neuronal reactions such as glial cells activation, negative neuronal regulation, vesicle-mediated transport in synapses and calcium-mediated signaling (CLU MAPT, SNCA, STAP1, RNF6 GAK, INPP5F, MAP4K4)

Immunological component that is macrophage and microglial activation and influence on Immunoglobulin A production (LA-DQB1, HLA-DRA, AICDA)

Future use of this data may help to determine (in silico and by experiment) main molecular genetic development mechanisms and possible ways of therapeutic correction of Alzheimer's disease and Parkinson's disease.

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Vulvodynia – a multidisciplinary problem

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ABSTRACT

This literature review is devoted to the problem of vulvodynia. The article summarizes information about the etiology and pathogenetic factors of this syndrome, touches the aspects of diagnosis and treatment of this form of chronic genital pain. Despite the prevalence of this pathology, women with pain in the vulva often remain undiagnosed and do not receive adequate therapeutic and psycho-emotional support. Currently, the focus is in searching of the definition, classification, prevalence, pathophysiological factors of occurrence and adequate personalized therapy of this nosology.

Key words: vestibulodynia, dyspareunia, sexual dysfunction, provoked vulvodynia, unprovoked vulvodynia, generalized vulvodynia, clitorodynia

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Вульводиния – мультидисциплинарная проблема

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РЕЗЮМЕ

Обзор литературы посвящен проблеме вульводинии как мультидисциплинарной проблеме. В статье обобщены сведения об этиологии и патогенетических факторах развития заболевания, затронуты аспекты диагностики и лечения данной формы хронической генитальной боли. Несмотря на распространенность патологии, женщины с болью в области вульвы нередко остаются не диагностированными и не получают адекватную терапевтическую и психоэмоциональную поддержку. В на-

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стоящее время актуальность представляет поиск определения, классификации, распространенности, патофизиологических факторов возникновения и адекватной персонализированной терапии данной нозологии.

Ключевые слова: вестибулодиния, диспареуния, сексуальная дисфункция, спровоцированная вульводиния, неспровоцированная вульводиния, генерализованная вульводиния, клитородиния.

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

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INTRODUCTION

Vulvodynia is a chronic vulvar discomfort that lasts more than 3 months and is manifested mainly by symptoms such as burning sensation and itching, as well as pain. At present, vulvodynia is a common but still poorly understood problem with multifactorial etiology. According to literature sources, an increasing number of patients seek medical help with the symptoms of this disease annually, while it is quite difficult to determine the exact cause of their condition. The problem of treating vulvodynia is extremely urgent due to the chronic course of this process and the difficulty in achieving the therapeutic effect due to the presence of a psycho-emotional component. This pathology was first outlined in the literature in the 1880s as "excessive sensitivity of nerves innervating the mucous membrane of the vulva" [1] or "hypersensitivity of the vulva" [2]. According to the type of condition, professionals distinguish generalized or localized forms of vulvodynia, each of which in turn is classified into provoked, unprovoked, independent of the presence of an irritant, and mixed types. The localized form includes vestibulodynia and clitorodynia [3]. Portuguese scientists have proposed a classification of vulvodynia based on 3 main groups of trigger factors: 1) local inflammatory factors (urinary tract infections, candidiasis, herpetic infection); 2) general pain susceptibility (fibromyalgia, irritable bladder syndrome, etc.); and 3) pinched/damaged pelvic nerve (scoliosis, pelvic surgery). It is assumed that such systematization could explain the differences between generalized and localized vulvodynia, as well as the difference in treatment responses [4]. In recent years, the term "vulvodynia" has been increasingly used in domestic medicine. It means a chronic pain syndrome or discomfort of a different nature, such as burning or itching, which occurs in the vulvar area and the entrance to the vagina [5]. According to the classification of chronic pain syndromes, the so-called «gynecological pain» is distinguished, which includes pain syndrome associated with endometriosis, vaginal, vestibular, generalized and localized vulvar pain syndrome and pain in the clitoral area [6]. This classification defines vulvodynia as «a syndrome of unexplained pain in the vulva, sexual dysfunction, and mental instability". There are similar terms, such as vulvar dyskinesia, provoked vestibulodynia, and dyspareunia. However, all of them have nuances and do not characterize the whole concept of vulvodynia [7].

PREVALENCE

For a long time, vulvodynia had been considered a rare disease. However, recent large epidemiological studies speak of a high incidence (17%) and prevalence (7%) of this pathology in the population [8-9]. Vulvodynia affects women of all age groups, from adolescence to menopause [10]. In the United States, 8.3% to 16% of women suffer from vulvodynia. According to estimates by the National Institute of Health, about 13 million women may have symptoms of the disease at some point in their lives, and in 6% of women symptoms of the disease occur before the age of 25 years. These indicators are considered significantly underestimated due to the absence of visible pathologies of the vulva [9, 11]. In Portugal, according to studies by Vieira-Baptista [4], the prevalence rate is 16%. Spanish researchers speak about the prevalence of vulvodynia in 7–8% of women by the age of 40, while 30% to 48% of them have never sought medical help [12]. A study conducted in Michigan revealed an incidence rate of 4.2% among women, with a prevalence rate ranging from 3.3% for women over 60 to 7.6% for girls under 20; pain syndrome that does not meet the criteria for vulvodynia was observed in 11.5% of women [13]. According to Reed [9] et al., the prevalence of vulvodynia differs depending on ethnicity: 4.3% of African women report symptoms compared with 9.3% of European women and 15.6% of Hispanic women. Other sources speak of an equivalent risk of developing vulvodynia in European and African American women [8]. Difficulties in diagnosing vulvodynia, in general, are associated with possible ethnic differences in the level of pain threshold, pain perception, and description of symptoms, as well as with possible poor accessibility of medical care [14].

ETIOLOGY AND PATHOGENESIS

Vulvodynia has a multifactorial etiology, however, not all causes are known today. One can assume that somatic, psycho-emotional, and psychosexual aspects (high levels of anxiety, depression, disturbances in emotional and sexual relations with a partner, etc.), as well as their combination, take part in the development of vulvodynia [15]. Despite the multicomponent state, the emphasis is laid on the somatic causes of this pathology, among which chronic inflammation in the vulva and vagina, atrophic processes, chronic contact dermatitis of the vulva, dermatosis of the vulva (lichen sclerosus and lichen planus) are distinguished [16]. The root cause of vulvodynia may also be improper development during embryogenesis [17–18], immune/genetic factors [19–20], and abnormal development of the female genital organs and their innervating nerve pathways [21–24]. The influence of environmental factors is not excluded: infections (human papillomavirus, chronic candida process), irritating substances (oxalates and products of its metabolism), injuries and microtraumas in the pelvic area. Currently, most vulvologists do not share this view. However, the results of a cross-sectional study in Portugal indicate a direct positive correlation between oral contraceptives, the presence of chronic candidiasis, genital herpes, urinary tract infections, depression, premenstrual syndrome and the development of vulvodynia. Pregnancy and childbirth were not associated with this pathology [4]. Pathophysiological changes in the mucous membranes in vulvodynia manifest themselves in an increase in sensitive innervation and local blood flow, which suggests the presence of a neurogenic inflammatory process [25]. The trigger mechanisms in the development of the chronic inflammatory process of the nerves innervating the vulva are contact irritants, recurring vulvovaginal infections, hormonal changes, and chronic skin diseases. During the outcome of chronic inflammation, normal sensations are perceived as abnormal, which leads to the occurrence of hypersensitivity and pain [21–22]. Histological biopsies from the vulva revealed an increase in the number of mast cells and hyperproliferation of nerve receptors [26]. It is also believed that abnormalities in the development of the pelvic floor, in particular, weak muscular skeleton, can cause tension of the nerves that pass through this area. Due to pressure or friction of irritated muscles, pain can be radiated to the vulva [27]. Pelvic floor muscle dysfunction is almost always present in women with vulvodynia. The role of these disorders in the occurrence of pain in vulvodynia is also confirmed by the improvement of symptoms along with the normalization of the function of the pelvic floor through physiotherapy. The quality of the pain and its response to drug treatment indicate its relationship with neuropathic pain. Whether the pain is peripheral or central in origin is unknown.

NEURAL PROLIFERATIVE (VULVAR) FACTORS

Although hypersensitivity to the vulvar vestibule is one of the defining characteristics of vulvodynia, in particular, provoked vulvodynia, the mechanism underlying this allodynia was not clarified until 1998. Westrцm, Willйn, Bohm-Starke, et al. [21] used immunohistochemical (IHC) staining to visualize an increase in the density of nerve endings in the vestibule endodermis in women with provoked vulvodynia who underwent vestibulectomy compared with the control group. Additionally, the neural proliferation of nociceptors was investigated, which explained the onset of allodynia. Quantitative sensory testing by Pukall et al. [28], showed that women with provoked vulvodynia are more sensitive to tactile and painful stimuli of punctate in comparison with control women. The similar results are observed in response to other forms of stimulation, for example, thermal and pressure pain. Hypersensitivity is not limited to static stimuli or vestibule.

EMBRYOLOGICAL AND CONGENITAL FACTORS

Vulvodynia has been described in young girls with or without concomitant interstitial cystitis and irritable bladder syndrome. One of the possible explanations for the coexistence of vulvodynia and interstitial cystitis is that these two conditions are congenital endothelial disorders of the urogenital sinus. Some cases of provoked vulvodynia may be associated with a congenital defect in hyperplasia of the neurons of the vulvar tissue. Women with vulvodynia have significantly more nerve fibers in the vaginal vestibule compared with the control group [23]. It has also been hypothesized that there is a correlation between the number of vanilloid receptors (VR1) and the further development of vulvodynia [29].

GENETIC FACTORS

Several studies have suggested a genetic predisposition to the development of provoked vulvodynia (for example, Babula et al. [30], Lev-Sagie et al. [31], Foster et al. [26], Gerber et al. [32], Goldstein et al. [33]). Genetic studies have focused on three possible (but potentially overlapping) mechanisms: genetic polymorphisms that increase the risk of developing chronic candida and other infections [31], genetic changes that affect the nature of inflammation [32] and increased susceptibility to hormonal changes caused by oral contraceptives. The genetic predisposition to vulvodynia associated with the polymorphism of interleukin (IL)-1β genes, which affects the nature of the inflammatory reaction, is proved. Women with vulvodynia are more often homozygous for the second allele of the IL-1β gene and the second allele of the IL-1β receptor antagonist gene compared to healthy women [32, 34].

HORMONAL FACTORS

It has been believed for decades that the vulvar and vaginal tissues respond to and depend on steroid hormones to ensure proper health and functioning, and that circulating estrogen deficiency leads to anatomical and physiological changes in the vagina. There are many reasons for the decrease in sex steroids, natural and iatrogenic, leading to physiological changes and symptoms of the disease. The most common cause of decreased sex steroids in women is menopause. Other natural causes of anovulation include lactation or anorexia, hypothalamic amenorrhea due to biological stress factors, such as excessive physical activity or physiological stress and hyperprolactinemia [35]. Iatrogenic causes of decreased circulating sex steroids include surgical factors such as ovariectomies and hysterectomies [36], combined oral contraceptives (COCs) (estrogen and progestin-containing oral contraceptives), used by 82% of North American women at some point in their lives [37]. It has been shown that COCs induce morphological changes in the mucous membrane of the vestibule, increasing its vulnerability to mechanical deformations [38]. In addition, the use of COCs is associated with a decrease in the pain threshold, a decrease in the size of the clitoris, the thickness of the labia, and introital diameter. The latest prospective randomized experimental study by Battaglia et al. [39] showed desensitization of orgasm, a decrease in lubricant production, and an increase in dyspareunia associated with COC intake. Bazin et al. [40] in a controlled study showed that women who started taking COC before age 17 had a relative risk of developing provoked vulvodynia. In addition, Bouchard et al. [41] and Harlow et al. [42] confirmed that early use of COCs significantly increases the risk of vulvodynia. Greenstein et al. [43] reported that the use of COCs containing ethinyl estradiol in a dose not exceeding 20 mg significantly increases the risk of provoked vulvodynia. Goldstein et al. [44] revealed polymorphism in androgen receptors, which significantly increased the risk of developing COC-induced provoked vulvodynia. Many medical practitioners believe that hormonal changes affect vulvodynia. Symptoms often occur just before menstruation, and there is evidence that early use of hormonal contraception may predispose to vulvodynia. However, other studies and clinical experience do not support this hypothesis [45]. Starting or stopping oral contraceptives and increasing estrogen levels usually do not improve symptoms. The study by Reed also disproved the hypothesis that oral contraceptives are associated with the further development of vulvodynia in women under 50 [46].

PSYCHO-EMOTIONAL FACTORS

Patients do not consciously associate vulvodynia with their psycho-emotional problems. There are several pathogenetic variants, depending on the ratio of somatic and mental factors:

1. Vulvodynia as a somatoform disorder – the predominance of psychogenic mechanisms in the absence of organic changes in the vulva or their

inconsequentiality. In this case, the presence of concomitant pain syndromes, such as fibromyalgia, irritable bladder syndrome, irritable bowel syndrome, is often observed;

2. Vulvodynia as a psychogenic reaction due to the effect on the patient's mental status of gynecological diseases and related symptoms. Ineffective therapy, relapses of the disease, anxiety often become trigger factors for the deterioration of the patient's psycho-emotional status and, as a consequence, the development of vulvodynia;

3. Vulvodynia as iatrogeny, often a similar condition develops against the background of the presence of sexually transmitted diseases in patients;

4. Vulvodynia as a psychosomatic disorder – the presence of a set of symptoms, including the presence of both mental and somatic pathologies [47].

CLINICAL EVIDENCE

Vulvodynia is characterized by the presence of burning, itching, pulsation, pain in the vulva. The pain may be constant or intermittent, localized or diffuse. Symptoms occur during friction on underwear or physical exertion, and in a sitting position or during rest [48]. The classification of vulvodynia is based on the location of the pain: localized and generalized. The localized form of vulvodynia and vestibulodynia occurs in contact with a sensitive area of the vulva. Usually, this form is projected in the vestibular gland in the vestibule of the vagina, possible irradiation into the clitoris (clitorodynia), and unilateral localization (hemivulvodynia). Pain is described as a sensation of burning, throbbing, tearing, "razor blade". Often, women with localized vulvodynia complain of dyspareunia and avoid sexual intercourse; pain can last from several hours to a day after intercourse, during it and when the penis penetrates the vagina. The use of tampons, playing sports or other active activities, wearing tight clothing can cause discomfort. Localized vulvodynia is divided into primary, when pain occurs after the first penetration into the vagina, and secondary, arising after a while [49]. Generalized vulvodynia is characterized by the localization of pain in the area of the vulva or around it, including the pubic area, the labia, the vestibule, and the perineum. Pain can be permanent or intermittent in nature and range from a sensation of slight discomfort to unbearable pain, greatly reducing the quality of life. Symptoms can be diffuse or localized in several areas at once, occur alternately, fade and worsen, and not depend on external irritation. The nature of pain does not differ from those with a localized form. Often there is dyspareunia, painful urination, a change in the nature of vaginal discharge [50]. A study by Sadownik et al. [51] revealed that 71% of women have dyspareunia, 64% have recurrent candidiasis, 57% and 46% have a sensation of burning and itching in the vulva, and 33% have sexual dysfunction. A study by Brown et al. [52] showed that European women describe the symptoms of burning pain 19 times more often than African-American women who describe the pain as "aching", which is less consistent with the classic manifestation of vulvodynia. Gansky et al. [53], while studying a group of women with fibromyalgia, found that European women have a lower pain threshold and a predominantly localized nature of the spread of pain compared to African-American women, mainly complaining about the generalized nature of the pain syndrome. Along with vulvodynia, the presence of other pain syndromes is often observed. Reed and his colleagues examined 24 women in his study for interstitial cystitis, irritable bowel syndrome, fibromyalgia, and vulvodynia. The incidence ranged from 7.5% to 11.8%; in 27% of patients, the presence of vulvodynia was significantly associated with other pain syndromes [54]. The chronic nature of vulvodynia can negatively affect a woman's self-esteem and cause depression and anxiety disorders. Vulvodynia is not considered a psychopathological condition. However, Tribo et al. [55] reported anxiety syndrome in more than 50% of women. Further studies have shown that women with anxiety disorders are 4 times more likely to develop vulvodynia. In addition, women can experience significant sexual and psychological problems in their relationship with a partner. Many of them use psychological support, sex therapy and/or counseling from a psychotherapist or sexologist. Pain caused by vulvodynia can ultimately lead to decreased sexual activity; fear and expectation of pain during intercourse can lead to sexual dysfunction such as disorders of sexual arousal, decreased libido, problems with orgasm/anorgasmia, phobic avoidance of sexual activity [56].

DIAGNOSTICS

One of the diagnostic methods for detecting vulvodynia is the "Cotton swab test" used to identify sensitive areas in the vulva. The test is carried out strictly sequentially, starting from the outer edge of the thigh to the inside, from top to bottom along the labia majora, and then the labia minora, to the frenum of the labia, perianal region and ending with the vestibule of the vagina and the area of periurethral and Bartholin glands. The right and left side of the vestibule should be examined separately. During the test, the patient evaluates each of the studied areas on a 10-point Likert pain scale, where 0 is its absence, and 10 is severe pain. A localized form of vulvodynia can be diagnosed if the patient experiences discomfort at individual points, and a generalized form – if pain covers a vast area. If the patient does not experience a burning sensation or pain in any of the areas, then vulvodynia cannot be considered as a differential diagnosis [48]. Friederich proposed local diagnostic criteria for vulvodynia, including soreness during the Q-tip test (point symmetrical palpation of the vaginal area with a cotton swab), erythema of the vulva of varying severity and pain when touching the vestibule of the vagina. However, only the last criterion is often positive and is the only diagnostic symptom. In addition, IMMPACT recommendations [57] are of great significance in the diagnosis of vulvodynia, used to evaluate such criteria as pain, physical/sexual functioning, emotional functioning, treatment efficacy and treatment satisfaction, symptoms and adverse events, and patient compliance.

DIFFERENTIAL DIAGNOSIS

Vulvodynia is a diagnosis of exclusion. The main diseases of differential diagnosis include atrophic vaginitis, introital or vaginal lichen planus, desquamative inflammatory vaginitis. The symptoms of genital neuralgia are similar to vulvar pain, however, pain, in this case, has characteristic clinical signs. The patient feels better in a standing position while sitting or lying there is great discomfort. Candidiasis infections, with the exception of *Candida albicans*, in particular, *Candida glabrata, Candida krusei (Issatchenkiaorientalis)* can cause itching and feeling of abrasion than classical itching but are usually asymptomatic [45]. The diagnosis of vulvodynia requires the exclusion of an obvious somatic pathology that can cause pain. The identification of the psychogenic component through the collection of anamnesis, including a pain history of life, is important; the sexual function, the level of stress, depressive episodes, if any, are studied. The clinical signs in the presence of a psychogenic component are characterized by a mismatch of the nature of pain and clinical manifestations, the dynamics of the pathology non-typical for gynecological diseases – sudden appearance and disappearance, changes correlated with relationships with a partner, sexual function (conflicts with a partner, lack of awareness of one's own sexuality, sensation of sexual inferiority), the level of mood and the lack of effect of the therapy [25]. For differential diagnosis, the pelvic floor muscles are examined by palpation in the projection of the muscle that raises the anus and the internal obstructive muscle for painful sensations [58].

TREATMENT

Therapy of vulvodynia requires an interdisciplinary approach and combines an individually selected ratio of drug therapy, psychopharmacotherapy, psychotherapy, sexuality counseling, and surgical interventions. It is important to understand the presumed root cause of the symptoms and perform a comprehensive differential diagnosis. The creation of awareness is important in the treatment of vulvodynia. Confidence and awareness of patients about their problem and further therapy helps to cope with the psycho-emotional aspects of vulvodynia and leads to recovery. Women need to realize that vulvodynia is a separate nosology, neither contagious nor associated with serious or life-threatening conditions, such as oncology, sexually transmitted diseases, or immunodeficiencies. However, it is worthwhile to inform women in advance that vulvodynia usually responds to treatment, but cannot be completely treatable, and requires high patient compliance. Lack of compliance nullifies the effect of the therapy. More often, improvements come slowly, by trial and error with an individually selected treatment program. Psychotherapeutic measures include cognitive-behavioral therapy, analysis of interpersonal relationships, and relationships with a partner. Often, training in relaxation techniques, self-examination with the study of the pelvic floor muscles and the techniques of their training, consultations of a psychotherapist and sexologist, including paired methods of sexual therapy give good results.

NON-SPECIFIC METHODS OF TREATMENT

Local care of the affected area and avoidance of potential irritants can improve the quality of life. Occasionally, a significant improvement in condition occurs when you refuse to use non-specific detergents, excessive washing, taking medications and local anti-candidiasis therapy and lubricants. 2% Lidocaine gel is a safe and non-irritating local anesthetic that can relieve discomfort [45]. Non-pharmacological treatment also includes physiotherapy of the pelvic floor muscles. Muscle relaxation subsequently leads to a decrease in pain. However, according to research, from 60% to 80% of successful physiotherapeutic outcomes are achieved only with treatment by pelvic-focusing physiotherapists [59]. Common methods of therapy include soft tissue mobilization, ultrasound, surface electromyography, and the use of vaginal dilators. As additional methods, electrical stimulation can be used; some involve electrical stimulation, exercises for the pelvic floor muscles (including Kegel), methods of thermal exposure and neuromodulation of the sacral plexus, and electric muscle stimulation, devices [60]. Hypnosis has been used with some limited success [61]. A study by Schlaeger et al. [62] addressed the issue of acupuncture in women with vulvodynia. 36 women received acupuncture 2 times a week for 5 weeks. It was found that pain and dyspareunia reduced significantly, while the sexual activity of women, on the contrary, increased. Coady et al. [63] put forward a hypothesis about the relationship of vulvodynia with impingement syndrome of the hip joint. 26 patients with generalized unprovoked vulvodynia or clitorodynia underwent arthroscopy of the hip. The study revealed significant symptomatology improvements in women under 30 almost 3–5 years after surgery. A review by Frigo N.V. [64] reported successful therapy with Neogyn vulvar soothing cream. 24 women aged 53–80 years were examined and treated with Neogyn cream for 12 weeks; after the therapy, 60.9% of patients had a significant decrease in discomfort, dyspareunia, and 65.26% improved the quality of their sexual life. G. Donders et al. [65] conducted a placebo-controlled, cross-sectional study of 30 patients with vulvodynia who were prescribed this cream. These studies showed a decrease in symptoms of provoked vestibulodynia. Narcotic pain killers should be used with caution; the combination of tramadol and hydrocodone was used in the short term for acute pain [50].

SURGICAL TREATMENT

Currently, vestibulectomy is the gold standard for the treatment of vestibulodynia, but only if the pain is localized only in the vestibule. The treatment efficacy ranges from 65% to 90%. Very often surgery causes damage to the vaginal mucosa, leading to the removal of a more extensive area than the focus of pain; in the postoperative period, symptoms may be preserved due to incomplete preoperative diagnosis and inaccurate pain map. Taking neuropathic pain killers usually continues after surgery to maximize the quality of life and promote the recovery of sexual functioning. Novocaine blockade is feasible only when other treatment methods do not provide positive dynamics to relieve pain and improve the patient's quality of life [66].

PAIN MODULATORS

The use of tricyclic antidepressants, such as Amitriptyline or Desipramine, can help reduce chronic neuropathic pain due to the central mechanism of action, which alters the transmission of pain impulses to the brain through the spinal cord. Brown et al. [67] studied the effect of Amitriptyline C with/without Triamcinolone; the efficacy of this method has not been proven. A randomized controlled trial by Foster et al. [68] showed that the effects of oral Desipramine were not superior to those in the placebo group. The efficacy of other antidepressants, such as Duloxetine and Venlafaxine, has also not been proven. A randomized controlled trial of Gabapentin – a drug that helps control epileptic seizures, has been shown to trigger symptoms of vestibulodynia. However, studies of Pregabalin anticonvulsant drug showed improvement in symptoms, and the use of Lamotrigine reduced pain within 8 weeks [69]. Hydroxyzine and Cetirizine have been used to reduce itching. Combinations of neuropathic pain killers (for example, Amitriptyline, Gabapentin, Pregabalin) can also be used for some women since they have different mechanisms of action [50].

CONCLUSION

Vulvodynia is a relevant multidisciplinary problem, causing both physical and psychological discomfort. This pathology reduces the quality of life not only of the patient herself but also of her partner, contributing to the development of a wide range of psycho-emotional and sexual problems. Vulvodynia requires careful diagnosis; often being a symptom of a number of serious diseases of the vulva, vulvodynia can also act as an independent nosology, and it can be difficult to establish the exact cause of this condition.

Treatment of vulvodynia must be client-oriented, in accordance with the potential predictors of the onset of the disease. It is very important not only to choose the appropriate therapy for a particular woman but also to provide psychological support in order to achieve the highest possible increase in the quality of life of such patients and their partners.

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The role of long, non-coding RNA in the biology of tumors

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ABSTRACT

One of the most significant events in recent years in the field of molecular biological research has been the recognition of the biological significance of non-coding ribonucleic acid (RNA). It turned out that a significant part of the non-coding part of the genome, which constitutes 98% of the genome, is rewritten. In addition to small RNAs (such as microRNAs (miRNA)), long non-coding RNAs (lncRNAs), which are a large group of non-coding RNAs (ncRNAs) over 200 nucleotides in length, have been discovered. They play a role in the regulation of a number of basic molecular processes (cell division, chromatin function, microRNA activity, etc.). Many of these long non-coding RNAs were expressed in tumors compared with healthy tissues, for example, H19, HOX antisense intergenic RNA HOX (HOTAIR), Metastasis Associated Lung Adenocarcinoma Transcript 1 (MALAT1). A large amount of evidence revealed their roles at all stages of carcinogenesis and in modulating metastasis through regulatory networks. Aberrant expression of lncRNAs has been observed in cancer patients. In this context, lncRNAs can regulate the main characteristics of cancer cells by controlling gene expression programs associated with their suppressive and oncogenic functions. Therefore, they can be excellent biomarkers and therapeutic targets for tumors.

Keywords: genome, long non-coding RNA, tumor, miRNA.

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Роль длинных некодирующих РНК в биологии опухолей

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РЕЗЮМЕ

Одним из наиболее значительных событий в последние годы в области молекулярно-биологических исследований стало признание биологической значимости некодирующих РНК. Оказалось, что значительная часть некодирующей части генома, которая составляет 98%, перезаписана. Помимо небольших РНК (таких как микроРНК) известно о длинных некодирующих РНК (lncRNAs), которые являются большой группой некодирующих РНК (ncRNAs) длиной более 200 нуклеотидов. Они играют роль в регуляции ряда основных молекулярных процессов (деление клеток, функция хроматина, активность микроРНК и т.д). Многие из этих длинных некодирующих РНК были экспрессированы

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в опухолях по сравнению со здоровыми тканями, например H19, HOTAIR, MALAT1. Большое количество исследований выявило их роль на всех стадиях канцерогенеза и в модулировании метастазирования через регуляторные сети. Была замечена аберрантная экспрессия lncRNAs у больных раком. В этом контексте lncRNAs могут регулировать основные характеристики раковых клеток, контролируя программы экспрессии генов, связанные с их супрессивными и онкогенными функциями. Следовательно, они могут быть отличными биомаркерами и терапевтическими мишенями при лечении опухолей.

Ключевые слова: геном, длинная некодирующая РНК, опухоль, микроРНК.

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

Источник финансирования. Авторы заявляют об отсутствии финансирования.

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INTRODUCTION

In recent years, we have witnessed radically new directions in the field of molecular biology. Among them, a new interpretation of the world of ribonucleic acids (RNA) is of great importance, in which new regulatory mechanisms led to the discovery of previously unknown aspects of the genome, cells, and organism [1, 2]. The wider use of bioinformatics methods is significant in expanding our knowledge. In addition to protein-based messenger RNAs (mRNAs), RNAs that do not encode proteins are becoming increasingly important. Epigenetic pathways that are not associated with a change in the nucleotide sequence play a primary role in the molecular mechanisms of these molecules. Among non-coding RNAs, there is growing evidence of the importance of small RNAs, including microRNAs (they are mature, 18–24 nucleotide single-stranded RNA fragments: important elements of post-transcriptional regulation). Their various expressions have been described in many diseases, and the role of miRNAs in the molecular biological aspects of neoplasms has already been proven [3]. In addition to microRNAs, it is known that a number of other small RNAs play a key role in supporting basic molecular processes, such as splicing during mRNA maturation, telomere maintenance, centromere control, and genome stability. The former "central dogma" of molecular biology has been changed in several respects, and the role of RNA in the regulation of cell and genome functions can be shown at many levels [2]. One of the most shocking results of the next generation sequencing methods, which revolutionized research in the field of molecular biology (deep sequencing), was that a significant part (70-90%) of the non-coding part of the genome is transcribed into RNA [4, 5]. For example, an RNA aggregate transcribed from the mouse genome (transcript) may consist of 180,000 RNA molecules, of which only 20,000 encode proteins [1]. The functions of most non-coding RNAs are currently largely unknown. Non-coding RNAs can mainly be divided into two groups: small RNAs (microRNAs, transport RNAs associated with PIWI RNAs, telomeric RNAs associated with the RNA promoter, small nucleolar RNAs, etc.); and a class of long non-coding RNAs up from a size of 200 nucleotides. Although there is a lot of data on small RNAs, especially microRNAs, much less is known about the importance of long non-coding RNAs [5–7]. In this short review article, we are trying to present a very new and rapidly developing direction. Unfortunately, for most of the terms mentioned, there are currently no Russian equivalents, so we used English terminology.

CHARACTERISTIC OF lncRNAs

The biological function of several lncRNAs, which play a role in the regulation of basic molecular biological processes, is described. They include regulation of gene expression and genome function, which can be positively and negatively affected by protein binding, including transcription factors, to affect chromatin structure and function. Regulation of the ratio of transcriptionally active euchromatin to inactive heterochromatin, modification of histone proteins (including methylation and phosphorylation) give greater importance to lncRNAs. They can participate in these processes as molecular scaffolds [5]. In addition, lncRNA genes express themselves weaker than the coding genes, and their expression is particularly specific for certain tissues. Depending on their position relative to the coding genes, lncRNA can be divided into two broad categories: intergenic lncRNAs and intragenic lncRNAs. Intergenic localized by definition in unannotated regions of the genome are commonly referred to as lincRNA. On the other hand, intragenic IncRNAs can be subdivided depending on how they overlap the coding genes or their orientation in relation to them (antisense, intron, etc.). Many lncRNAs have an antisense sequence complementary to other sequences, and it is also known that some of the promoters that regulate gene expression also allow bidirectional transcription [4]. The binding of antisense lncRNA to complementary RNA can lead to the formation of double-stranded RNA, which is the fundamental substrate for the RNA interference process and can lead to the appearance of small interfering RNA (siRNA) during the general maturation of miRNAs. SiRNAs, like their endogenous analogues, have a mechanism similar to miRNAs for post-transcriptional regulation of gene expression [4]. In humans, the number of lncRNAs is estimated to a range from 5,000 to 7,000, but it is expected that this number will increase in the future [8]. However, the number of experimentally confirmed and known functions of lncRNAs is only about 100 [9]. One of the first known lncRNAs was XIST (X inactive specific transcript), responsible for inactivation of the X chromosome. XIST binds to proteins, of which the protein group PRC (polycomb repressor complex) should be noted. Modification of histones and chromatin leads to inactivation of the X chromosome. PRC proteins also play a role in the regulation of chromatin structure due to the expression of lncRNAs [10].

THE FUNCTIONS OF lncRNAs IN PHYSIOLOGICAL PROCESSES

lncRNAs mainly act by modulating gene expression [11]. This function can be performed locally when lncRNAs act on neighboring genes in the cis-position, or distally when their functions are performed regardless of the location of the target genes. In particular, there is a class of lncRNA with enhancer-like activity that can transcriptionally activate neighboring genes [12]. More generally, studies of the functions of lncRNAs have shown that they are potentially involved in various biological processes in mammals [11]. These processes include, for example, maintaining the pluripotency of embryonic stem cells, cell differentiation, cell cycle regulation, and the immune response. LncRNAs can regulate gene expression through various mechanisms. The molecular aspects of these mechanisms have been described in detail in a recent review [11]. LncRNAs can potentially bind DNA, proteins or other RNAs, forming networks and, thus, providing an interaction between different functional molecules. Some lncRNAs can change the chromatin context near their target genes by a set of transcription factors, histone modification factors, thereby stimulating or inhibiting the transcription of target genes, depending on the context. Among the lncRNAs that were functionally characterized, XIST, which gene is located on the X chromosome [13], is directly involved in the inactivation of the X chromosome in women. After transcription, XIST is retained in the nucleus and covers the inactive X chromosome. In addition, it interacts with the Polycomb 2 inhibitor complex (PRC2), which makes it possible to purposefully recruit this complex and thereby contributes to maintaining the inactivation of the X chromosome [13]. Interestingly, XIST, in turn, is regulated by other lncRNAs, such as TSIX (Transcription silencing inactivation of X) and XITE (X-inactivation intergenic transcription element) [13]. Other lncRNAs, such as AIRN, H19, and KCNQ1OT1, also participate in the inactivation of gene expression through their association with chromatin-associated inhibitor complexes. HOTAIR lncRNA, whose gene is located in the HOXC locus, will serve as a framework for the PRC2 and LSD1 complexes (lysine-specific demethylase 1), two complexes associated with transcription inhibition, and facilitate their recruitment within the HOXD locus [14, 15]. In contrast, Mistral and HOTTIP lncRNAs will facilitate the expression of HOXA genes by recruiting the WD5 / MLL epigenetic complex [16, 17]. LncRNAs are also significantly involved in post-transcriptional processes associated with mRNA biogenesis, such as splicing, transport, translation, and mRNA degradation. For example, UCHL1-as, an antisense LncRNA that partially overlaps the 5 ‘end of the UCHL1 gene, promotes translation of the mRNA of the UCHL1 gene [18]. In addition, lncRNAs can act as “sponges” to prevent miRNAs from binding to their mRNA targets. CDR1-as /

ciRS-7 (sponge for miR-7), circular lncRNA expressed in humans, has 70 binding sites for miR-7 [19, 20]. In addition, some non-coding RNAs, called enhancer RNAs (eRNAs), are formed from distal cis-regulatory elements [12]. Currently, the role of these eRNAs in the transcriptional activity of the target gene has not yet been determined, since they can also be simply by-products of active regulatory elements. In this sense, it has recently been demonstrated that divergent transcribed lncRNA / mRNA pairs reflect a specialized transcriptional regulation mechanism involving bidirectional promoters.

lncRNAs IN TUMORS

lncRNAs can affect many aspects of tumor formation, such as stimulating cell division, eliminating effects that inhibit cell growth, inducing unlimited ability to replicate, stimulating invasion and metastasis, enhancing neovascularization and inhibiting apoptosis [8]. One of the earliest identified lncRNAs with biological significance in the tumor was H19. H19 is 2.3 kb lncRNA. This is a highly conserved imprinted gene that is expressed only in the maternal allele. This phenomenon, in which the alleles of the father and mother behave differently and only one allele is expressed, is called genomic imprinting. Another important gene involved in the H19 regulatory system is IGF-2, an insulin-like growth factor type 2, which is expressed only in the paternal allele. Increased expression of IGF-2 has been reported in several tumors, including adrenal cancer. In Beckwith-Wiedemann syndrome (hemihypertrophy, neonatal hypoglycemia, omphalocele, adrenal cancer, etc.), increased expression of IGF-2 is a fundamental indicator [21]. Increased expression of H19 is observed in cancer of the bladder, breast and hepatocellular carcinoma. It has been shown that the proto-oncogene c-myc stimulates H19 expression [22]. A close relationship between large and small non-coding RNAs is indicated by the fact that the first exon H19 encodes miRNA-675, which inhibits mRNA, the tumor suppressor Rb (retinoblastoma) [23, 24]. However, in many experiments, a decrease in H19 expression was associated with an increase in the tumor [6].

SRA (steroid receptor RNA activator), identified as a co-activator of steroid receptors (estrogen, progesterone, glucocorticoids and androgens), is also lncRNA. It has a transactivation effect through its AF-1 domain. Breast tumors have been shown to have increased SRA expression, which may play a role in tumor formation [25]. However, recent evidence suggests that SRA can not only function as lncRNA, but also can encode a transcriptional protein. The formation of non-coding and protein-coding RNA can be controlled by alternative splicing [26]. In the regulation of cell division in the aging process of cells, telomeres located at the end of chromosomes and the telomerase enzyme that regulates their length are of great importance. The telomerase enzyme complex itself also contains non-coding RNA called TERC (telomerase RNA component) and TERT (telomerase reverse transcriptase) activity. According to recent data, another lncRNA also plays a role in regulating telomerase activity called TERRA (telomeric repeat-containing RNA). Some evidence suggests that TERRA expression in tumor cells is reduced and that increasing TERRA expression may be a possible method of inhibiting tumor growth [27].

Among lncRNAs involved in metastatic processes, HOTAIR and MALAT1 should be distinguished. The expression of HOTAIR in primary and metastatic breast cancer is significantly increased [28]. Increased HOTAIR expression can be assessed as a prognostic signal associated with metastasis and decreased survival [6, 28]. PRC2, already mentioned in the XIST case, plays a role in its molecular mode of action. The PRC2 complex inhibits the transcription of a number of genes, including the fundamental processes of cell division and differentiation [29]. HOTAIR binding to PRC alters the inhibitory effect of PRC, restraining the inhibition of several genes' transcription. Increased expression of lncRNA MALAT1 (metastasis associated lung adenocarcinoma transcript 1) was observed in non-small cell lung cancer [30]. In addition to non-small cell lung cancer, increased expression was observed in cancer of the prostate, breast, liver and uterus. MALAT1 is important for the regulation of cancer cell invasiveness, but its exact mechanism of action is unknown. Some data suggest that mRNA maturation may play an important role in alternative splicing processes [31]. Like XIST and HOTAIR, ANRIL (antisense non-coding RNA in the INK4 locus) also binds to the PRC complex. Significance in the biology of tumors of the INK4b-ARF-INKa locus is indicated by its deletion in several tumors [32]. ANRIL inhibits the expression of several genes (including tumor suppressor genes p15 and p21) through complexes of PRC1 and PRC2 [33, 34].

MEG3 was the first lncRNA originally identified as a tumor suppressor. Like H19, MEG3 has only the maternal allele [35]. By analogy with H19, one can also interpret that MEG3 encodes miR-770, whose gene is located in the intron at the 3'-end of RNA [36]. MEG3 is most strongly expressed in pituitary and brain tissues, and its expression in pituitary adenomas is associated with increased methylation of the regulatory region of MEG3 expression [35]. Activation of p53 pathways is a priority in the suppressor effect of MEG3. P53 itself induces the expression of certain lncRNAs, among which the tumor suppressor lncRNA-p21 should be noted [37]. GAS5 (growth-arrest specifc 5) is another tumor suppressor. Like the previously mentioned SRA, GAS5 also plays a role in the regulation of glucocorticoid receptor activity, by inhibiting glucocorticoid activity [38, 39]. Increased expression of GAS5 leads to inhibition of cell proliferation in breast and prostate cancer cell lines [40]. Apparently, an extremely interesting molecular mechanism is that lncRNAs can bind complementary miRNAs and, thus, inhibit their action ("sponge" miRNA). MicroRNA binding is also tested for inhibition of the action of miRNAs with artificial nucleic acids, since several miRNAs can be inhibited simultaneously [41]. An example is lncRNA HULC (highly upregulated in liver cancer), which exhibits increased expression in hepatocellular carcinoma [42]. A mechanism similar to HULC-mediated miRNA inhibition has been described for tumor suppressor PTEN (phosphatase and tensin homolog). PTEN, a tumor suppressor gene, has the pseudogen PTENP1. PTENP1 and PTEN compete for binding of inhibitory miRNAs. Under normal conditions, lncRNA PTENP1 allows expression of PTEN by binding of miRNAs [43]. However, in tumors, somatic miRNA binding sites result in a loss of ability to bind PTENP1. Therefore, the expression of PTEN is reduced, which can lead to an increase in tumor growth [44]. lncRNA, called αHIF, plays a central role in the regulation of HIF1α (hypoxia-induced factor 1α) specifically in neovascularization processes. αHIF is an antisense lncRNA that is complementary to the 3 'untranslated portion of HIF1α mRNA. Increased expression of αHIF leads to inhibition of HIF1α and thereby to inhibition of angiogenesis [45]. The expression of αHIF has been described in many tissues and, interestingly, its expression in breast cancer is a poor prognostic factor [46].

THE MOLECULAR MECHANISM OF lncRNA IN TUMOR METASTASIS

The metastatic cascade is a coordinated sequence of cell-biological events that includes local cell invasion and allows cancer cells to exit the primary site, develop new blood vessels (angiogenesis), migrate and penetrate the microenvironment, perform intravation and extravasation, survive in circulation and colonize distant organs [47]. There is increasing evidence for the role of lncRNAs at every stage of metastasis. Let us examine the role of lncRNAs in cell invasion.

lncRNA IN CELL INVASION

In order to spread to distant organs, cancer cells must separate from the primary tumor using extracellular proteases to destroy the extracellular matrix (ECM) and invade the adjacent parenchyma. Then metastasis occurs when invasive cancer cells enter the blood and lymph vessels, pass through the bloodstream and enter the endothelium, eventually settling in a distant organ and creating a secondary tumor [48]. The epithelial-mesenchymal transition (EMF) is one of the central and important processes that allows epithelial cells to acquire migration ability and penetrate into tissues and organs [49, 50]. EMF is performed by activating a number of transcription factors (EMF-TF), mainly the ZEB, SNAIL, and TWIST families [51]. Many research groups have reported that lncRNAs are the main regulators of invasion. We summarized the most thoroughly studied lncRNAs involved in the regulation of EMF-TF to stimulate metastasis. Several lncRNAs, including lncRNA-ATB21 and HOTAIR, function as miRNAs to modulate ZEB and SNAIL levels in cancer [52]. Other lncRNAs are also involved in the epigenetic regulation of EMF-TF expression, such as TRERNA1 as an enhancer of the recruitment of SNAI1 and ZEB1-AS1p300 to the ZEB1 promoter [53, 54]. LncRNAs also function through RNA and protein interactions to regulate metastasis. For example, GAPLINC stimulates the expression of SNAI2 by binding to PSF and the NONO protein [55]. In addition to the regulation of SNAIL and ZEB, Hu et al. revealed more than 99 lncRNAs involved in EMF processes induced with TWIST [56]. Detailed mechanisms of how lncRNAs bind to TWIST / EMF signaling pathways have also been confirmed by other groups of scientists. TWIST binds to lncRNA HOTTIP, which recruits and directs WDR5 to the HOX cluster and induces HOXA9 expression [57]. High levels of HOXA9 correlate with an aggressive cellular phenotype in prostate cancer. It was shown that in addition to direct binding to TWIST, LncRNA CHRF regulates the TWIST / EMF signaling pathway, acting as miR-489 [58]. CHRF inhibits the expression of TWIST and further inhibits the progression of EMF in CRC [58].

DIAGNOSTIC AND THERAPEUTIC VALUE OF lncRNAs

The role of miRNAs in the diagnosis of tumors is confirmed by several experimental results. Their applicability is greatly enhanced due to their stability, so that they can be safely detected not only in frozen tissue samples, but also in body fluids and secretions. lncRNAs, despite their larger size, can also be found in body fluids, such as, for example, in blood samples of patients with hepatocellular carcinoma (HULC) [41]. The detection of PCA3 (prostate cancer gene 3) in urine has been described in some studies as a more sensitive biomarker compared to the prostate-specific antigen (PSA) [59]. Detection of some lncRNAs in tissue has a predictive value. For example, in hepatocellular carcinoma, increased MALAT1 expression is associated with poor prognosis and decreased survival after liver transplantation [60]. Although we are just beginning to learn the biology of lncRNAs, and there are many more questions that need to be clarified, it is possible that they may become a therapeutic target in the future, based on their significance in tumor biology.

CONCLUSION

Thus, acquaintance with long non-coding RNAs is a new chapter in research in the field of molecular biology of tumors, which contributes to a better understanding of tumor development processes. Clarification of the functions and mechanisms of these lncRNAs in biological systems under normal and pathological conditions may lead to potential opportunities for the identification of biomarkers and new therapeutic targets for tumors. To date, only a very small number of lncRNAs have been studied for their effect on the pathological process of neoplasms. Studies on lncRNAs require more sensitive detection methods than those on proteins and other RNAs due to lncRNAs lower expression. With an increased understanding of the role of lncRNAs in tumor biology, we can expect to identify new diagnostic biomarkers in the future. A clear understanding of how lncRNAs regulate many mechanisms in metastasis may lead to the emergence of new therapies for cancer patients. The lncRNA research area is expected to continue to improve in the near future.

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Chronic heart failure: syndrome or disease?

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ABSTRACT

The authors of the study analyze various definitions of chronic heart failure (CHF). CHF, having many faces, despite the consensus concerning the paradigm of its pathogenesis, is given different definitions, using both the syndromic and nosological approaches. Most authors share a view of CHF as the final stage (outcome or complication) of many diseases in which there is impairment of ventricular filling or ejection of blood, i.e. as a syndrome, and not an independent nosological form. Nevertheless, at the beginning of the XXI century leading Russian specialists in heart failure presented a reasoned point of view on CHF not only as the final stage of the cardiovascular continuum, complicating the course of a disease of the cardiovascular system, but also as an independent nosological form. This approach, which contradicts the standard rules for the formulation of the final clinical and pathological diagnoses, as well as the agreed positions of the International Statistical Classification of Diseases and Related Health Problems, has been the subject of reasonable criticism. Since the identification of the underlying cause of heart failure is crucial for therapeutic reasons, the only correct view is that of CHF as a syndrome, the detailed description of which in clinical diagnosis is an important intranosological characteristic that allows building the most effective differentiated therapy and accurately determining the prognosis of the disease.

Key words: chronic heart failure, definition, International Statistical Classification of Diseases and Related Health Problems, syndrome, disease, rules of diagnosis.

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Хроническая сердечная недостаточность: синдром или заболевание?

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РЕЗЮМЕ

В лекции проанализированы различные определения хронической сердечной недостаточности (ХСН). Многоликой ХСН, несмотря на консенсус, касающийся парадигмы ее патогенеза, дают различные определения, используя как синдромальный, так и нозологический подход. Большинство авторов объединяет взгляд на ХСН как на финальную стадию (исход, осложнение) многих болезней, при которых нарушается способность желудочка наполняться кровью или изгонять ее, т.е. как на синдром, а не на самостоятельную нозологическую форму. Тем не менее в начале XXI в. ведущие российские специалисты по сердечной недостаточности представили аргументированную точку зрения на ХСН не только как на конечный этап сердечно-сосудистого континуума, осложняющий течение того или иного заболевания кардиоваскулярной системы, но и как на самостоятельную нозологическую форму. Такой подход, противоречащий стандарту правил формулировки заключительного клинического и патологоанатомического диагнозов, а также согласованным позициям Международной статистической классификации болезней и проблем, связанных со здоровьем, стал предметом обоснованной критики. Так как идентификация причины, лежащей в основе сердечной недостаточности, принципиальна для выбора терапии, единственно правильным является взгляд на ХСН как на синдром, развернутая характеристика которого в клиническом диагнозе представляет собой важную интранозологическую характеристику, позволяющую построить наиболее эффективную дифференцированную терапию и точно определить прогноз заболевания.

Ключевые слова: хроническая сердечная недостаточность, дефиниция, Международная статистическая классификация болезней и проблем, связанных со здоровьем, синдром, болезнь, правила формулировки диагноза.

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

CHF, having many faces, despite the consensus concerning the paradigm of its pathogenesis, is given different definitions using both the syndromic and nosological approaches [1–6]. Most authors share a view of heart failure as the final stage (outcome, complication) of many diseases that affect the heart, i.e. as a syndrome, and not an independent nosological form. In particular, experts from the American College of Cardiology and the American Heart Association, in a lapidary and yet comprehensive definition that has not been revised since 2001, interpret CHF as a complex clinical syndrome that can be caused by any structural or functional heart disease that interferes with the ability of the ventricle to be filled with blood or expel it [7]. Similar definitions are given in the European, Canadian, British, Korean, Australian-New Zealand, Indian and other guidelines [8–13].

Nevertheless, at the beginning of the XXI century leading Russian experts on heart failure presented a reasoned point of view on heart failure not only as the final stage of the cardiovascular continuum, complicating the course of a particular cardiovascular system disease, but also as an independent nosological form: "... a disease with a complex of characteristic symptoms (shortness of breath, decreased physical activity, swelling, etc.), which are associated with inadequate perfusion of organs and tissues at rest or during exercise, and often with fluid retention in the body. The root cause is deterioration in the ability of the heart to fill or empty due to damage to the myocardium, as well as an imbalance of vasoconstrictor and vasodilating neurohumoral systems" [14, 15].

The formal basis for designating CHF as an independent nosological form is the existence of a corresponding heading ("Heart failure" – I50) in the International Statistical Classification of Diseases and Health Problems (10th revision, ICD-10); and informal basis is the idea shared by many cardiologists that CHF develops according to uniform laws, regardless of etiology. It is well known that at the late stage of any disease of the cardiovascular system, "remodeled heart syndrome" pushes into the background the importance of the etiological factor and can independently determine the quality of life and prognosis of the patient [16–18].

Recognizing the formal logic of the arguments presented, we allow ourselves to criticize the nosological interpretation of CHF. Firstly, the presence of the appropriate code in the ICD-10 does not necessarily justify the possibility of its use for encoding the underlying disease. For example, pulmonary embolism (I26) or acute renal failure (N17) is indicated in the diagnosis under the heading "complications of the underlying disease", since replacing the underlying disease with its complication or one of the manifestations is not allowed by the standard rules for formulating the final clinical and pathological diagnoses [19]. However, doctors who have been making diagnostic mistakes for years, ignoring key postulates of the rules for formulating final clinical and pathological diagnoses and calling this clinical experience, are not very rare [2]. Even if we displace from our consciousness the provisions of the rules for the formulation of the diagnosis, the clinician must understand that the diagnosis of "heart failure" (by the way, according to ICD-10, it excludes: conditions caused by arterial hypertension – I11.0, including with kidney damage - I13. -, the consequences of heart surgery or in the presence of a heart prosthesis – I97.1, as well as heart failure in a newborn – P29.0) may be at least some alternative to a detailed diagnosis, say, "ischemic cardiomyopathy" or "dilated cardiomyopathy", only when it is first formulated at the bedside "without past medical history" with symptoms and signs of heart failure, the corresponding stage III classification of N.D. Strazhesko and V.Kh. Vasilenko, i.e. in a situation where it is extremely difficult to make a reasonable conclusion about the nature of the disease and (or) this, unfortunately, is almost meaningless.

It is another thing when it comes to a patient with initial, yet reversible manifestations of heart failure. In this case, the development of an effective program of rehabilitation treatment is not conceived without an accurate and timely recognition of the etiological essence of the disease underlying heart decompensation. No one will doubt that the management of patients with common occlusive coronary atherosclerosis, mitral stenosis, hemochromatosis or amyloidosis in a cardiology clinic will differ significantly, even if the stage and functional class of heart failure are completely the same, since the best treatment is always etiotropic [2, 20].

Indeed, one of the main target issues of medical diagnosis is the rationale for treatment. Let us turn to the section "Diagnosis, diagnostics" of the Big Medical Encyclopedia [21], in which V.Kh. Vasilenko writes: "For the possibility of a real impact on the patient's condition, knowledge of not only the general nature of the process and its initial and final links, but also the entire chain of phenomena in their sequence and interdependence (pathogenesis) is necessary; only then does it become possible to break the chain in the most accessible and decisive link" [19]. Therefore, the clinical diagnosis should be not only pathogenetic (contain additional intranosological characteristics of the pathological process), but also nosological (meet the requirements of international classifications and the nomenclature of diseases, taking into account the peculiarities of domestic classifications).

Another objective of the diagnosis is a unified statistical study of morbidity and mortality. Let us return to the currently relevant revision of the International Statistical Classification of Diseases and Health Problems. For obvious reasons, the possibility of using the code I50.0 ("Congestive heart failure" – right ventricular failure secondary to left ventricular heart failure), which some experts usually insist on, is limited only to clinical cases with a far advanced stage (IIB–III) of CHF. Therefore, using this code, only part (and not the largest one) of patients with heart failure will be taken into account in statistical reports.

Be that as it may, the experts of the Society of Heart Failure Specialists, the Russian Cardiology Society and the Russian Scientific Medical Society of Therapists in the section "Determining Heart Failure" of the 4th revision of the National Guidelines for the Diagnosis and Treatment of CHF no longer consider the latter as a nosological unit, rightly indicating that "from a practical point of view, heart failure is a syndrome characterized by certain symptoms (shortness of breath, swelling of the ankles, fatigue) and clinical signs (swelling of the cervical veins, small bubbling rales in the lungs, displacement of the apical impulse to the left) resulting from a violation of structure or function of the heart "[22]. Howeve, apologists for the nosological approach to heart failure, while maintaining their beliefs in clinical practice, continue to formulate a diagnosis of heart failure without reference to its etiology.

Such rigidity of thinking requires educational effort. First of all, the fundamental difference between the nosological form and the syndrome regarding their nature should be recalled. In accordance with the industry standard "Terms and definitions of a standardization system in healthcare" (All-Union Standard No. 91500.01.0005-2001, enforced by order of the Ministry of Health of the Russian Federation No. 12 dated 01.22.2001), in the first case, albeit with certain reservations, about monocausal pathology: "... a combination of clinical, laboratory and instrumental diagnostic signs that make it possible to identify a disease... and attribute it to a group of conditions with a common etiology and pathogenesis, clinical manifestations, unified approaches to the treatment and correction of the condition". Whereas the syndrome is "a condition that develops as a consequence of a disease and is determined by a combination of clinical, laboratory, instrumental diagnostic features that allow it to be identified and assigned to a group of conditions with different etiologies, but common pathogenesis, clinical manifestations, general treatment approaches that also depend on the diseases underlying the syndrome."

Persistent in the nosological independence of CHF, they are under the illusion that the cause of CHF does not matter or the treatment is the same in all cases: whether it is coronary heart disease, heart disease or tachycardiomyopathy. At the same time, they forget that any structural or functional heart disease underlying CHF has its own specificity, which leaves an imprint on the course of heart failure and prognosis, and identification of the cause underlying CHF is crucial for the choice of therapy (for example, valvuloplasty or valve prosthetics for malformations, surgical or endovascular revascularization of the ischemic myocardium or rhythmic therapy for tachycardiomyopathy) [8, 23].

Those persistent in the nosological independence of CHF are under the illusion that the cause of CHF does not matter or the treatment is the same in all cases: whether it is coronary heart disease, heart defect or tachycardiomyopathy. At the same time, they forget that any structural or functional heart disease underlying CHF has its own specificity, which leaves an imprint on the course of heart failure and prognosis, and identification of the cause underlying CHF is crucial for the choice of treatment (for example, valvuloplasty or valve prosthetics for defects, surgical or endovascular revascularization of the ischemic myocardium or rhythm-reducing therapy for tachycardiomyopathy) [8, 23].

CONCLUSION

Thus, the only correct view on CHF is as a syndrome, a detailed description of which in the clinical diagnosis is an important intranosological characteristic that allows for the construction of the most effective differentiated therapy and accurately determining the prognosis of the disease [24].

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Virtual patients as a format for simulation learning in continuing medical education (review article)

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ABSTRACT

The use of virtual patients for students and for advanced training of medical doctors is a definite pedagogic innovation. The computer-based interactive multimedia simulations of scenarios for diagnosis and treatment allow for the avoidance of the risk of improper actions in regard to a real life patient, to repeat the clinical situations an unlimited number of times, and to standardize the tasks and criteria of their completion. Virtual patients represent a factual basis of problem-based learning. This review article focuses on the use of this educational technology for the development of medical decision making skills internationally, on its pedagogical effectiveness, and on the variants of the linear and branching scenarios. Meta-analyses demonstrate the pedagogical effectiveness of virtual patients and an interest of the trainees. An integration of the virtual patients into the learning contributes to clinical training gamification, which inspires the students and medical doctors to engage in interactivity and teamwork. The creation of a repository or a web-service of multimedia virtual patients in the tradition of national clinical school is of great current interest for implementation in the system of higher and continuing medical education.

Key words: case technologies, problem-based learning, gamification, standardization, multimedia, Web-service.

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Виртуальные пациенты как формат симуляционного обучения в непрерывном медицинском образовании (обзор литературы)

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РЕЗЮМЕ

Использование виртуальных пациентов для обучения студентов и повышения квалификации врачей в России является определенной педагогической инновацией. Компьютерные мультимедийные интерактивные симуляции сценариев диагностики и лечения позволяют избежать риска неправильных действий в отношении реального больного, повторять клинические ситуации неограниченное количество раз, стандартизовать задания и критерии оценки их выполнения. Виртуальные пациенты являются фактологической основой проблемно-ориентированного обучения.

В обзоре рассмотрены применение этой образовательной технологии для формирования навыков принятия врачебных решений за рубежом, ее педагогическая эффективность, варианты использования линейных и разветвленных сценариев. В метаанализах показана педагогическая эффективность виртуальных пациентов и заинтересованность обучающихся. Интеграция виртуальных пациентов в образовательный процесс способствует геймификации клинической подготовки, что стимулирует студентов и врачей к интерактивности и командной работе. Весьма актуально создание репозитория или Web-сервиса мультимедийных виртуальных пациентов в традиции отечественной клинической школы для использования в системе вузовского и непрерывного медицинского образования.

Ключевые слова: case-технологии, проблемноориентированное обучение, геймификация, стандартизация, мультимедиа, Web-сервис.

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

Источник финансирования. Автор выражает благодарность Российскому фонду фундаментальных исследований за финансовую поддержку работы в рамках выполнения гранта № 19-013-00231 А «Информационно-коммуникационные технологии формирования и совершенствования клинико-диагностических компетенций обучающихся в системе послевузовского медицинского образования».

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INTRODUCTION

One of the main problems of modern medical education is the difficulty of forming decision-making skills among specialists, both when performing manipulations and in clinical and diagnostic setting. In Russian higher medical education and abroad, various formats of simulation learning and virtual modeling are actively used. These educational technologies involve the use of various training devices and simulators that allow the student to play the role of a professional of the healthcare system and to develop manual skills and methods for making medical decisions in a safe situation.

The main incentive for the development of various simulators is to bridge the gap between students' theoretical knowledge and their clinical decisions without risking harm to real patients. The second reason for the introduction of these technologies is the need to standardize the assessment of clinical and diagnostic competencies of doctors and the possibility of repeating the clinical situation as many times as necessary, exploring various strategies and options for action [1–3]. One of the formats of simulation learning is the use of virtual patients (VP).

VIRTUAL PATIENTS IN HIGHER MEDICAL EDUCATION

In the scientific literature, one can find a different understanding of the term "virtual patient": computerized robotic simulators, standardized clinical cases performed by actors, and computer multimedia simulations of clinical situations.

Different medical proxy dummies have been used for a long time in medical education. For example, in 1968, the famous Harvey mannequin was designed to develop skills in diagnosing the state of the cardiovascular system. The model reproduced various types of respiration, pulse, blood pressure, murmurs and cardiac sounds corresponding to 25 diseases of the cardiovascular system. In the 1980s an android was created with an integrated model of physiological parameters of the cardiovascular system, controlled by one of the first personal computers with 35 scenarios of clinical situations [4]. Simulation learning centers are being intensively developed all over the world, equipped with mannequins of various levels of complexity, including an information model for the scenario of a clinical case and feedback from the student's actions [5]. The use of actors instead of patients was tested in 1963 as part of a training program for neurologists [4]. Currently, standardized patients are widely used in Europe and the USA to assess the clinical competencies of doctors, including as part of state licensing programs.

The scope of this review is, in a sense, the "polar" format of the simulation, used not for developing manual techniques, but for the formation of clinical thinking and medical decision-making skills. The results of the first attempts to use computer simulations for the training of doctors, nurses and students were published in the 60–70s of the 20th century [6–8]. Since then, these technologies have been applied in different fields of medicine and for different groups of students, but systematic use began in the USA and Western Europe only in the 1990s.

The term "virtual patient" has become widely used in foreign scientific publications after a series of works [2, 9–11]. The literature review [12] discusses the meaning of this term used in 536 articles: 37% of the articles dealt with interactive simulations of treatment and diagnostic process robots, 19% dealt with computer simulations of clinical situations, and 16% dealt with "standardized patients" performed by actors. Continuing this work, the authors analyzed 185 definitions of VP and built a conceptual map of this subject area [13].

In the future, by the term "virtual patient" we will understand the educational technology of the clinical areas of knowledge, namely, computer multimedia interactive simulations of scenarios for diagnosing and treating patients [10, 14].

The process of solving situational problems during the study of individual diseases has always been part of the training of future doctors. In an expanded form, they are cases that contain multimedia data from a patient examination and serve to analyze information when solving medical and diagnostic problems [15, 16]. The case method provides the strength and consistency of knowledge, a process approach to decision making. Important components of the case method are the assessment of the student's actions and the explanation of the mistakes made [17]. It is this approach that is more often mentioned in the domestic scientific literature than the virtual patient, as its factual basis. Educational technologies using clinical cases are considered in a number of domestic works both at the level of conceptual models and practical use [16, 18, 19].

A report by the European Regional Bureau of the World Health Organization (2016) states: "Schools... should make greater use of e-learning in medical education and continuing education for health professionals." Despite the rapid development of Russian e-health over the past 7–8 years, this process has weakly affected the educational medical space. Separate experiences of the development and use of electronic medical history for classroom work and self-preparation of students in clinical disciplines are described. An elective course "Electronic Medical Record" was created at Stavropol Medical University [20]. The concept of developing a training version of a medical information system and the experience of its application in the educational process are described in a number of works. Virtual modeling of professional tasks is used in dental education, teaching of general medicine, and training of surgeons [21–24]. For these purposes, both widespread software products [25] and specially designed software shells [26] can be used. Ways to integrate simulation technologies and techniques into medical education are considered in the article by K.A. Muravyova et al. (2011). It was concluded that the necessary educational and methodical work and experiments in teaching technologies are needed [27]. However, the vast majority of work on the creation of virtual patients and their use in medical education has been carried out abroad.

THE PEDAGOGICAL EFFECTIVENESS OF VIRTUAL PATIENT TECHNOLOGY

Implementing e-learning technologies in clinical medicine is more difficult than in the technical and scientific fields of knowledge, and the virtual patient is the main option for their practical implementation [28]. The use of VP has become one of the few digital technologies that have significantly changed the educational process. As the main component of lectures and seminars, VPs are the basis for training based on case study and problem-oriented learning and can partially replace traditional teaching methods in clinical disciplines [7, 10, 29, 31, 32].

VPs provide standardization, efficiency, interactive training, and links to evidence-based information sources, contribute to the study of rare cases of the disease, reduce the intensity of the teacher's work and increase student autonomy, and are ideal for developing clinical decision skills that are largely unformalized [14]. A number of reviews, including systematic ones, are devoted to the methodology and technology for developing VP [1, 33, 34].

In their foundational article, J. Bateman et al. (2013) try to answer the question of how the characteristics of VP affect the effectiveness of the formation of clinical competencies of students [35]. The authors developed a model of student interaction with the VP as an educational tool. The model includes three main aspects: clinical (content of the case), pedagogical (sequence and form of presentation of the content, a way to evaluate the student's interaction with the content), digital (the software used, user interface features). Educational experiments were conducted in different institutions; students had different clinical and educational experience and skills, different attitudes to e-learning. During the experiments, the impact of using VP on the students' competencies, their further preferences and motivations was evaluated.

The use of VP in medical education has a significant history: not only have recommendations been developed for their creation [9, 10], but specific proposals have been formulated for the development of clinical thinking skills based on the technology of VP [37]. Experienced educators, in accordance with the recommendations of the MedBiquitous consortium, created two cases of VP verified by six doctors. These cases included a history of life and disease, instrumental and laboratory studies, results of a physical examination of patients, a user-driven tree-like and linear trajectory of providing content, various types of questions, and probabilistic approaches to assessment. Additionally, a demonstration of examples, a list of differential diagnoses, and a help system with the search for pros and cons, and other reference resources were used. Both cases after working with them in an hour-long discussion were evaluated by 46 students of the second and fourth years of study. Students were informed about the experiment, participated voluntarily, without payment; the assessment of VP was carried out according to the recommendations [38]. Compared to traditional methods of presenting cases with paper case histories, virtual patients stimulated interaction between students and their active involvement in the educational process. When using VP, students identify well the key characteristics of the problem; questions for the Bayesian approach to solutions are useful for them. A branching path makes the VP more realistic, but complicates the task and increases the error rate. The solving process is also made more difficult by the interface with scrolling, the lack of audio, video files and feedback from the VP.

A systematic review and meta-analysis of D. Cook et al. (2010) show that the use of virtual patients has a significant educational effect [8]. Comparing the different properties of VP, the authors come to the conclusion about the educational effectiveness of a structured menu system and an interface in a native language for learning; the connection of the text or voice form of presentation of information with the success of training is not so obvious. Thoughtful feedback from the student is undoubtedly important for positive effect, as well as repeated solutions on one VP, although the latter option can cause a negative reaction of students. The long time provided for completing the task using the VP generally reduces the proportion of correct decisions, but this depends on the complexity of the case and the status of the student.

A meta-analysis of 12 randomized controlled trials showed a pronounced positive effect of using VP in learning [39]. The advantages of using the VP in the educational process are not in doubt [40], however, specific methods of integrating the VP with traditional curricula require further research. It is also recommended that fundamental biomedical knowledge be integrated with clinical descriptions of cases of the VP [41].

At the University of St. George (London) a problem-oriented training for medical students is implemented. To use this educational technology on the basis of a "paper" description of completed cases of the disease, conceptual maps of each case were developed, and then virtual interactive patients for on-line work were created in the OpenLabyrinth software application [2]. Ten groups of students worked with five virtual cases in a linear and branched scenario. Both students and teachers note the prospects of this approach, as well as the need for parallel full-time clinical classes.

A study of family medicine knowledge in a group of students who studied in the traditional way (48 people) and using virtual patients (51 people) showed no differences [42]. The authors conclude that the new approach is highly effective, taking into account the absence of risk for real patients. Advice on how to develop the professional competencies of doctors using VP is given by S. Murphy et al. (2016) [43]. Among them are the use of professional nuances in the VP scenario (informed consent, confidentiality, etc.), the need for teamwork predefined by the script, debriefing of the cases worked out and a collective analysis of making stage decisions, taking into account the educational level of students.

VP testing at the University of Bogotб was carried out with the participation of 216 students who noted the positive and systemic impact of VP technology on the individual learning process and the correspondence of the socio-cultural context of clinical practice cases [44]. Testing of acquired skills, from the students' point of view, was qualitatively different from the usual exam and strengthened the motivation for learning. Students consider virtual patients an important educational tool, especially in the field of development of medical logic and decision making.

N. Berman et al. (2009) for 2 years studied the effect of various methods of integrating VP into the clinical disciplines program on the perception and satisfaction of 545 students [32]. The questionnaire was validated; the results were processed by factor analysis. Students rated the effectiveness of VP higher than traditional methods. The integration of VP into the learning process directly affects its effectiveness and student satisfaction, can be combined with the elimination of some traditional educational approaches. However, from the students' point of view, the opposition of e-learning to traditional learning is not constructive; these approaches should complement each other.

A group of students who used typical cases of VP in the study of rheumatology to substantiate medical decisions was studied. Students perceived VP in connection with real patients and the clinical context of training, evaluated them as an integration of biomedical knowledge and clinical experience, as an aid in structuring the available clinical diagnostic information. This integration provided the basis for decision-making in a loosely structured clinical environment in the absence of stress of real actions. Along with this, students lacked emotional interaction with patients and the complexity of real work. The effectiveness of the integration of complex cases of VP into medical education depends both on their technological features and on the clinical situation [45, 46]. Among the adverse effects of the use of VP, it is also worth mentioning the limited control over the distribution of depersonalized clinical diagnostic information, the difficulties of editing the VP, the insufficient validity and reliability of the content [13]. Taking into account the very different implementations of VP, their use does not allow us to evaluate the learner's cognitive skills, as well as to diversify patients according to ethnic and socio-cultural characteristics [47]. Lack of emotional contact and feedback with the virtual patient makes learning difficult [35, 45].

VPs are used to form not only the clinical thinking of students, but also effective laboratory diagnostics [48]. LabCAPS software was used to create eight virtual patients, among which were implemented similar in characteristics, but contrasting in required solutions, which is extremely useful for the formation of recognition of stable clinical and laboratory patterns in students. Students praised the logic of the VP interface, its learning effect, and differentiated cases with confidence.

If the twentieth century was dominated by content learning models, now they are giving way to models of different types of activity of students and teachers. The use of VP implies the active work of the student, which is expected to lead to greater learning efficiency [49]. A number of activities are built into the VP during development (methods for students to obtain information and ensure interactivity); others are associated with the use of VP (work in groups, independent preparation of students, the context of achieving different educational goals), or with the inclusion of VP in the curriculum of the discipline [7]. Evaluation of the role and pedagogical effectiveness of VP technology depends on what activities we are considering. Therefore, the literature discusses not only VP technology, but the roles that these computer simulations can play in the medical education of the future [50].

DIVERSIFICATION OF VIRTUAL PATIENTS

VP scenarios can be divided into those predetermined by a specific completed case and aimed at solving a medical problem as a whole, without a clearly defined sequence of actions [51]. The type of scenario, the amount of data and the form of their presentation, the availability of evidence sources, and the potential for further use of the acquired competencies in the clinic influence the process and the result of learning using VPs. There are different versions of VP models: static and dynamic, linear and branched, with interactivity and without it. Most VP now implements a linear scenario, with the ability for the student to answer questions and make certain decisions [1]. To improve communication skills with patients, static VPs are more often used, while dynamic clinical decision-making skills are used [40]; branched VP scenarios suggest a high level of interactivity with the student [13]. Creation of a computer database of completed cases of the disease for demonstration to students is equivalent to static VP without pronounced interactivity; creation of a computer database of multimedia clinical diagnostic tasks is equivalent to dynamic interactive VPs.

In a number of works by M. Toro-Troconis et al. the process of using VP as a game format for medical education has been studied in detail. From the authors' points of view, the linear VP scenario is more suitable for primary students, and branched scenarios better develop the cognitive skills of future doctors in senior courses [52, 53]. The sex of the student does not affect their attitude to the VP, although this issue needs to be studied in more detail on a large sample of junior students. Students are advised to spend sufficient time daily in this "virtual world", and they are willing to complete tasks, provided that there is time for clinical preparation with real patients.

At the same time, the use of VP in the pedagogical process requires serious methodological support and guidance on the game approach to medical education. The introduction of this format will allow supporting and developing the joint work of various specialists within the framework of one team, including, for example, pharmacists, physiotherapists, and nurses. VPs, as a digital gamified representation of real clinical cases, have a high educational potential for a large number of students, the gain from which significantly exceeds the costs of their development [53].

It was assumed that the relevance of the language in which the virtual patient is presented is of great importance. However, the staff of the medical faculty of the Romanian University did not find statistically significant differences in the students' ability to make a correct diagnosis and make a therapeutic treatment plan for four identical VPs in Romanian (136 students) and in English (144 students). The inclusion of VP in the program of the Romanian medical university in English the authors consider an acceptable and economically viable option for the globalization of medical education [54].

Although the creation of virtual patients requires significant time and financial resources, this technology in terms of cost-effectiveness and educational effectiveness has significant advantages relative to standardized patients and other simulation techniques. The possibilities of the widespread use of VP in the global network, the use in distance and continuing medical education make this approach unique [13, 14].

VIRTUAL PATIENT REPOSITORIES

At the end of the twentieth century virtual patients were included in the USMLE exam program and are used in the educational process in all developed European countries. The effectiveness of VP in medical education was highly appreciated not only by foreign, but also by domestic scientists and teachers [55]. Despite promising characteristics, VPs are slowly being introduced into the curricula of medical universities, although the optimism of teachers and administration remains. The cost of development (the preparation of one case of VP is estimated at 10-50 thousand dollars) is one of the limiting factors; however, the cost of organizing training centers equipped with robotic mannequins is several orders of magnitude higher [1].

To expand the use of virtual patient technology in medical education, it is natural to create VP repositories [44]. The Aquifer Consortium brings together manufacturers of virtual patients; teachers and students using this technology [56]. The users of Aquifer virtual training courses are most US medical schools and international medical programs. The base of VP of this organization has a multi-parameter search system for virtual patients, depending on the disease, the field of health, and educational tasks. In the 2017–2018 school year, more than 67,000 students accessed the Aquifer VP base about 1.4 million times. The systematic work on introducing VP into practice, including the preparation of a standard for the development and use of VP for educational purposes, is carried out by the Association of American Medical Colleges [57].

The created VP resources are actively used in foreign educational institutions. In particular, the European Commission supported the eViP project aimed at creating a database of virtual patients and clinical diagnostic situational tasks [58]. Universities and medical faculties of Great Britain, Sweden, Germany, Holland, and Romania participate in this project. As part of the project, ClujNapoca University of Medicine and Pharmacy (Romania) and Karolinska University (Sweden) developed computer applications for creating VP in various clinical areas [44, 59]. The basis was the software tool used since 2005 at the University of Bogotб (Spain) for the use of VP in medical education [60]. This Web application allowsthe creation of linear interactive scripts based on real, completed cases.

In 2017, the American Medical Association considered the use of virtual electronic health records (EHR) as a platform for clinical training in medical schools [61]. EHR is used in 90% of medical practices, but students and young doctors are little trained in this technology. The Regenstrief Institute is developing an EHR platform for clinical training. The virtual patient database of this institute includes more than 10,000 real depersonalized cases of the disease and is presented in its own information system [62]. This base is used to develop clinical thinking and decision-making skills in students of more than 30 educational medical institutions in the United States.

CONCLUSION

Currently, a significant positive effect of the technology of virtual patients in medical education and advanced training of doctors has been proved, their economic efficiency has been demonstrated in comparison with simulation centers of robotic mannequins and the use of standardized patients, the possibilities of remote use of VPs for the formation and improvement of competencies in clinical diagnostic decisions are obvious. It is time to create a repository or Web service for multimedia VPs within the framework of domestic clinical schools for use in the system of university and continuing medical education.

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Association between cholangiocarcinoma and liver flukes: review of epidemiological studies

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ABSTRACT

According to official medical statistics, liver fluke infections caused by Opisthorchis felineus, *Opisthorchis viverrini* and *Clonorchis sinensis*, are reported annually in the Southeast Asia, European countries and the Russian Federation. These infections are the main cause of digestive system diseases in the population of endemic regions. The aim of the review is to analyze the findings of epidemiological studies and to assess the relationship between liver and bile duct cancer and *Opisthorchiidae* liver fluke infections.

Materials and methods. The authors reviewed original studies published in 1974–2019 via the MEDLINE databases and the eLIBRARY scientific digital library.

Results. The studies have shown that cholangiocarcinoma is a significant medical and social problem in the trematode-endemic areas of Southeast Asia due to the absence of specific symptoms, long asymptomatic course, resistance to therapy and high mortality of patients. Long-term infection caused by trematodes *Opisthorchis viverrini* and *Clonorchis sinensis* is associated with a significant risk of developing cholangiocellular cancer. An epidemiological multicenter study is required to establish the relationship between the *Opisthorchis felineus* infection and cholangiocarcinoma in the population of endemic regions in the Russian Federation.

Key words: cholangiocarcinoma, *Opisthorchis viverrini, Clonorchis sinensis, Opisthorchis felineus,* liver cancer, review, epidemiological study.

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Анализ эпидемиологических исследований взаимосвязи холангиокарциномы и печеночных трематодозов

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РЕЗЮМЕ

Согласно официальной медицинской статистике, случаи инвазии, вызываемые печеночными трематодами Opisthorchis felineus, *Opisthorchis viverrini* и *Clonorchis sinensis,* ежегодно регистрируются как в регионах Юго-Восточной Азии, так и в европейских странах, в Российской Федерации, являясь причиной заболеваний органов пищеварительной системы у населения эндемичных регионов. Цель обзора – анализ результатов эпидемиологических исследований, посвященных оценке взаимосвязи злокачественных новообразований гепатобилиарной системы и печеночных трематодозов, вызванных гельминтами семейства *Opisthorchiidae*.

Изучены оригинальные исследования, опубликованные за период 1974– 2019 гг. и размещенные в базах данных MEDLINE и научной электронной библиотеки eLIBRARY. Проведенные исследования свидетельствуют, что холангиокарцинома является значимой медико-социальной проблемой в эндемичных по трематодозам регионах Юго-Восточной Азии ввиду отсутствия специфических симптомов, длительного бессимптомного течения, резистентности к терапии и высокой смертности пациентов. Длительная персистенция в организме печеночных трематод *Opisthorchis viverrini* и *Clonorchis sinensis* ассоциирована со значительным риском развития холангиоцеллюлярного рака. Требуется проведение эпидемиологического многоцентрового исследования для установления взаимосвязи инвазии *Opisthorchis felineus* и холангиокарциномы у населения эндемичных регионов Российской Федерации.

Ключевые слова: холангиокарцинома, *Opisthorchis viverrini, Clonorchis sinensis, Opisthorchis felineus,* рак печени, обзор, эпидемиологическое исследование.

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

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INTRODUCTION

At present, helminthiases are spread worldwide, causing significant medical and social damage [1]. According to official medical statistics, cases of infection caused by liver flukes *Opisthorchis felineus (O. felineus), Opisthorchis viverrini (O. viverrini)* and *Clonorchis sinensis (C. sinensis)* are reported annually in the hyperendemic regions of Southeast Asia (Thailand, Vietnam, China, Korea), and in European countries (Greece, Italy, Spain, Portugal, Germany, Poland), being the cause of diseases of the digestive system in 40 million people [2–5]. Moreover, more than 600 million people are at risk of infection each year [6–8].

Based on studies conducted in Southeast Asia, the International Agency for Research on Cancer (IARC) has included hepatic trematodes *O. viverrini* and *C. sinensis* in the registry of biological carcinogens. The mentioned studies have shown a relationship between *O. viverrini-* and *C. sinensis-caused infection and cholangiocarcinoma* (CCA) [9–11].

CCA is one of the most unfavourable diseases in relation to the prognosis of malignancies [12]. The high incidence of bile duct cancer is observed in Northeast Thailand, as well as in China and South Korea [13–15].

In the Russian Federation, the study of the liver fluke infections caused by *O. felineus* and associated chronic diseases remains relevant. Over the past 20 years, there has been a significant increase in the incidence of opisthorchiasis in the endemic regions of Western Siberia and spread of invasion in several other regions due to increased population migration [16, 17].

The aim of this review was to analyze the results of epidemiological studies on the relationship between malignancies of the hepatobiliary system and hepatic trematodoses.

DATA SOURCES

The authors analyzed epidemiological studies on the relationship of malignant neoplasms of the hepatobiliary system and hepatic trematodoses caused by *Opisthorchiidae* family worms *(C. sinensis, O. viverrini and O. felineus)* from publications in the MEDLINE databases via the PubMed electronic search engine (https://www.ncbi.nlm.nih.gov/pubmed/) and the scientific electronic library eLIBRARY (https://elibrary.ru/). The review used original articles published over the period from January 1, 1974 to December 31, 2019, grouped according to the following algorithm.

Keyword preliminary search. To search for foreign publications in the PubMed database, the keywords "epidemiological study, *Opisthorchis viverini* infection, *Clonorchis sinensis infection, Opisthorchis felineus* infection, cholangiocarcinoma, liver cancer, malignant diseases" were used. In the eLIBRARY electronic library, the same following terms in Russian were studied. Also, search for studies corresponding to the above-mentioned terms was carried out among the references in the selected publications. In total, 889 publications from PubMed and 4 papers from the scientific electronic library eLIBRARY were studied.

Abstract analysis (summary) of the selected publications. This stage excluded experimental studies, review publications that are not epidemiological studies, as well as articles lacking required data in the text of the abstract. All in all, 374 publications were analyzed, of which 48 were selected.

Analysis of full-text publications. The publications studied had to have the following features as a selection criteria: original epidemiological study, completeness of the description of the study design, including the size and characteristics of the sample, selection criteria; open access to the full text of the article; compliance with the criteria for the diagnosis of "cholangiocarcinoma" (histological examination of the tumor); and description of the diagnostic method for verification of infection. The exclusion criteria were the absence of the required data in the text of the article (sample size, description of the diagnostic method for CCA and opisthorchiasis).

As a result, 14 full-text publications met the inclusion criteria and were included in this review.

EPIDEMIOLOGICAL CHARACTERISTICS OF CHOLANGIOCARCINOMA

CCA is a heterogeneous group of liver tumors characterized by damage to the intra- and / or extrahepatic bile ducts, high mortality due to its aggressiveness, the absence of specific symptoms and / or prolonged asymptomatic course, and resistance to therapy [12, 13, 18]. CCA is the second most common type of liver tumor and the most common cause of death from cancer. It also accounts for about 3% of all neoplasms of the digestive system. The incidence increases with age; with women developing CCA more often than men [19, 20].

According to the anatomical classification, CCA is divided into intrahepatic, perihilar and distal. It is believed that intrahepatic CCA accounts for about 10% of cases of primary liver cancer, of which 50% are Klatskin tumors, 40% of them are distally localized tumors, and intrahepatic lesions occur in 10% of cases. CCA has a poor prognosis, with the median survival of 24 months. The only treatment methods are first-line (Gemcitabine, Cisplatin) and second-line (Oxaliplatin, Fluorouracil according to the FOLFOX scheme) chemotherapy, targeted therapy with pembrolizumab in the presence of MSI-H / dMMR and / or surgical intervention in the early stages of the disease [21–23].

The incidence of CCA in the world is about 5.9 cases per 100 thousand people annually. The highest incidence is recorded in Northern Thailand (more than 80 cases per 100 thousand population), China (more than 7.5 cases per 100 thousand population) and South Korea (more than 8 cases per 100 thousand people). On the contrary, the incidence rates of CCA in the European countries or Americas do not exceed 0.7-3.36 and 0.3-1.67 per 100 thousand, respectively [24].

The average incidence of CCA in the Russian Federation is about 4.8 per 100 thousand annually. According to the official medical statistics for 2011-2013, in the Russian Federation, the highest incidence of CCA was noted in the Republic of Sakha and Tomsk Region (14.5 and 9.3 per 100 thousand population, respectively) [17, 25].

It is important to note that in Europe, America and Africa, hepatocellular carcinoma dominates in the histological profile of liver cancers, while in regions endemic for liver flukes, cholangiocellular cancer predominates (more than 80 cases per 100 thousand in Northern Thailand) [26]. The highest incidence of hepatocarcinoma (more than 20 per 100 thousand) is recorded in China, Mongolia, Southeast Asia, as well as in countries of West and East Africa, located south of the Sahara Desert [12, 26].

CCA refers to multifactorial diseases, the development of which involves many risk factors, including genetic, infectious, environmental, and epidemiological ones. The significant risk factors include primary sclerosing cholangitis / ulcerative colitis, chronic viral hepatitis C and B, long persistence of the Epstein-Barr virus, non-alcoholic fatty liver disease, cholelithiasis and / or malformation of the biliary system, and deposition of radiopaque substances (Thorotrast) in the bile ducts [27, 28]. There is experimental evidence that N-dinitrosodimethylamine can serve as an inducer of carcinogenesis in the bile ducts [29, 30].

One of the most important and significant risk factors for CCA is prolonged persistence in the body of liver flukes *O. viverrini* and *C. sinensis,* included in the register of Group 1 biological carcinogens with proven oncogenicity for humans based on the results of studies conducted in Southeast Asia [11]. According to the 2019 report of IARC, *O. felineus* is still a biological agent with unproven carcinogenicity in humans (Group 3) due to the insufficient number of meaningful epidemiological multicenter studies [31–34].

ASSOCIATION BETWEEN CHOLANGIOCARCINOMA AND *O. VIVERRINI* INFECTION

We reviewed eight full-text articles on epidemiological relationship between CCA and *O. viverrini* infection (table).

O. viverrini infection is a significant public health problem in the Mekong River Basin countries of Southeast Asia such as Thailand, the Lao People's Democratic Republic, Vietnam and Cambodia [35–37]. According to official medical statistics, Thailand (north-eastern province) has the highest prevalence of *O. viverrini* infection, where at least 6 million people are infected by opisthorchiasis. It also has the highest rates of bile duct cancer and cholangiocarcinoma (more than 90 cases per 100,000 men and 38.3 cases per 100,000 women) [14, 38–40].

The first significant study conducted in Northeast Thailand (table 1) showed that patients with *O. viverrini* (stool and/or bile microscopy) had significantly higher frequency of CCA diagnosis than non-infected individuals [41].

The case-control study conducted in 1987–1988 found a statistically significant association between CCA and the presence of antibodies to *O. viverrini* in serum. The results showed that men and regular users of betel nut (mostly women) had higher risk of CCA development. A possible mechanism is increased exposure to nitrosamines [42].

In the case-control study conducted in 1990–1991 in 85 villages of Northeast Thailand, 12,311 people aged 24 years and over were screened for *O. viverrini* (microscopy of stool). Ultrasonography of the hepatobiliary system was performed in individuals with different intensity of invasion, 15 patients were diagnosed with CCA. The highest prevalence of CCA was found in the group with the highest intensity of *O. viverrini* infection [43].

The case-control population study conducted in 1999–2001 showed that increasing antibodies to

O. viverrini was the most significant risk factor for CCA development. Eating fermented fish, alcohol consumption and smoking increased the risk of CCA. The role of alcohol may be explained by its influence on the metabolic pathways of endogenous and exogenous nitrosamines [44].

The case-control study conducted as part of a cohort study in 1990–2001 showed significant relationship between development of CCA and detection of *O. viverrini* eggs in stool samples. Also the study found that individuals who consumed fruits and vegetables 3–4.6 times a day had significantly lower risk of CCA. Nevertheless, eating meat more often than 0.45 times per day significantly increased the risk of CCA [45].

The case-control study conducted in 1999–2001 showed that risk of CCA in patients with high levels of antibodies to *O. viverrini* and chronic viral hepatitis B and/or C was significantly higher [46].

The case-control study conducted in 2011 established that consumption of alcohol, raw freshwater fish and beef sausages increased the risk of CCA, while fruits and/or vegetables consumption reduced this risk. A relationship between a decrease in the MTHFR gene expression and consumption of raw freshwater fish infected with *O. viverrini* and meat was identified. These dietary items are source of nitrosamines, folates and antioxidants that may cause carcinogenesis along with *O. viverrini* infection [47].

In the case-control study conducted in 2009–2012, the data on increased risk of CCA in patients who had family history of cancer and increased level of immunoglobulin G to *O. viverrini* were obtained again. The consumption of alcohol more than three times a week and uncooked meat (beef, pork) also increased risk of CCA [48].

It is important to note that according to the U.S. government data, more than 700 Vietnamese war veterans were diagnosed with CCA over the past 15 years. In analysis of 50 serum samples, antibodies to *O. viverini* were found in 20% of them. Infection may be linked with consumption of heat- untreated river fish during a stay in the endemic region of South-East Asia. The long-term asymptomatic course of liver fluke infection resulted in development of bile duct cancer diagnosed at the last stage of the disease [49, 50].

Association between cholangiocarcinoma and *C. sinensis* infection

We reviewed six full-text publications on epidemiological relationship between CCA and *C. sinensis* infection (table).

According to the medical statistics, infection caused by *C. sinensis* is the most common in Asia, especially in China, Taiwan, Korea, Japan and Vietnam: more than 15 million people are infected, and 200 million are at constant risk of infection [51]. China has the highest incidence of clonorchiasis with more than 13 million people, accounting for 85% of the total number of cases [52, 53].

The first meaningful epidemiological studies carried out in South Korea (table 1) showed an increased risk of CCA in patients with positive status of *C. sinensis* infection [54, 55].

The case-control study carried out in 1990–1993 showed that the detection of *C. sinensis* eggs in samples of stool and alcohol overuse were the most significant risk factors for CCA [56].

According to the case-control study carried out in 2000-2004 in Seoul, *C. sinensis* infection was significantly related to intrahepatic CCA. The important risk factors for CCA also include chronic viral hepatitis B, liver cirrhosis, alcohol consumption, diabetes mellitus, and choledochal cysts [57].

The case-control study conducted in 2003–2004 in the same region found that eating raw freshwater fish and positive antibodies to *C. sinensis* in the serum were associated with an increased risk of CCA. Significant risk factors for distal CCA were the radiological signs of *C. sinensis* and history of raw freshwater fish consumption [58].

In the case-control study conducted in 2011 in China, association between intrahepatic CCA and *C. sinensis* infection as well as cholelitiasis, chronic viral hepatitis B and liver cirrhosis was detected [15].

ASSOCIATION BETWEEN CHOLANGIOCARCINOMA AND *O. FELINEUS* INFECTION

At present, there is deficiency of significant epidemiological multicenter studies in the Russian Federation to establish the relationship between CCA and *O. felineus* infection. According to the pilot analysis of official medical statistics data, the incidence of liver and bile duct cancer in Russia is about 4.8 cases per 100,000 population annually. Our pilot analytical study of official medical statistics data in all regions of the Russian Federation in 2011–2013 showed a significant relationship between the incidence of *O. felineus* infection and malignancies of the hepatobiliary system. Thus, the incidence of hepatobiliary malignancies is significantly higher in regions with high level of opistorchiasis among the population (≥ 50 cases per 100,000) [17].

At present, the results of some domestic studies based on autopsy materials and surgical protocols demonstrating carcinogenic potential of *O. felineus* have been published.

This way, among patients that underwent surgery at the Tomsk Hepatology Surgery Centre from 1980 to 2000, 13% (n =152) of 1170 patients with *O. felineus* infection suffered from hepatic, pancreatic and duodenal cancer: liver or bile ducts – in 61 cases, pancreas – in 66 cases, gallbladder – in 15 cases and major duodenal papilla – in 10 patients [34].

In the analysis of autopsy materials of 44 individuals who died of liver cancer, CCA was established in 80% and hepatocellular carcinoma in 20% of cases [59].

In 2019, two cases of CCA in patients with *Opisthorchis felineus* infection have been published. Patients lived in the endemic rural area, practiced fishing and undercooked cyprinoid fish consumption. They had been infected by liver fluke *Opisthorchis felineus* for a long time and did not receive antihelminthic treatment. Both cases presented unspecific symptoms at the onset of the disease at the stage when already severe pathological changes had occurred, and patients died of multiorgan dysfunction syndrome during six months after CCA diagnosis verification [60].

CONCLUSION

Liver trematodes *Opisthorchis viverrini* and *Clonorchis sinensis* are significant biological carcinogens associated with a significant risk of developing cholangiocellular cancer. *O. felineus* is still a biological agent with unproven carcinogenicity in humans (Group 3) due to insufficient epidemiological data. However, current experimental and clinical data indicate significant carcinogenic potential of *O. felineus*. An epidemiological multicenter study is required to establish the relationship between the *Opisthorchis felineus* infection and cholangiocarcinoma in the population of endemic regions of the Russian Federation. The study results are necessary for elaborating guidelines on screening programs for early CCA diagnosis as well as for preventing and treating hepatobiliary cancer, which is socially important in endemic regions for opisthorchiasis in the Russian Federation.

Table

Epidemiological studies assessing the risk of cholangiocarcinoma development in *O. viverrini* and *C. sinensis* infection

Author, year	Country	Study design	Study sample (n)	Risk of CCA development
Study of *O. viverrini* infection				
Kurathong et al., 1985 [41]	Thailand	Case-control	551	OR = 1.21 95% CI: 0.36–4.06
Parkin et al., 1991 [42]	Thailand	Case-control	206	OR = 5.0
Haswell-Elkins et al., 1994 [43]	Thailand	Cross-sectional	12 311	OR =14.1
Honjo et al., 2005 [44]	Thailand	Case-control	258	OR = 27.09
Poomphakwaen et al., 2009 [45]	Thailand	Case-control	216	OR = 2.99 95% CI: 1.04–8.62
Srivatanakul et al., 2010 [46]	Thailand	Case-control	212	OR = 25.04 95% CI: 5.81–07.91
Songserm et al., 2011 [47]	Thailand	Case-control	657	OR = 2.0 95% CI: 1.14–3.48
Manwong et al., 2013 [48]	Thailand	Case-control	146	OR = 3.09 95% CI: 1.04–9.16
Study of *O. sinensis* infection				
Kim Y. et al., 1974 [54]	Korea	Cross-sectional	1402	OR = 6.5 95% CI: 3.5–112.04
Chung C. et al., 1976 [55]	Korea	Case-control	595	OR = 6.0 95% CI: 2.82–12.04
Shin H.R. et al., 1996 [56]	Korea	Case-control	609	OR = 2.7 95% CI: 1.13–6.46
Lee T. et al., 2008 [57]	Korea	Case-control	2488	OR = 13.6 95% CI: 6.1–30.31

End of table

Author, year	Country	Study design	Study sample (n)	Risk of CCA development
Choi D., Lim J.H., Lee K.T. et al., 2006 [58]	Korea	Case-control	244	OR = 8.62 95% CI: 5.05–16.06
Peng N.F., Li L.Q., Qin X. et al., 2011 [15]	Korea	Case-control	294	OR = 3,55 95% CI: 1.6–7.89

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MicroRNAs and small interfering RNAs as tools for the directed regulation of cellular processes for cancer therapy

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ABSTRACT

MicroRNAs and small interfering RNAs (siRNAs) belong to an extensive class of small non-coding RNAs and play an important role in gene expression regulation in cells. It is shown that changes in the amount or activity of these molecules may lead to the development of various diseases, including cancer. This made it possible to consider them as promising diagnostic and prognostic markers, as well as tools for the directed regulation of protein synthesis in the cell and targets for therapy. This review summarizes the basic knowledge about the biogenesis, distribution and the mechanisms of action of microRNA and siRNA, as well as currently used ways of target genes expression management with their help. Possible methods of these molecules delivery into the cell *in vitro* and *in vivo* are considered.

Key words: small non-coding RNA, gene expression regulation, target therapy, cancer.

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МикроРНК и малые интерферирующие РНК как инструменты направленной регуляции клеточных процессов для терапии онкологических заболеваний

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РЕЗЮМЕ

МикроРНК и малые интерферирующие РНК (миРНК) относятся к обширному классу малых некодирующих РНК и играют важную роль в регуляции экспрессии генов в клетках. Показано, что изменения в количестве или эффективности воздействия этих молекул могут сопровождать развитие различных заболеваний, включая онкологические. Это позволило рассматривать их как перспективные диагностические и прогностические маркеры, а также инструменты для направленной регуляции синтеза белков в клетке и мишени для терапии. В данном обзоре суммированы основные знания о биогенезе, распространении и механизмах воздействия микроРНК и миРНК, а также способы направленного влияния на экспрессию генов с их помощью, используемые в настоящее время. Рассмотрены возможные варианты доставки молекул в клетку *in vitro* и *in vivo*.

Ключевые слова: малые некодирующие РНК, регуляция экспрессии генов, направленная терапия, онкологические заболевания.

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INTRODUCTION

MicroRNAs and small interfering RNAs (siRNAs) belong to the large and heterogeneous class of small non-coding RNAs, an important function of which is the regulation of gene expression in the cell. The history of the study of non-coding RNA began in 1993 with the publication by Victor Ambros and colleagues of data on the discovery of short RNA molecules that affect the translation of the lin-14 protein of the nematode Caenorhabditis elegans [1]. Since then, studies of small non-coding RNAs have been very intensive. But it was microRNAs and siRNAs that attracted the most attention and were studied more thoroughly. The accumulated knowledge allows the use of these molecules to affect living cells and the directed regulation of cellular processes. They have been used both in research projects and in the development of drugs in practical medicine. This is especially true in the treatment of malignant neoplasms, where the transformation of a cell into a tumor is accompanied by a significant shift in gene expression.

MicroRNA AND SiRNA

MicroRNA is a class of non-coding protein RNA molecules with a length of 18–24 nucleotides. They are important participants in the process of gene expression, regulating its intensity. Today, several thousand different microRNAs are known, each of which is able to control the synthesis of one to several hundred proteins. As a result, more than 60% of human genes are expressed with the participation of microRNAs.

A change in the level or activity of microRNAs can cause disturbances in the synthesis of certain proteins, which can lead to the development of a disease. It has been shown that a number of diseases are accompanied by deviations in the work of various microRNAs. A detailed study of the relationship between the work of individual microRNAs and the pathophysiology of diseases suggests the possibility of their use as molecular markers for diagnosing and predicting the course of the disease, as well as targets for targeted therapy.

SiRNAs are largely similar to microRNAs. These are molecules of 21–23 nucleotides in size that have a similar maturation path with microRNAs and a similar principle of action, but at the same time possess a number of features that separate them into a separate class [2]. In general terms, differences between microRNAs and siRNAs are presented in Table 1, and are described in more detail below.

Table 1

Differences in microRNAs and siRNAs		
Characteristic	MicroRNA	SiRNA
Molecule size	18–24 nucleotides	21–23 nucleotides
Structure	Single stranded	Double stranded
Beginning of biogenesis	From introns or individual sections of their own DNA (endogenous path)	From RNA of viruses or bacterial plasmids introduced into the cell, artificial vectors, etc. (exogenous pathway)
Immunogenicity	Own molecules, but artificially synthesized microRNAs can elicit an immune response	May elicit an immune response
Target Complementarity	Partial complementarity (the presence of a key «seed» region)	Complete complementarity
Targets	DNA, mRNA	mRNA
Specificity	One microRNA molecule regulates many DNA / RNA molecules, one DNA / RNA molecule can be a target for several microRNAs	Highly specific, one siRNA molecule binds one portion of mRNA, blocking the synthesis of one protein
Activity Result	Activation or repression of translation or transcription, mRNA degradation is possible	MRNA degradation, gene silencing
Activity sites	Cytoplasm, nucleus	Cytoplasm

BIOGENESIS OF microRNAs AND siRNAs

Understanding the biogenesis of microRNAs and siRNAs is very important for the possibility of influencing it. According to the canonical representation (Fig. 1), microRNA molecules are transcribed in the nucleus by RNA polymerase II from DNA regions that can be located both inside the genes encoding proteins (in introns) and in isolated regions of the genome under their own promoter. The resulting RNA transcript is called primary microRNA (pri-miRNA, pri-microRNA) and forms a secondary stem-loop structure with the presence of 7-methylguanosine molecules at the 5'-end, and at the 3'-end of poly (A)-"tail". After interacting with an enzyme complex consisting of RNase III (Drosha) and its companion DGCR8 (Pasha), pri-microRNA is converted to a microRNA precursor (pre-miRNA, pre-microRNA), consisting only of a stem-loop structure. Using exportin-5, pre-microRNA is transported from the nucleus to the cytoplasm, where the loop region is cleaved by another RNase III–Dicer, leaving a microRNA duplex consisting of two fully or partially complementary RNA strands 18–24 nucleotides in size. Subsequently, one of the duplex strands (host) forms a complex with proteins called the RNA-induced silencing complex (RISC), while the other ("passenger" strand), as a rule, is destroyed.

SiRNAs in the process of maturation pass a path similar to microRNAs. And although, unlike the latter, siRNA synthesis in mammalian cells does not begin from its own genome, but from vector molecules introduced into the cell from the outside (by bacteria, viruses or artificially), the same proteins (Dicer) participate in its biogenesis, and maturation is completed the formation of the RISC complex. In addition, siRNA can form in the cell as a result of cleavage of the RNA of the virus in a human cell. The mature siRNA molecule preserves the structure of the duplex.

TRANSPORT AND LOCALIZATION OF microRNAs IN THE BODY

After maturation, microRNA molecules can be transferred to different parts of the cell or beyond (Fig. 2). Some of them remain in the cytoplasm, where they interact with messenger RNA at various stages of translation, regulating protein synthesis. Another part of the molecules is transported into the intercellular space and circulates freely in the body in the form of ribonucleoprotein complexes or in exosomes [3, 4]. Exosomes are excreted by the cell and spread throughout the body, found in the extracellular space (extracellular matrix), blood plasma, synovial fluid, cerebrospinal fluid, saliva, urine and other liquid media and transferring microRNA from cell to cell. Exosomal microRNAs can act as markers for diagnosing or predicting the course of malignant neoplasms [5]. Another direction of movement of mature microRNAs is reverse transport to the nucleus. It has been shown that most microRNAs are capable of such reverse transfer and are detected both in the nucleus and in the nucleoli [6]. In addition, gene expression is regulated in the nucleus with the participation of the RISC complex, for which the proteins involved in this process (AGO, TRPB, Dicer, TRNC6A) are freely transferred from the cytoplasm to the nucleus [7].

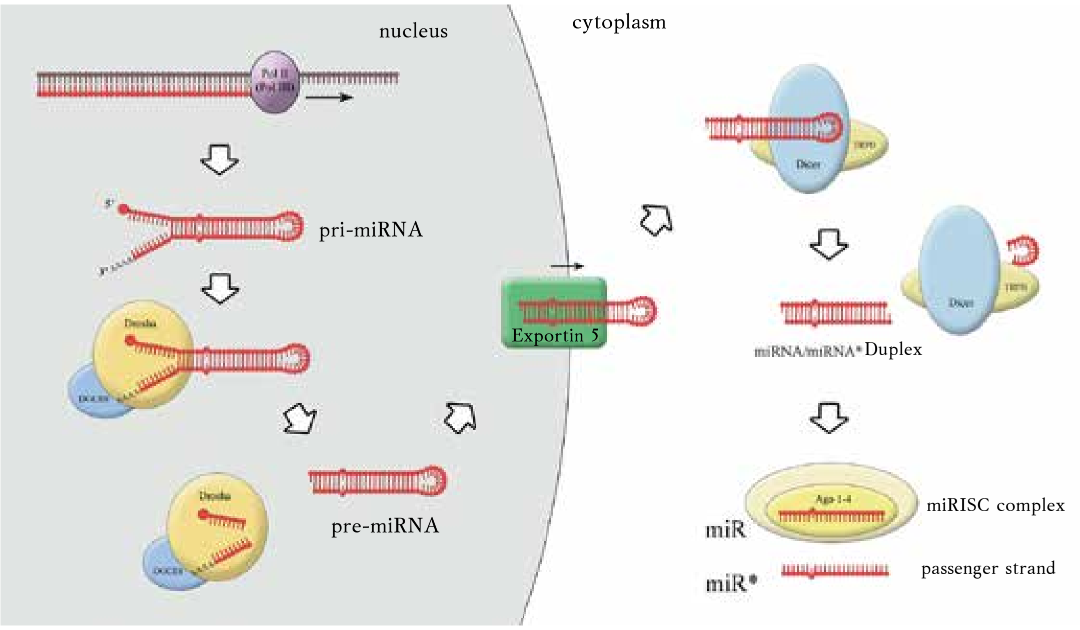

Fig. 1. The canonical scheme of microRNA biogenesis

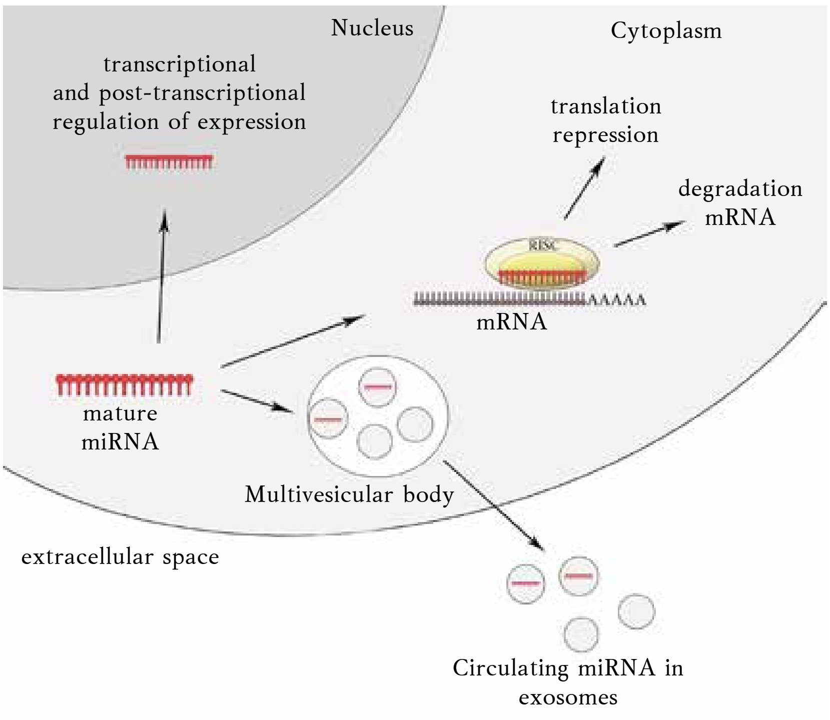

Fig. 2. Transport of mature microRNA in the body

When ingested or formed in a cell, siRNAs are usually stored in the cytoplasm, regulating via interaction with mRNA. In 2004, H. Kawasaki and K. Taira showed that siRNAs are able to induce cell DNA methylation by interacting with CpG islands in the promoter part of the gene. However, free penetration and the constant presence of siRNAs in the cell nucleus were not proven [8]. The mechanism of siRNA delivery to genomic DNA is still not fully understood.

WAYS OF REGULATING GENE EXPRESSION INVOLVING microRNA

The most studied method for regulating protein synthesis is the interaction of microRNA with messenger RNA in the cell cytoplasm. MicroRNAs bind to mRNA in specific complementary regions (target sites). Most often, these sites are found in the 3’-untranslated region of the mRNA (3’-UTR), but, in general, are found in the coding sequence (CDS), and even in the 5’-UTR [9]. As a rule, the binding site on the mRNA molecule is highly conserved so that random mutations or polymorphisms do not interfere with protein synthesis. Moreover, one mRNA can be a target for many microRNAs, and their joint participation determines the degree of suppression of protein synthesis.

Unlike plants, in animals, complete complementarity of microRNAs to the target gene is practically not found, but it has been proved that in this case, for the effective binding of the RISC complex, it is sufficient to complement the target molecule with only the second to eighth nucleotide region at the 5’-end of the microRNA molecule, called the key (“seed”) region. Although there are microRNAs that regulate in a different way: it has been proven that miR-24 is involved in the regulation of cellular processes by acting on target genes that do not contain a site complementary to its “seed” region [10]. In any case, the result of microRNA attachment is the effect of the protein part of the RISC complex on the target mRNA.

The main protein of the RISC complex in humans is an enzyme of the Argonaute AGO2 family, which is a structural analogue of RNase H, and therefore has the ability to cleave mRNA molecules directly at sites determined by microRNAs. Due to its action, degradation of the mRNA molecule is possible after meeting with microRNAs. However, it was shown that this occurs only in 29% of cases, whereas in approximately half of the cases (48%), interaction with microRNAs results in repression of translation without matrix destruction, and in 23% there is a simultaneous course of these two processes [11]. It is assumed that the method of regulation may depend on the site of landing of microRNAs. The binding of microRNAs to mRNAs in the 3’-non-coding region (3’-UTR) is more likely to lead to the degradation of target RNA due to deadenylation of the poly (A) “tail” and, as a result, destabilization and rapid degradation of the molecule [12–14] . Landing on the coding part of the gene is more likely to suppress the synthesis of the polypeptide on the ribosome [15]. And when binding to a 5’-non-coding region (5’-UTR), messenger RNA can result in mRNA degradation due to preliminary decapping of the 5’ end [16], as well as activation of translation [17]. Switching the microRNA-protein complex from the inhibition function to translation activation may depend both on the action of specific factors (for example, eIF4E) and on the state of the cell or phase of its cell cycle [18]. For example, miR-206 inhibits KLF4 protein synthesis in proliferating epithelial cells, but it activates in immortalized epithelial cells of the MCF10A line [19]. Like repression, activation can range from mild stimulatory effects to a significant increase in polypeptide synthesis.

Nuclear microRNAs are capable of influencing gene expression at post-transcriptional or transcriptional levels. Thus, they are able to control the synthesis or cause degradation of other microRNAs or long non-coding RNAs [20]. The ability of microRNAs to bind to single or even double stranded DNA leads to inhibition of [21] or activation of [22] gene transcription. It is noteworthy that in the nucleus, microRNA also acts in combination with the Argonaute protein, as well as other components of the RISC complex.

SiRNA REGULATION

The binding of RNA to the target mRNA molecule is its main difference from microRNA. The siRNA molecule does not have a “seed” region, but has complete complementarity to the mRNA molecule. For this reason, its action very specifically extends to the synthesis of only one protein. The result of its binding is the cleavage of the target with proteins of the RISC complex in the region between the 10th and 11th nucleotides of siRNA and complete cessation of translation [23].

DISORDERS OF microRNA IN MALIGNANT NEOPLASMS

Disorders associated with the development of malignant neoplasms can occur at the stages of biogenesis or work of microRNAs. These can be mutations of genes encoding microRNAs themselves or proteins important for their synthesis. Despite the general conservatism, polymorphisms in mRNA are also possible at the sites of binding to microRNAs. Also, the development of a malignant neoplasm can be associated with a change in the levels of certain microRNAs in the cell.

Table 2 presents examples of various abnormalities associated with biogenesis and the action of microRNA in the cell.

Table 2

Various forms of microRNA disorders with malignant neoplasms

Type of disorder	Diseases	Source
Polymorphisms and mutations in the genes of proteins associated with biogenesis and the action of microRNAs	Dicer1 syndrome: the presence of mutations in the *Dicer1* gene leads to a change in the structure and functions of the protein and, as a result, to a disruption of the synthesis of various microRNAs. The result is malignant neoplasms: pleuropulmonary blastoma, Sertoli-Leydig cell tumor, neuroblastoma, rhabdomyosarcoma, etc.	[24–26]
Change or loss of the gene encoding microRNA during chromosomal rearrangements	Deletion of a 13q14 chromosome fragment in chronic B-cell lymphoblastic leukemia leads to the loss of microRNA miR-15 and miR-16 coding regions, which are negative regulators of BCL2 protein synthesis. The result is a decrease in the ability of cells to apoptosis	[27, 28]
Mutations and polymorphisms in a gene encoding microRNA	Single nucleotide substitution G> C (rs2910164) in the gene encoding miR-146a leads to a change in the production of this microRNA and is associated with an increased risk of developing renal cell carcinoma, glioma, early manifestation of familial breast or ovarian cancer	[29–31]
Mutations and polymorphisms in mRNA at sites of interaction with microRNAs	*HIF1A* gene polymorphism in the region near the binding of the “seed” region of miR-199a leads to increased protein synthesis, which is associated with poor prognosis for the ductal adenocarcinoma of the pancreas. Single nucleotide substitution in the 3’ non-coding region of the *SET8* gene disrupts the miR-502 binding site, which increases the risk of early development of breast cancer	[32, 33]
Reduced microRNA	MiR-143 and miR-145 have a reduced level in the cells of cancer of the gastrointestinal tract and exhibit oncosuppressive properties upon endogenous administration	[34]
Increased amount of microRNAs	MicroRNA miR-21 has a relatively high level in six types of solid tumors (breast, lung, prostate, stomach, pancreas and rectum cancer), as well as glioblastoma	[35, 36]

To correct molecular processes in the cell using microRNA, it is possible to regulate both the number of regulatory molecules and the quality of regulation by controlling the ability of the RISC complex to bind specifically to the target site.

WAYS OF TARGETED REGULATION OF GENE EXPRESSION

Imitators (mimics) of microRNA. To artificially increase the level of a given microRNA in a cell, synthetic copies of this molecule are introduced into it – imitators, or mimics (replacement therapy). These can be mature molecules,direct copies of microRNAs that have the same properties of binding molecules as their prototype, or its predecessors, and even genes encoding it. The introduction of mature microRNA is a more convenient and quick method of exposure. Due to its small size, the molecule easily penetrates through the cell membrane as part of RNA-lipid complexes (lipid transfection, or lipofection). Using mature mimics, for example, the possibility of suppressing the proliferation of gastric and colorectal cancer cells using miR-375 *in vitro* has been demonstrated [37, 38]. However, in the case of using microRNA mimicry to enhance the effect of repression of protein synthesis, it is important to remember that for active operation of microRNA it must form the RISC ribonucleoprotein complex. It was shown that the introduction of individual microRNA molecules into the cell, as a rule, meets a limited protein pool, and to form the ribonucleoprotein complex, they are forced to compete with the endogenous microRNA of the cell for the protein components of the RISC complex and can deplete their reserves. As a result, this can lead not only to an insignificant effect of suppressing translation of target mRNA, but also to an increase in the translation of other proteins due to impaired regulatory functions of the cell's own microRNAs [39]. At the same time, even small amounts of exogenous microRNA are introduced into the cell together with plasmids expressing AGO2, leading to a significant effect of its activity [40].

The introduction of microRNA precursors allows at least partially solving the problem with the formation of the RISC complex: the synthesis and maturation of a molecule in a cell is more likely to lead to its natural encounter with AGO2. It was even shown that the introduction of a microRNA duplex consisting of the leading and passenger strands is more likely to lead to the formation of an active microRNA molecule than the introduction of a mature single-stranded molecule [41]. The possibilities of introducing pre-miRNA or pri-miRNA into the cell are also described.

In general, the use of imitators opens up wide possibilities for substitution therapy for the fight against malignant neoplasms. Thus, increased miR-4779 microRNA activity due to the use of mimics led to the suppression of tumor growth, blocking of the cell cycle and stimulation of cancer cell apoptosis in colorectal cancer [42]. In another study, targeted management of the miR-29b simulator allowed the suppression of the development of acute myeloid leukemia [43].

MicroRNA inhibitors. The antisense inhibitor is an RNA oligonucleotide complementary to the target microRNA. Upon their binding, a sufficiently strong duplex is formed, which prevents the microRNA from landing on mRNA and thereby removes the ban on translation. One of the natural regulators of microRNA activity in the body is competitive endogenous RNA (ceRNA), including long non-coding RNA molecules (long ncRNA, lncRNA) [44], ring RNA [45], pseudogenes. Due to the presence of microRNA landing sites in their nucleotide sequence, these molecules are capable of acting as a so-called molecular sponge, which assumes an attack of microRNAs and thereby removes the block from real messenger RNA [46]. With the help of such a "sponge", almost complete removal of active miR-221/222 from the cell was achieved, which led to increased apoptosis of squamous cell carcinoma of the oral cavity [47].

Exogenous inhibitors also show effective inhibition of the activity of target microRNAs. But at the same time, it becomes necessary to deliver the molecule into the cell through the membrane and maintain its stability in the cytoplasm, since an exogenous RNA molecule that does not have protection can be rapidly destroyed by RNases. These problems are partially solved by one of the latest generations of microRNA inhibitors, created on the basis of "closed" ("locked") nucleic acids, or LNA-inhibitors (locked nucleic acid). They are oligomers 12–14 nucleotides long, having a methylene bridge between the 2'-O and 4'-C ribose rings in the nucleotide part. As a result, such an LNA molecule is more resistant to the action of endonucleases, forms a stronger duplex with the RNA or target DNA, and also more easily penetrates the cell membrane (due to its small size) [48], and does not show significant toxicity to the body in experiments on mice [49]. All this makes LNA inhibitors promising for the development of drugs based on the principle of inhibition of microRNA activity. It was with their help that it was possible to degrade miR-21 and thereby achieve increased apoptosis and suppressed proliferation of hepatocellular carcinoma cells [50].

SYNTHETIC REGULATORS

Sometimes, when researching or developing a method for treating a disease, it becomes necessary to affect certain regions of messenger RNA that do not have a natural site for regulatory molecule landing. For example, if an oncogenic gene mutation occurs that leads to a change in the structure of the protein, it would be convenient to block the synthesis of the mutant form of the protein, while maintaining the normal protein. This requires regulation with recognition of mutations in messenger RNA. In this case, artificial microRNA molecules come to the rescue. In 2017, M. Acunzo and colleagues vividly demonstrated the feasibility and benefits of creating artificial microRNAs for targeted inhibition of translation of the *KRAS* gene containing the G12S point mutation to increase the sensitivity of lung cancer cells (cell line A549) to treatment with gefitinib. It was demonstrated that even a single nucleotide substitution changes the regulatory effect [51]. However, since microRNAs can affect several mRNAs at the same time, this can lead to undesirable side effects and make it difficult to use for one purpose. A more specific option is the use of siRNA molecules. But the complete complementarity of siRNA makes it less sensitive to polymorphisms and mutations. In addition, siRNA activity *in vivo* may be limited by the occurrence of an immune response. For example, when studying the effect of siRNAs on the growth of cells carrying the V617F mutation of the *JAK2* gene, a significant decrease in the effect of inhibition by cytokines was shown [52].

Another type of synthetic microRNA inhibitors is peptide nucleic acids (PNA). These are chemical compounds that are linear polymer molecules similar to DNA, but with N- (2-aminoethyl) glycine instead of sugar. The advantage of PNAs is their resistance to degradation by nucleases and proteases, as well as the independence of hybridization with DNA or RNA from the concentration of salts in the medium [53]. Another important property of PNA is sensitivity to non-complementary bases. Even one mismatching nucleotide is capable of changing the melting temperature of the PNA-DNA duplex to 15 ° C, which makes them promising molecules for the selective inhibition of targets containing single nucleotide mutations [54]. In addition, it was shown that PNAs can be modified in such a way that their penetration into the cell is ensured without the participation of an additional transfection reagent [55]. To date, this type of molecule has been successfully used to inhibit microRNAs *in vivo* [56].

Proper design and optimization of the conditions for the use of artificial molecules can achieve the desired effect of reducing or disabling protein synthesis in the cell, reduce nonspecific effects, and avoid toxicity or immunogenicity of the introduced substance. This makes these molecules promising therapeutic agents in medicine in general and oncology in particular.

DELIVERY OF SMALL NON-CODING RNA TO CELLS

The delivery of microRNAs and siRNAs to cells *in vitro* and *in vivo* is still a challenge and is a field of active development. First, free RNA molecules without any protection or modification are easily destroyed by nucleases, and in the animal body they are also excreted by the kidneys and liver [57] or are retained in non-target organs. Secondly, other tissues and structures (blood vessel walls, connective tissues, tumor microenvironment), which have different permeability, as a rule, arise on the way to the tumor cell in the body, which can significantly complicate the delivery. Thirdly, foreign RNA molecules can initiate an immune response and cause unwanted side effects [58]. Fourthly, even in an *in vitro* culture, where the achievement of the goal is possible by introducing molecules into the nutrient medium, the membrane barrier is required to penetrate the cell. Finally, a molecule entering the cell may be denied access to the target as a result of inclusion in the endosome [57, 59] or non-specific interaction with non-complementary or partially complementary RNA molecules.

Various methods are currently being developed for delivering RNA molecules to tumor cells: chemical, physical, biological. Chemical methods include the use of polymer complexes (polyethyleneimine), lipid nanoparticles (liposomes), dendrimers, inorganic compounds (iron oxide, gold, silicate nanoparticles) [60, 61], etc. It is believed that chemical delivery methods initially have low transfection efficiency in comparison with biological. This is partly due to the short lifespan of molecules *in vivo*, their ability to bind to serum proteins in the blood. However, modifications of chemical compounds make it possible to overcome these difficulties by creating stable constructs that provide more specific delivery of microRNAs to cells. Examples are nanostructured lipid carriers that have a positive charge on the surface of the bilipid layer, or molecules recognized by cell receptors that have been successfully used to deliver microRNAs to cell cultures and *in vivo* in mouse models [62, 63]. Chemical delivery methods can also include modifications of the RNAs themselves, which increase the stability of molecules, reduce their toxicity and (or) facilitate penetration into the cell (LNA, PNA). Chemical methods are effectively used in *in vivo* studies.

Physical delivery methods are suitable for *in vitro* cultures and include magnetofection, biobalistics, electroporation, sonoporation, laser irradiation, etc. The most common method is electroporation, which allows an electrical impulse to break a gap in the membrane, thereby ensuring direct penetration of nucleic acids into the cell cytoplasm, or placing the desired molecule in the exosome for subsequent delivery to the cells. The use of exosomes increases the stability of molecules and facilitates the *in vivo* delivery method [64], while direct delivery to cells in *vitro* by electroporation is highly regarded for its simplicity and efficiency [65].

Chemical and physical delivery methods provide, as a rule, the transient nature of gene expression, having a relatively short life span of the introduced molecules. For a longer effect, biological methods are used, namely delivery as part of viral vectors (transduction). The DNA of adenoviruses, retroviruses, lentiviruses can be used as a vector.

Adenoviral vectors are double-stranded DNA molecules, are characterized by relative ease of use and are successfully used for fast and short-term introduction of molecules, since they are not able to introduce the desired gene into the genomic DNA of a eukaryotic cell and ensure constant synthesis of a given RNA. However, another important advantage is their ability to introduce foreign DNA fragments up to 38 kb into the cell. In contrast, retroviral RNA vectors contain no more than 8 kb of a foreign nucleotide sequence. However, it is introduced into the genome of the host cell at the stage of mitotic division. Lentiviral vectors are similar to retroviral ones, but differ in their ability to introduce a foreign sequence into the genome of both dividing and non-dividing cells located in the postmitotic period or at the stage of terminal differentiation. Retroviral and lentiviral vectors are used for stable transfection of dividing cells *in vitro* and *in vivo*, demonstrating the high efficiency of introducing both mimics and microRNA inhibitors [66]. The disadvantage of viral vectors is the high immunogenicity and potential toxicity of molecules, as well as the instability of the viral genome and the likelihood of the virus reversing to the "wild" type with the loss of the insert of interest [67]. Recent developments are aimed at modifying designs to reduce or eliminate the negative effects of these vectors [68], which opens up wide possibilities for their use in science and clinical practice for the treatment of diseases.

CONCLUSION

The use of regulatory microRNA and siRNA molecules to control gene expression in a cell seems to be a powerful technology for studying normal cellular processes and pathology, as well as for treating diseases, in particular, malignant neoplasms. Patents have already been registered and a number of microRNA or siRNA-based drugs are being registered for the treatment of diseases, including chronic lymphocytic leukemia (regulation of the *BCL-2* gene), liver cancer (regulation of *VEGF* and *KSP* expression), and other solid tumors, including later stages of progression [69]. Therapies due to the directed regulation of gene expression using microRNAs and siRNAs predict a great future, calling these drugs new-generation drugs. However, much of the mechanisms of such regulation remains incompletely studied, and the world of regulatory molecules still requires a deep and multifaceted study.

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Assessment of calcification of the coronary arteries and long-term prognosis of cardiovascular disease

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ABSTRACT

Vascular calcification is a distinctive feature of cardiovascular diseases of atherosclerotic origin. Visualization of calcifications is carried out by invasive and non-invasive methods. Knowledge of the presence and degree of calcification can predict clinical outcomes in patients at high risk of coronary events, help in the prevention and treatment of coronary heart disease.

The article presents a brief description of the methods of visualization of vascular calcium and a review of studies on the relationship of calcification with the risk of long-term adverse cardiovascular events.

Key words: calcification, coronary atherosclerosis, methods of assessing vascular calcification, cardiovascular disease

List of abbreviations: IVUS - intravascular ultrasound, CHD – coronary heart disease, CAG – coronary angiography, MSCT – multispiral computed tomography, OCT – optical coherence tomography, CVD – cardiovascular disease.

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Оценка кальцификации коронарных артерий и отдаленный прогноз сердечно-сосудистых заболеваний

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РЕЗЮМЕ

Сосудистая кальцификация является отличительной чертой сердечно-сосудистых заболеваний атеросклеросклеротического генеза. Визуализация кальцификатов осуществляется инвазивными и неинвазивными методами. Знания о наличии и степени кальциноза могут предсказать клинические исходы у пациентов с высоким риском коронарных событий, помочь при проведении профилактики и лечении ишемической болезни сердца.

В статье представлены краткая характеристика методов визуализации сосудистого кальция и обзор исследований по изучению связи кальцификации с риском отдаленных неблагоприятных сердечно-сосудистых событий.

Ключевые слова: кальцификация, коронарный атеросклероз, методы оценки кальцификации, сердечно-сосудистые заболевания.

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INTRODUCTION

Coronary heart disease, which is based on atherosclerosis, remains one of the main causes of morbidity and mortality in all countries of the world. Vascular calcification is a hallmark of the atherosclerotic process. Despite the abundance of clinical data, the fundamental role of calcification in rupturing an unstable atherosclerotic plaque is still unclear. Visualization of the structural features of calcification in coronary arteries using invasive and non-invasive methods can be important for predicting plaque rupture, and knowledge of the presence / degree of calcification can provide an idea of the level of risk of cardiovascular disease and can help in the prevention and treatment of coronary heart disease (CHD) [1–3].

CALCIUM ASSESSMENT METHODS

The total indicator of coronary artery calcium, determined using invasive and non-invasive imaging methods, provides additional value compared with traditional risk indicators for identifying patients with a high risk of cardiovascular disease [4, 5]. Invasive methods include x-ray coronary angiography (CAG) and optical coherence tomography (OCT). Non-invasive methods include radiography, multispiral computed tomography (MSCT), single-photon emission tomography, positron emission tomography and others. These methods have their advantages and disadvantages.

INVASIVE METHODS

The main methods for assessing damage to the coronary arteries are endovascular methods. The advantages of these methods are the direct visualization of the lumen of the vessel, the installation of sensors near the atherosclerotic plaque, which allows the most reliable assessment of the severity of deposition of atherosclerotic masses and their calcination.

Coronary Angiography. Coronary angiography is still one of the leading methods for the diagnosis and treatment of coronary insufficiency. Coronary angiography allows for the obtainment of the most complete picture of the anatomy and degree of damage to the coronary artery bed. The disadvantages of this research method include the use of x-ray radiation and the introduction of contrast agents. In addition, coronary angiography can provide only a two-dimensional outline of the coronary lumen and cannot fully demonstrate the complex nature of atherosclerotic plaques, which are responsible for the relationship between angiographic data and clinical outcome [6]. To identify the morphological features of calcified plaques, 3D virtual intravascular endoscopy is used, which, as shown, provides additional information about the wall of the coronary arteries and plaques [7].

Recently, classical CAG has been replaced by intravascular ultrasound and optical coherence tomography.

Intravascular ultrasound. One of the most informative and specific methods for determining atherocalcinosis is intracoronary ultrasound using high resolution sensors. This technique allows you to clarify the degree of the initial lesion of the coronary bed, to carry out effective control during x-ray endovascular interventions. In contrast to X-ray contrast angiography, intravascular ultrasound examination makes it possible to obtain a section of the vascular wall in several planes, allowing quantitative and qualitative assessment of the lumen of the vessel, the area of distribution of the plaque and its calcification, and the presence of a pronounced acoustic shadow. Factors limiting the use of IVUS include a feature of the coronary artery topic, impeding sensor advancement, and microvascular lesion nature. The possibilities of IVUS are significantly expanded by means of spectral analysis of the data obtained, in which the 4 main components of the atherosclerotic plaque are assigned the corresponding color coding. Such an analysis is called virtual histology. IVUS with virtual histology can detect the most dangerous type of atherosclerotic plaque [8]. Using IVUS with virtual histology Noto T. et al. (2015) observing patients with acute coronary syndrome, showed that with a calcium content of > 3.4% of the area of atherosclerotic plaques, the frequency of coronary events increased by 4.4 times [9].

Optical coherence tomography. Optical coherence tomography is an intravascular light-optical imaging technique that uses laser radiation with a wavelength of 1300 nm to obtain information about the three-dimensional structure of the vascular wall. Recently, OCT has become the leading technology of intracoronary imaging with a higher resolution (10–20 μm) than IVUS (100–200 μm) [10, 11].

In contrast to intravascular ultrasound, OCT is able to estimate the thickness of calcium and, consequently, the area and volume. This method has a number of advantages, such as high resolution sensors, short-term investigation, and the possibility of reconstructing visual and easy-to-interpret images in various planes. However, OCT has limitations on the depth of penetration through tissues, which is <2 mm, and this significantly affects the role of OCT in the evaluation of plaques [7, 12].

A study by Habara M. et al. (2018) aimed at assessing the characteristics of vascular calcification in vivo using OCT as compared with histological data, showed that OCT does not allow microcalcifications to be seen [13], but can show spotty or speckled calcification, which is smaller than macrocalcification, but larger than microcalcification [14]. Also, using the OCT method it is difficult to detect calcifications located behind the necrotic nucleus of an atherosclerotic plaque [15].

NON-INVASIVE METHODS

Since invasive interventions have a number of contraindications and a high risk of complications, high-tech methods of non-invasive imaging are increasingly used in medical practice.

Radiography. The detection of coronary calcification using x-ray was already used in the 30s of the twentieth century. The technique has some limitations in its application, when conducting an x-ray of the chest organs, only large calcifications can be detected and their extent can only be estimated indirectly. The X-ray method is the main method of radiation diagnostics at the first stage of medical care, and subsequently requires more high-tech methods, such as computed tomography, magnetic resonance imaging, positron emission tomography, etc. However, in recent years, the introduction of digital technologies has increased the capabilities of radiography. Sultanova M.D. (2017) studied the possibilities of digital radiography in the diagnosis of coronary calcifications on 90 patients and compared the results with multispiral computed tomography. The author noted that the detection of coronary calcifications during digital radiography is limited to certain values of the calcium index and this method can be recommended as a screening diagnosis of the risk strategy for cardiovascular diseases [16].

CT scan. The main method for quantifying coronary artery calcification is computed tomography. When synchronized with electrocardiography, computed tomography can detect and quantify even small deposits of coronary calcium and, accordingly, assess the presence and severity of coronary atherocalcenosis. According to a standardized quantitative system for measuring coronary calcification, the amount of coronary calcium is expressed in units of the calcium index. The calcium index is calculated according to the standard Agatston method [17] and is determined by multiplying the area of calcified lesion by the density factor. The density factor is calculated by the peak density in the calcification zone and is 1 for calcinates with a density of 130–199 HU, 2 for lesions with a density of 200–299 HU, 3 for 300–399 HU and 4 for calcinates with a density of more than 400 HU. The total calcium index is calculated as the sum of the indices on all slices [18].

Positron emission tomography. Positron emission tomography (PET)/CT using ^{18}F-sodium fluoride (^{18}F-NaF) has the potential of non-invasively identifying microcalcification [19–21]. Irkle et al. (2015) have shown that ^{18}F-NaF is adsorbed by calcified deposits inside a high atherosclerotic plaque affinity and is selective and specific. In addition, PET/CT using ^{18}F-NaF makes it possible to distinguish between macro- and microcalcification regions [22]. Molecular imaging can diagnose atherosclerosis at an earlier stage, including in pre-symptomatic patients, and may be another option for detecting vulnerable plaques and predicting future adverse cardiovascular events [23].

ASSESSMENT OF CORONARY CALCIFICATION AND PROGNOSIS OF RISK OF LONG-TERM ADVERSE CARDIOVASCULAR EVENTS

Evaluation of coronary calcification is important for predicting the risk of long-term adverse cardiovascular events in patients with various forms of coronary atherosclerosis, including subclinical. Shaw L.J. et al. (2015) using electron beam computed tomography, determined the coronary calcification index in 9715 patients of different sexes and ages without the clinical manifestations of coronary artery disease, followed up for 15 years. High coronary calcification indices were associated with males, old age, and diabetes mellitus dyslipidemia and smoking. The authors noted that in patients with even a low level of arterial calcium, the overall risk of mortality is almost 70% higher than in those who did not have any calcium deposits, and in patients with the largest calcium deposits, this risk is six times higher. Researchers noted that the degree of calcification of the coronary arteries predicts 15 year mortality in asymptomatic patients [24].

Genereux P. et al. (2014) included in the study about 7000 patients with acute coronary syndrome who underwent coronary angiography. In 32% of patients, severe and moderate calcification of the infarction-associated artery was noted. It was these patients who had recurrent adverse cardiovascular events more often during the year after acute coronary syndrome. The authors noted that moderate / severe calcification was more common in older people, patients with hypertension and myocardial infarction with ST-segment elevation. The authors also noted gender differences; more pronounced calcification was in men [25].

Blaha M.J. et al. (2016) studied more than three thousand patients with an initial coronary calcification coefficient> 0. Observation was conducted for about 10 years. During this time, 368 cases of CHD and 493 cases of CVD were identified. The authors showed that taking into account the number of calcified vessels together with the determination of the overall calcification index can improve the prognosis of adverse cardiovascular events [26].

Based on the results of the CARDIA study, which included more than 5,000 young participants observed over 30 years, in which coronary calcium was measured 15, 20, and 25 years from the start of the study, Carr J.J. et al. (2017) concluded that the presence of coronary calcification among individuals aged 32 to 46 years is associated with an increased risk of fatal and non-fatal coronary artery disease during 12.5 years of follow-up. The authors noted that a coronary calcification coefficient of 100 or more is associated with early death, and study participants younger than 50 years old with any level of coronary calcium, even with very low rates detected by computed tomography, have an increased risk of clinical coronary artery disease, CVD and death [27].

Lutai M.I. and Golikova I.P. (2017) studied 142 people with coronary artery disease or suspected coronary artery disease. In order to detect coronary calcification, all patients underwent MSCT; the diagnosis was verified by coronary angiography. The authors also evaluated the degree of calcification of the aorta. The authors concluded that calcification of the coronary arteries and aorta significantly increases with age, higher in the presence of diabetes mellitus, arterial hypertension, and hypercholesterolemia. Patients with a high coronary calcium index were more likely to have had myocardial infarction and had a family history of CHD aggravated than patients with low calcium index [28].

The severity of calcification of the aorta and coronary arteries is an independent predictor of cardiovascular mortality. Hoffmann U. et al. (2016) studied coronary artery calcium, thoracic and abdominal aorta, mitral and aortic valve using computed tomography of the heart in people without cardiovascular disease at the time of observation. The study included 3217 participants, the average age of which was 50 years, half of which were women. Participants were observed for an average of 8 years. The authors evaluated the possibilities of predicting coronary heart disease, CVD, and mortality from all causes by the level of vascular calcification and compared with the data obtained using the Framingham scale. According to the authors, calcification of the abdominal aorta and coronary artery was most common, while calcification of the thoracic aorta and valves was less common. Coronary artery calcium was most strongly associated with CHD, CVD, and all-cause mortality, regardless of the risk factors of the Framingham scale. Moreover, with an increase in the initial level of calcium, the risk of adverse events increased. The modified

Agatston index 101 and higher indicated a significant risk of developing coronary heart disease and CVD. The degree of non-coronary calcification also identifies individuals with a higher risk of developing coronary heart disease and CVD, regardless of risk factors [29].

Lehmann N. et al. (2018) studied risk factors and performed CT at the beginning of the study and after five years in 3281 people, without cardiovascular events at the beginning and within 5 years of observation. Severe coronary and cardiovascular events, as well as general cardiovascular events, including revascularization, were recorded after a second CT scan for 10 years. The authors established a high prognostic value of coronary calcium in relation to coronary and cardiovascular events, as well as mortality from all causes. A particularly pronounced relationship was shown in patients with coronary calcium levels in the first stage of the study> 400. These patients had high rates of severe coronary and severe / general cardiovascular events (10-year risk: 12.0%, 13.5%, and 30.9%, respectively). An increase in coronary calcium> 400 at the second stage of the study led to an almost twofold increase in the risk of coronary and general cardiovascular events compared with individuals whose level of this indicator during repeated CT did not exceed 400. In patients with zero calcium level at two stages of CT 10- summer risk was 1.4%, 2.0% and 2.8% [30].

Paixao A.R. et al. (2015) in their study evaluated the effect of coronary artery calcification on predicting the risk of coronary heart disease in a younger population (44.4 ± 9.0 years). The study included 2084 patients without diabetes and cardiovascular disease. In a young multinational cohort, according to the authors, adding the definition of coronary calcium to a model consisting of traditional risk factors for coronary heart disease significantly improved discrimination and risk classification [31].

Criqui M.H. et al. (2017) having examined 6814 men and women who at the time of registration did not have clinical manifestations of cardiovascular diseases, concluded that the volume of calcification of the coronary artery is positively and independently associated with the risk of coronary artery disease and CVD, while the density of calcification for any volume is inversely proportional and reliably associated with the risk of coronary heart disease and CVD [32]. Forbang N.I. et al. (2016) using computed tomography in a multinational cohort of 997 participants with an Agatston index> 0 determined the volume and density of calcification of the abdominal aorta and coronary arteries. The observation was carried out for 9 years. They studied the association of the volume and density of calcification with CHD, CVD, and death from all causes. In models adjusted for factors of cardiovascular disease, an increase in the volume of calcification of the abdominal aorta was associated with an increase in mortality from all causes, and in coronary arteries, with coronary artery disease and CVD. Unlike previous researchers, Forbang's calcification density was not significantly associated with CVD [33].

Puchner S.B. et al. (2018) investigated using computed tomography of patients with and without acute coronary syndrome. The authors noted that a low level of local calcium indicates plaque instability, while a high level of high density calcium may be a marker of plaque stability, despite the fact that a higher overall score of the coronary calcification index is a marker of increased cardiovascular risk [34].

In addition to the volume and density of calcium in the coronary arteries, data on the structural characteristics of calcifications have predictive value. Studies show that microcalcification is more common in unstable plaques, while stable plaques are characterized by larger deposits [35, 36]. Kataoka Y. et al. (2014) by studying OCT patients with stable coronary artery disease who have clinical indications for percutaneous coronary intervention showed that spotted calcification indicates a greater vulnerability of plaques [37]. Later Sakaguchi M. et al. (2016) examined patients with acute coronary syndrome, with and without rupture of a plaque. The authors showed that more often spotted calcification tends to be located near the site of plaque rupture, while analysis of spotted calcification is an independent prognostic factor for plaque rupture [2].

CONCLUSION

Assessing coronary calcium, in addition to traditional risk factors, provides valuable, long-term prognostic information for assessing the risk of coronary heart disease and cardiovascular disease of atherosclerotic origin. In the future, the determination of calcium density indicators, regional and extra-coronary calcification, which are prognostic for the risk of coronary heart disease and CVD, regardless of the Agatston indicator, can further improve the risk assessment, which will allow the clinician to prescribe preventive pharmacotherapy taking into account the assessment of the 10-year risk of coronary heart disease in their patients. Knowledge of the morphological features of the plaque can affect treatment strategies for the prevention of acute coronary syndrome and may be useful in understanding the pathophysiological mechanisms of plaque rupture.

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Molecular genetic markers of atrial fibrillation

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ABSTRACT

Atrial fibrillation (AF) is the supraventricular form of tachyarrhythmia characterized by uncoordinated atrial stimulation and manifested in the increased frequency of their contraction. The frequency of this pathology directly correlates with the patients' age and reaches 50% in an older age group. This fact determines the need for search of any markers of individual AF risk, which may contribute to an increase in the effectiveness of preventive actions. Among such markers, polymorphic variants of genes involved in the pathogenesis of AF are the most promising markers. This review discusses the results of studying the genetic markers of the AF development, as well as the possibility of their use as predictors of this pathology.

Key words: atrial fibrillation, genetic polymorphism, cardiovascular diseases.

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Молекулярно-генетические маркеры фибрилляции предсердий

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РЕЗЮМЕ

Фибрилляция предсердий (ФП) – наджелудочковая форма тахиаритмии, характеризующаяся нескоординированным возбуждением предсердий, проявляющимся в увеличении частоты их сокращения. Частота возникновения данной патологии напрямую коррелирует с возрастом пациентов и достигает 50% в старшей возрастной группе. Необходимость увеличения эффективности профилактиче-

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ских мероприятий обусловливает поиск маркеров, позволяющих оценивать индивидуальные риски развития заболевания. Среди таких маркеров наиболее перспективными являются полиморфные варианты ключевых генов, участвующих в патогенезе ФП. В данном обзоре обсуждены результаты изучения генетических маркеров развития данной патологии, а также возможность их использования в качестве предикторов ФП.

Ключевые слова: фибрилляция предсердий, генетический полиморфизм, сердечно-сосудистые заболевания.

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INTRODUCTION

Atrial fibrillation (AF) is a subtype of supraventricular tachyarrhythmia, characterized by disorganized atrial activation with the atria's contractions occurring up to 350 times per minute. It significantly weakens the heart's pump function [1]. The prevalence of AF in the general population is constantly growing. It is estimated to affect 33.5 million people globally [2]. The relationship between AF and age has been well described in the medical literature. AF occurs in 0.5% of young adults aged less than 40 years and increases up to 25% in adults aged 40 to 70 years. Among the elderly, its incidence increases up to 50% [3–5]. AF is commonly considered as a severe risk factor contributing to the development of adverse cardiovascular events. Almost 12.5% of adverse events among elderly patients are associated with the presence of AF [6–8]. Long-term survival of patients undergoing mitral valve repair depends on the presence of concomitant AF that is commonly diagnosed in 40–60% of patients with mitral valve insufficiency. Odarenko et al. have reported a 17-fold increased risk of thromboembolism in patients with rheumatic mitral valve disease and concomitant AF in comparison with sinus rhythm patients. AF persisting after mitral valve repair was associated with atriomegaly and a long history of heart rhythm disturbances [9].

Myocardial pathology or alterations in the neurohumoral activation are generally considered as the main mechanisms contributing to the pathogenesis of AF [10, 11]. AF is secondary to arterial hypertension, coronary artery disease (CAD), hypertrophic and dilated cardiomyopathy, and congenital and acquired heart disease in almost 70% of cases. But in some cases, its etiology remains unknown and AF is considered as idiopathic or primary [12] and commonly referred to as familial AF [13]. Moreover, the genetic contribution should not be excluded from the list of the factors leading to the onset of secondary AF. The probability of its onset varies even if the underlying diseases are of similar severity.

There are two groups of risk factors contributing to the development of AF: traditional risk factors (age, ischemia, diabetes mellitus, metabolic syndrome, binge alcohol consumption, etc.) and genetic risk factors (gene polymorphisms involved in the pathogenesis of AF). Genetic risk factors are currently on the rise as they may provide novel insights into drug targets for therapy and give clues how to improve the prognosis. Over 30 genetic loci have been recently determined that are involved in the pathogenesis of AF [14]. Today, molecular genetic studies of AF are focused either on identifying mutations in the genes linked to the onset of AF or determining gene polymorphisms that indirectly affect myocardial function (the renin-angiotensin-aldosterone system gene, inflammatory response, etc.).

Thus, the identification of candidate genes associated with an increased risk of AF is the most important direction of modern genetics. These studies are aimed at defining the triggers that are responsible for the onset of various forms of AF, and the factors relevant to the chronicity of this pathology [5, 14].

MOLECULAR GENETIC MARKERS ASSOCIATED WITH AN INCREASED RISK OF AF

While the role of traditional risk factors in the development of AF has been studied well, the genetic contribution to its onset has recently received recognition worldwide. In 2004, Lubitz et al. showed that the presence of AF in parents increased the risk of AF developing in their children by 40% [15]. Investigators from Iceland found that the risk of AF reduced with increased genetic distance between relatives [16]. In addition, the risk of developing AF in both monozygotic twins was higher than in dizygotic twins [17]. All these studies have confirmed the significance of genetics in the development of AF, giving the green light to molecular genetic studies. Over 3,800 studies have been performed examining the associations of genetic polymorphisms and phenotypic traits (genome-wide associations studies, GWAS) by the end of December 2018 [18]. In 2007, investigators from Iceland reported that two independent single nucleotide polymorphisms (SNP) on chromosome 4q25 were associated with AF [19]. Other studies in the independent samples from Caucasian [20], Chinese [21] and African American [22] populations confirmed the role of this locus in the development of AF. Since 2006, genome-wide association studies (GWAS) allowed defining additional 23 loci related to AF. What is more, the role of most of them in the pathogenesis of AF has been described for the first time [14, 23–27].

ION CHANNEL GENES

GWAS have indicated that the development of familial AF is caused by the *KCN (KCNA5, KCND3, KCNE1, KCNE2, KCNH2, KCNJ2, KCNJ5, KCNN2, KCNN3 and KCNQ1), SCN (SCN5A and SCN10AJ), J1AJ, G1, JAA, GJ, G1, JA, GJ*, and G (*CAV1* and *CAV2*) gene mutations [5, 14]. These genes encode proteins of potassium and sodium channels, connexin and calveolin that are involved in the membrane transport. They play an important role in the function of the myocardium. Mutations in these genes have been shown to affect the function of ion channels and the conformation of proteins, which affects the state of the myocardium. In 2012, Danish researchers examined the relationship of the KCNE1 gene mutation, encoding the potassium channel beta subunit protein, with AF. They bidirectionally sequenced the entire coding sequence of the *KCNE1* gene in 209 patients with early-onset AF (aged under 40 years) and in 216 healthy individuals. Patients with AF were heterozygous carriers of the *KCNE1* c.74 G> T and *KCNE1* c.179 G> A. In addition, functional analysis of polymorphic variants showed that mutations in c.74 G> T and c.179 G> A were associated with an increase in the flow of potassium ions across the membrane, thereby confirming the role of this process in the pathogenesis of AF [23].

In 2014, the coding regions of *KCNE2* and *KCNE3* were bidirectionally sequenced in 192 patients with early-onset AF. Two missense mutations, M23L (c.67A>T) and I57T (c.170T>C), were identified in *KCNE2*. These mutations were absent in the control group without AF. There were no AF-specific mutations in *KCNE3*. Thus, researchers reported two mutations in KCNE2, encoding potassium channel β-subunit protein, as possible genetic substrates for early-onset AF [24].

Li et al. assessed the associations of polymorphisms on *KCNE1, KCNQ1, KCNH2* gene with the risk of AF in Chinese population. The study included 438 AF patients and 450 healthy subjects, ensuring high reliability of the results. Among the nine SNPs, only three SNPs were associated with an increased risk of AF. Polymorphism of rs1805127*G allele in *KCNE1* was significantly associated with the risk of AF as compared to A allele (A/G vs. A/A, OR 1.56, p = 0.049; G/G vs. A/A, OR 1.59, p = 0.044; dominant G/G + A/G vs. A/A, OR 1.57, p = 0.036) as well as rs2283228*C allele (A/C vs. A/A, OR 1.62, p = 0.001; C/C vs. A/A, OR 1.73, p = 0.012; C/C + A/C vs. A/A, OR 1.64, p < 0.001) and rs1057128*A allele on KCNQ1 (A/A vs. G/G, OR 1.92, p = 0.013; A/A + A/G vs. G/G, OR 1.78, p <0.025). The polymorphism of rs1805120*T allele on *KCNH2* was associated with a lower risk of AF. Five other SNPs (rs2237892, rs2237895, rs2237897, rs2070357 and rs2070356) showed no significant association with the risk of AF (p > 0.05) [25].

The role of mutations in the *KCNQ1* gene has also been studied in vitro. In induced atrial repolarization, the S140G mutation was associated with an almost four-fold increase in positive repolarization currents and peak potential during membrane repolarization, compared with carriers of the major allele. In induced ventricular repolarization, the peak potential in mutant cells was three times higher than that of carriers of the major alleles. In general, the total peak potential was higher compared to that in the experiments with atrial repolarization, regardless of allelic carriage. The change in potential, in turn, led to a shortening of the QT interval, which in patients with AF with the S140G mutation of the *KCNQ1* gene can be a predictive factor [26]. Similar results were obtained using in silico modelling [27].

Several studies have shown the effects of nonsense mutations in the *KCNA5* gene, encoding the Kv1.5 transmembrane protein. This protein forms sodium channels that are activated by changing the membrane potential in the region near the channel. This mutation leads to the synthesis of a defective protein and is associated with an increased risk of AF [19–30].

KCND3 gene encoding the Kv4,3 transmembrane protein is another well-studied gene associated with AF. In 2000, German researchers found that patients with AF showed a 61% decrease in mRNA expression of the gene compared with sinus rhythm patients (p <0.001), despite similar expression of mRNA of KC*NA4* and *KCNA5* genes in both groups [31]. A similar trend has been reported by Brundel et al. The expression of *KCND3* and *KCNJ5* genes was 35% and 47% lower in patients with chronic AF compared with those in the control group. Significant differences in mRNA expression were obtained only for the KCND3 gene among patients with paroxysmal AF. Likely to the previous study, similar expression of *KCNA5* gene was observed in both groups. Interestingly, the expression of *KCND3* and *KCNJ5* proteins was significantly reduced in chronic and paroxysmal AF [32]. In 2013, the role of KCND3 c.1633G>C [33] and g.112392360 G> T [34] in the development of early-onset AF was described in 209 young adults with AF (aged under 40 years).

Recently, Danish investigators have examined 14 genes encoding the proteins that make up the ion channels. One substitution in *GJA5, KCND3, KCNE5* genes, two substitutions in *KCNE1, KCNE2, SCN2B* genes, three substitutions in *KCNA5*, *KCNQ1*, *SCN3B* genes and eight substitutions in SCN5A gene were associated with early-onset AF [35].

ADRENERGIC RECEPTOR GENES

Adrenergic receptors are a class of receptors that are localized on the outer cell membrane. They are responsible for the recognition and binding of epinephrine, norepinephrine and synthetic analogues of catecholamines and mediating their physiological and pharmacological effects. Adrenergic receptors are divided into several classes based on their location and function: 1) α1A are expressed in the bladder neck, urethra and prostate; 2) α2β are expressed in arterioles (their stimulation and narrowing leads to an immediate increase in arterial blood pressure); 3) α2 are expressed in neuromuscular synapses; 4) β1 are expressed in the myocardium and kidneys (their stimulation increases both the heart rate and the strength of the heart muscle, followed by an increase in conduction velocity of the nerve impulse); 5) β2 are expressed in bronchioles and liver; and 6) β3 are expressed in adipose tissue. Based on their biological function, the genes encoding β1- and α2β-adrenergic receptors (ADRB1 and ADRA2B, respectively) are of the greatest interest to researchers examining genetic predictors of AF.

The *ADRB1* gene is located on the long arm of chromosome 10 (11q23-q25) and encodes a protein consisting of 477 amino acid residues. The available data on its role in the development of AF are contradictory. In 2014, a large prospective study in 947 adult Americans who underwent cardiac surgery (coronary artery bypass grafting, heart valve replacement, congenital heart surgery) was performed in the period from 1999 to 2005. The associations of two major SNPs, Arg389Gly (rs1801253) and Ser49Gly (rs1801252) in the *ADRB1* gene with a risk of AF in the postoperative period (14 days after the indexed surgery) were examined. Atrial fibrillation was recorded in 239 (25.2%) patients. Carriers of the Gly389Gly genotype (rs1801253) showed a two-fold increased risk of AF (OR 2.63, p = 0.008), compared with carriers of the Arg389Arg genotype. Patients who did not receive β-blocker therapy demonstrated further risk increase (OR 7.00, p = 0.005). Ser49Gly polymorphism was not associated with the risk of developing AF [36], as opposed to the results of the study in the Russian population. The latter reported that the heterozygous genotype of the rs1801252 (Ser-49Gly) of the *ADRB1* gene was associated with an increased risk of both primary and secondary AF [37]. However, carriers of the Arg389Arg genotype (rs1801253) of the *ADRB1* gene with a confirmed diagnosis of AF were less sensitive to the therapy for heart rate control and required higher dosages of drugs (atenolol, carvedilol, metoprolol, diltiazem, verapamil) compared to heterozygous carriers [38]. Importantly, a 40% decrease in mortality was observed among patients with this genotype who received bucindolol compared with carriers of the Gly allele [39].

The *ADRA2B* gene is located on the long arm of chromosome 2 (2q11.2) and encodes the α2β-adrenergic receptor. The relationship between the inheritance of certain genetic variants of the *ADRA2B* gene and the risk of familial AF was examined in the Russian population [100 probands with diagnosed AF and three siblings (n = 144)]. Three types of genotypes were identified: homozygous I/I genotype, heterozygous I/D genotype, and homozygous D/D genotype. Homozygous I/I genotype prevailed among AF patients compared with the control group (43.7% vs. 25.2%, respectively, p = 0.034). Patients were then subdivided into the primary AF group and secondary AF group. Homozygous I/I genotype was commonly found in patients with primary AF (42.2%) compared with the control group (25.2%). However, there were no significant differences found in the frequencies of genotypes in AF patients and their relatives as well as healthy volunteers. Researchers concluded that the I/I genotype of the *ADRA2B* gene could be a risk factor for primary AF [40].

GENES OF THE RENIN-ANGIOTENSIN SYSTEM

The renin-angiotensin system (RAAS) is a hormone system that regulates blood pressure through the effects on vascular tone. The peptide hormone angiotensin II plays the key role in this process. Angiotensinogen is a precursor to angiotensin II. Angiotensinogen is cleaved to form an inactive angiotensin I peptide, followed converted by the angiotensin-converting enzyme (ACE) to the active angiotensin II. The RAAS plays an important role in the pathogenesis of AF [41]. Recent studies have identified the contribution of the *ACE*, *AGT*, and *AGTR1* genes to the genetic susceptibility to AF.

In 2004, Taiwanese researchers examined the insertion/deletion (I/D) polymorphism of the *ACE* gene, 6 allelic variants of the *AGT* gene (T174M, M235T, G-6A, A-20C, G-152A, G-217A), and *AGTR1 A1166C* gene polymorphism in 250 AF patients. The *AGT* gene haplotypes significantly differed between the groups of patients with AF and the control group (250 people). In addition, reliable associations of multiple AGT gene polymorphisms, M235T, G-6A, and G-217A, with a risk of AF were reported [42]. Later, these findings were confirmed by Topal et al., Zhao et al. The T allele and the T/T genotype of M235T polymorphism, the G allele and the G/G genotype of G-6A polymorphism were associated with an increased risk of AF [43, 44]. In 2008, the same research group recruited 1,236 patients to identify associations between individual polymorphic variants of the studied genes and the risk of AF. While none associations were determined, the differences in haplotypes between AF patients and healthy subjects were confirmed. Moreover, intergenic interactions were found between the I/D polymorphism of the *ACE* gene, A1166C polymorphism of the AGTR1 gene, and AGT haplotypes [45].

Danish researchers examined the AGT gene polymorphism (A-20C, G-6A, T174M and M235T) and the I/D ACE gene polymorphism in 9,253 patients. Out of them, 968 patients suffered from AF. They found that carriers of the A/C and C/C genotypes of the AGT gene had a higher risk of AF compared with carriers of the A/A genotype (OR 1.1 and 1.5, respectively). Moreover, the combination of the I/D and D/D genotypes of the ACE gene synergized to increase the overall risk of AF (OR 1.2 and 2.4, respectively) [46].

In 2011, a meta-analysis examining the I/D polymorphism of the ACE gene, covering 18 studies (a total of 7,577 AF patients) was published. Researchers concluded that there were no evidences to confirm the presence of the relationship between this polymorphism and the risk of AF. But they hypothesized that the ACE gene and AF in patients with arterial hypertension were linked [47]. Then, a number of studies of the I/D polymorphism of the ACE gene were performed. The results of most studies confirmed previous data on the association of the ACE gene I/D and D/D genotypes with an increased risk of AF [43, 48, 49]. However, one of the recent studies in the Russian population has reported that carriage of the homozygous D/D genotype may have a relatively protective effect on the development of AF [50].

Feng et al. have recently showed the associations of rs1492099 polymorphism in the AGTR1, gene encoding the angiotensinogen receptor, (the frequency of the minor allele in the patient group was 14.2% vs. 8.8% in the control group, OR 1.727) and rs6632677 polymorphism of the ACE2 gene (the frequency of the minor allele was 16.3% in male patients vs. 9.1% in healthy men, OR 1.954) with AF in Chinese population [51].

NITRIC OXIDE SYNTHASE GENE

Various vasodilation factors that regulate vascular tone can modulate contractile activity of the myocardium, thus participating in the pathogenesis of AF. These factors include nitric oxide (NO), which prevents the tonic contraction of blood vessels originated by endocrine, neuronal or local sources followed by NO synthase (NOS) formation. A decrease in the production of NO synthase can cause oxidative stress and provoke changes in the myocardial conduction system, leading to the development of AF [52]. NO synthase is encoded by the *eNOS* gene located on chromosome 7. Russian investigators examined 100 probands and their three siblings and compared the findings with those obtained for 91 healthy subjects recruited in the control group [53]. The homozygous G/G genotype (G894T) prevailed in AF patients (58.5%) compared with the control group (39.6%; p = 0.039). Patients with AF were further subdivided into the groups with primary and secondary AF. The homozygous G/G genotype was found only in patients with primary AF compared with the control group. Patients with secondary AF did not report any statistically significant differences in the frequencies of alleles and genotypes. Thus, the G allele has been shown to serve as a predisposing factor for atrial fibrillation.

The relationship of G894T, T786C, and 4b/a in the *eNOS* gene and AF in patients with heart failure was also studied. The G allele (G894T polymorphism) was more common in the group of patients with AF. Carriers of the G/G genotype demonstrated almost a three-fold increased risk of AF [54, 55]. Other polymorphic variants were not associated with AF [54, 57]. Nevertheless, recent evidences have suggested the protective effects of T786C polymorphism on the risk of AF [56].

G PROTEIN-COUPLED RECEPTOR GENES

G protein-coupled receptor kinases (GRKs) are a family of protein kinases that phosphorylate the intracellular domains of G-protein coupled receptors (GPCRs) and regulate their activity. Phosphorylation occurs after ligand receptor binding and G-protein dissociation. In addition, GRK kinases regulate the cellular response independently of their kinase activity. The *GRK5* gene is an important regulator of GPCR function that maps on chromosome 10 at the region of q24 (10q24). Recent studies have shown that the polymorphism of this gene is associated with a decrease in mortality among African Americans with heart failure and coronary artery disease [58].

In 2014, a study in 563 patients undergoing coronary artery bypass grafting, of whom 111 patients developed postoperative AF, was performed. A total of 492 SNPs in 10 genes were analyzed. Four polymorphic variants of the *GRK5* gene were associated with an increased risk of postoperative AF (rs3740563, OR = 2.75; rs4752292, OR = 2.21; rs11198893, OR = 2.51; rs10787959, OR = 1, 72). The meta-analysis showed that the polymorphic variant rs3740563 played a key role in the formation of individual sensitivity to AF. Thus, the genetic variation of the *GRK5* gene was associated with postoperative AF in patients who underwent coronary artery bypass grafting, despite preoperative therapy with β-blockers [59].

A similar study was conducted in Chinese population in 2015. The study included 1,348 patients. Nine SNPs were examined. Of them, six SNPs were assessed in another group of 2,000 patients to validate the results. Only two variants of the *GRK5* gene (rs4752292 and rs11198893) were associated with an increased risk of AF (OR for the minor allele was 1.32 and 1.47, respectively) [60].

CONCLUSION

Our review confirms the genetic contribution of various systems in the pathogenesis of AF (ion channels, adrenergic receptors, the renin-angiotensin system, NO synthase, and GRK kinase receptors) and formation of increased individual risks of its onset. The available data have indicated a fairly extensive panel of genes that can serve as potential molecular genetic markers of AF development, as well as stated the need to study intergenic interactions between potential candidate genes. The presented inconsistencies on the association of some genes with AF as well as interpretative variations depending on the studied population have set the rationale for further studies in different ethnic groups.

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Historical and modern aspects of surgical treatment of Ebstein's anomaly

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ABSTRACT

Congenital heart defects (CHDs) are recognized as the most common type of congenital pathology. The frequency of CHDs reaches 2.4–14.2 % per 1000 newborns. Ebstein's anomaly is a rare and complex pathology that can be manifested clinically at any age. Drug treatment is ineffective in patients with this pathology. Preference in this case should be given to surgical treatment. Cardiac surgeons must know basic methods of correction of this pathology, their advantages, and disadvantages. The literature review shows the evolution of Ebstein's anomaly surgical correction techniques from the middle of the XX century to the present moment. The description of the main tricuspid valve repair techniques, which had an impact on the development of Ebstein's anomaly surgery, is given. The volume of flap tissue that can be separated from the wall of the right ventricle is the key to successful valve repair. A case of tricuspid valve repair and replacement is given. "Cone" reconstruction is the most promising modern technique. There is also a need in new techniques and modification of the existing ones. The works aimed at their improvement and elimination of imperfections are promising.

Key words: congenital heart defects, Ebstein's anomaly, tricuspid valve replacement, tricuspid valve repair.

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Исторические и современные аспекты хирургического лечения аномалии Эбштейна

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РЕЗЮМЕ

На сегодняшний день врожденные пороки сердца (ВПС) занимают одно из ведущих мест среди всей врожденной патологии. Частота ВПС достигает 2,4–14,2% на 1000 новорожденных. Аномалия Эбштейна – редкая и сложная патология, клиническая картина которой может проявляться в любом возрасте. Медикаментозное лечение пациентов с этой патологией малоэффективно. Предпочтение в данном случае следует отдать хирургическому лечению. Для кардиохирурга важно знать основные методы коррекции этой патологии, их преимущества и недостатки.

В литературном обзоре показана эволюция методов хирургических коррекции аномалии Эбштейна с середины XX в. по настоящий момент. Дано описание хирургической техники основных способов пластики трикуспидального клапана, оказавших влияние на развитие хирургии аномалии Эбштейна. Ключевым моментом для успешной пластики клапана становится объем ткани створки, который возможно отделить от стенки правого желудочка. Приведен опыт выполнения протезирования и пластики трехстворчатого клапана. Наиболее многообещающим методом на сегодняшний день является «конусная» реконструкция. Таким образом, существует необходимость поиска новых методов и модификации уже имеющихся. Работы, направленные на их усовершенствование и устранение недостатков, являются перспективными.

Ключевые слова: врожденные пороки сердца, аномалия Эбштейна, протезирование трикуспидального клапана, пластика трикуспидального клапана.

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

Ebstein's anomaly (EA) is a rare congenital heart disease of the "blue" type, the incidence of which is 5.2 cases per 100,000 newborns, which is about 1% of all congenital heart defects [1-3]. Wilhelm Ebstein first described this pathology in 1866 during an autopsy of a nineteen-year-old patient, Josef Prescher, who died of chronic heart failure. Working as a physician's assistant and a prosecutor, he published his article describing this clinical case [4]. In our country, the first description of this pathology belongs to A.A. Elashevich (1925). The term "Ebstein's anomaly" as a designation of a nosological unit was first introduced into the literature in 1937 by Yater W. and Shapiro M., who described 16 clinical cases of this defect in their article [5]. According to various authors, the average life expectancy in the natural course of the disease is up to 50 years, with 80–87% of deaths occurring at the age of 30–40 years. In recent years, the interest of the surgical community specifically in plastic surgery in EA has increased, so the purpose of the literature review is to describe possible surgical approaches to correct this anomaly.

To date, there is no unequivocal opinion regarding the etiology of the disease. Most researchers are inclined to the multifactorial nature of this disease. Embryonic development of the tricuspid valve (TV) begins with the 5th week of intrauterine development. It has been established that precisely during this period disruption of the laying of the cardiac jelly between the ventricular myocardium and the endocardium occur, which leads to disruption of the delamination process, that is, embryonic separation of the tissue of the cusp from the tissue of the right ventricle (RV) [6].

EA is characterized by the following characteristics (Fig. 1) [7].

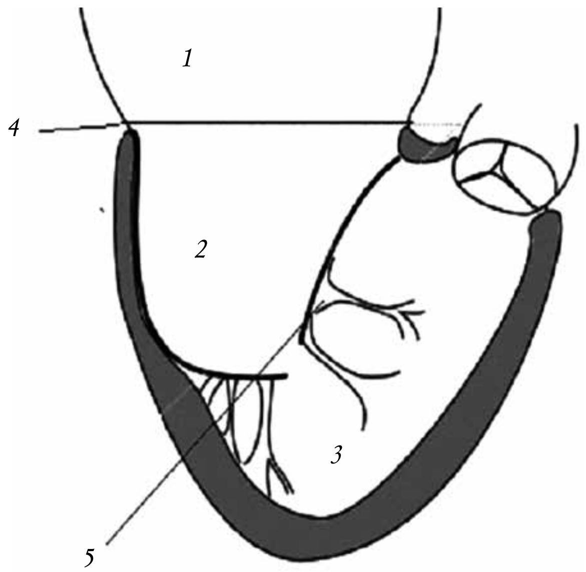


Fig. 1. Scheme of the structure of the right ventricle with Ebstein's anomaly: *1* – right atrium; *2* – atrialized part of the RV; *3* – the functional part of the RV; *4* – anatomical ring of the TV; *5* –functional ring of the TV

The first characteristic is the fusion of the cusps with the ventricular myocardium. As a rule, the anterior cusp is delaminated more from the ventricular wall, while the posterior and septal cusps are delaminated minimally. In the most severe cases, the septal cusp is a ridge of connective tissue. The second is the anterior-apical displacement of the functional ring of the TV (the place of transition of the atrialized part of the ventricle to the true right ventricle with normal delamination of the cusps) towards the outflow tract of the RV. The third characteristic is the redundancy of the tissue of the anterior cusp (sail-shaped cusp), its fenestration and limitation of mobility between the valve cusp and the RV due to chord connection. The fourth characteristic is the presence of a thinned, dilated and akinetic part of the RV (atrialized part), and the number of myocardial fibers in this area is much less than normal [8]. The fifth sign is the expansion of the true (anatomical) fibrous ring of the TV. As the severity spectrum increases, the fibrous transformation of the cusps from their muscle precursors increases during embryogenesis [9, 10]. In EA, the structure of the RV myocardium with a smaller number of myocyte fibers was altered, the nuclei of myocytes were displaced under the sarcolemma, and the Z-strips of sarcomeres were broken, so this anomaly can be considered a type of ventricular myopathy [11].

The most common concomitant defect is an atrial septal defect (42–60% of cases), while ventricular septal defect, transposition of the great vessels, and pulmonary stenosis are less common [10]. Due to the underdevelopment of the true fibrous ring, additional pathways function that are clinically manifested by Wolf – Parkinson – White syndrome in 7–30% of cases [12, 13].

In 1988, A. Carpentier [14, 15] (Fig. 2) proposed the following classification of Ebstein's anomaly.

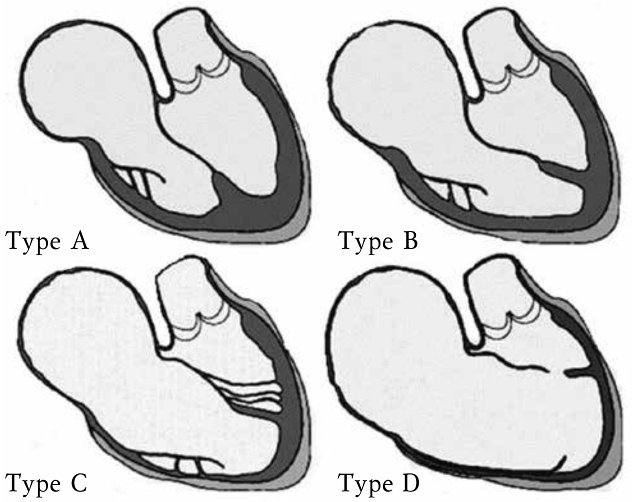


Fig. 2. Types of Ebstein's anomaly [14]: type A – true RV volume is satisfactory; type B – here is a large atrialized component of the RV, and the anterior cusp of the TV moves freely; type C – the anterior cusp of the TV is limited in its movement and can cause obstruction in the outflow tract of the RV; type D – almost complete atrialization of the ventricle with the exception of a small part of the outflow tract of the RV

In the Russian clinical guidelines, the extreme form of AE is additionally highlighted – type E: the fused anterior, posterior and septal valve cusps together with the inflow section of the RV form a "tricuspid sac". The wall of the inflow section of the RV is thinned. The communication between the "tricuspid sac" (atrium) and the infundibulum (ventricle) is often formed by a narrow hole in the region of the so-called anterior-septal commissure.

The natural clinical course of this pathology depends on the degree of TV dysplasia and function of the RV. Despite the extensive experience in surgical treatment, indications for surgery are not defined. To date, indications for surgical treatment are as follows: decreased exercise tolerance; cyanosis; progressive dilatation of the right heart (cardiothoracic index over 60%); dysfunction of the right ventricle (ejection fraction below 30%, displacement of the interventricular septum towards the left ventricle); left ventricular dysfunction (decrease in ejection fraction below 50%, reduced end-diastolic volume); atrial arrhythmias.

Relative contraindications for correction according to J. Dearani et al. are [16]: age over 50; severe pulmonary hypertension; a significant decrease in left ventricular function (ejection fraction of less than 30%); a complete violation of the delamination of the septal and posterior cusps of the tricuspid valve, while the delamination of the anterior cusp is less than 50%.

But over the past few years, the approach to indications for surgical treatment has fundamentally changed. Since almost all patients require surgical intervention for this pathology sooner or later, the team of authors from the Mayo Clinic, led by J. Dearani, proposes to perform the operation as soon as the defect was diagnosed. This concept is based on the fact that with age, RV function deteriorates due to volume overload and the likelihood of right ventricular heart failure in the early postoperative period increases.

Ebstein's anomaly surgical correction options include:

1. Two-ventricular correction (valve prosthetics (biological or mechanical prosthesis), valve plastic).
2. One and a half ventricular correction (plastic or prosthetic valve with bi-directional cava pulmonary anastomosis).
3. One-ventricular correction (Starnes operation).

Until the middle of the last century, treatment of patients with EA was extremely conservative. The results of drug therapy were not significant; therefore, the main priority was for surgical care. Currently, all operations are performed on an open heart using a cardiopulmonary bypass and cardiac arrest.

Most babies in the newborn period do not require surgery. However, in extreme forms of the defect with severe clinical manifestations such as shortness of breath, cyanosis, and tachycardia and in the absence of the effect of prostaglandin E_1 therapy, surgical intervention is necessary. Right ventricular failure is also increased by the increased pulmonary vascular resistance, which persists in the first few weeks of life. To assess the survival of such patients in 1992, the GOSE scale (Great Ormond Street Echo) was developed [17]. The GOSE index is equal to the ratio of the sum of the sizes of the right atrium and the atrialized part to the sum of the left atrium, left ventricle and the functional part of the RV (the dimensions of the heart chambers are determined in the four-chamber position). Depending on the number of points, the risk of death in the natural course of the defect is determined.

Table 1

GOSE SCORE

Parameter	SCORE, in points	Mortality, %
GOSE I	<0.5	0
GOSE II	0.5–0.99	10
GOSE III	1–1.49	44
GOSE IV	>1.5	100

If the anatomy of the cusps and ventricle is not suitable for performing two-ventricular correction, one-ventricular correction is performed (Fig. 3). This technique was first introduced by V.A. Starnes in 1991, 27 patients with the Starnes procedure in 1989–2015 were operated on at Children's Hospital Los Angeles.

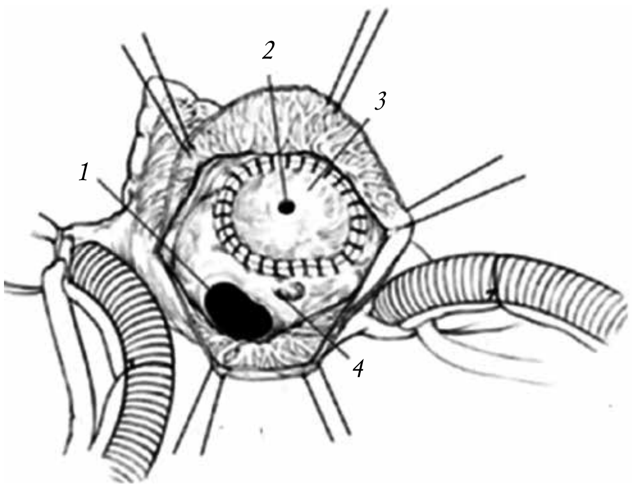


Fig. 3. Starnes operation. Image modified from [18]: *1* – dissected interatrial septum; *2* – fenestration PTFE patch 4 mm; *3* – PTFE patch; 4 – coronary sinus

An exception is made from the bloodstream of the RV by fixing a polytetrafluoroethylene patch with a fenestration of 4 mm to the true fibrous ring. In this case, the coronary sinus remained on the side of the right atrium. An atrial septum was additionally excised to form a single atrium. [18] Pulmonary blood flow was provided by a modified Blok – Taussig shunt. At the next stage of hemodynamic correction at the age of 3–6 months, a bi-directional cava-pulmonary anastomosis is performed; at 2–4 years old, total cava-pulmonary connection is performed. In newborns with an extreme form of Ebstein's anomaly, the right ventricle is dilated and is not able to function adequately to maintain cardiac output; therefore, it is necessary to exclude the RV from the bloodstream [19, 20].

The surgeon is most interested in two-ventricular correction by means of plastic surgery or valve prosthetics. The first prosthetics of TV in EA was performed by C. Barnard in South Africa (Cape Town) in 1963. Then the procedure was modified by cardiac surgeons D. Ross and J. Somerville, who used an aortic homograft in 1970 to avoid the need for anticoagulants [21]. In 1988, N. Kumar and B. Dubey used pulmonary homograft for implantation in the tricuspid position. In the USSR, a successful valve replacement was performed by G.M. Soloviev in 1964 [22].

Valve prosthetics are possible using a biological or mechanical prosthesis [23]. A characteristic of prosthetics is that the valve is located above the true fibrous ring of the TV. The tissue of the cusps, causing obstruction of the outflow tract of the RV, must be excised, and the true fibrous ring is narrowed to the size of the prosthesis. The atrialized part of the RV is also reduced. On the posterolateral wall, the tissue is usually thinned, so the suture line should be closer to the atrium to avoid damage to the right coronary artery. To avoid damage to the atrioventricular node, the suture line is located above the coronary sinus, thus, the drainage of venous blood will be carried out in the RV.

Among the methods of surgical treatment, valve replacement for this pathology has significant disadvantages compared with reconstructive surgery. According to M. Brown et al., the long-term results of 378 TV prosthetics operations are: 6% mortality in the early postoperative period and 17% in the first ten years after. The frequency of prosthetics was 41% over the next 20 years [24].

In 2007, H. Bartlett et al. in their study, they described the results of TV prosthetics in 97 patients whose average age was (2.9 ± 1.7) years. 44 children received a mechanical prosthesis and 53 received a biological prosthesis. As a result, 26 (27%) patients died in the early postoperative period. Among the complications in the early postoperative period, complete atrioventricular blockade was observed in 13 patients, which required the implantation of a pacemaker. In the group with a mechanical prosthesis, the frequency of obstruction was higher than in the group with a biological prosthesis (23 versus 6%). Valve thrombosis in the early postoperative period was observed in five patients. All these patients received a mechanical prosthesis.

TV prosthetics is associated with high mortality, especially in children under 1 year of age [25]. A significant disadvantage of prosthetics with biological valves in children is calcification, especially during the period of active growth and increase in the level of hormones in the blood. The need for continuous use of anticoagulants in case of implantation of a mechanical prosthesis gives serious complications and deterioration in the quality of life of young patients [26]. In addition, with the growth of the child, a discrepancy between the size of the prosthesis and the size of the chambers of the heart occurs, which again requires re-prosthetics with implantation of a larger prosthesis; the use of prostheses in newborns is impossible. According to Russian data of Yu.N. Gorbatyh et al., the need for prosthetics in a 10-year period reaches 45–60% [27].

TV prosthetics is justified only in cases of inefficiency or inability to perform plastic surgery; therefore, there was a great need for new valve plastic methods.

K. Hardy can be considered a pioneer in valve reconstruction with EA, who in 1964 first put forward the concept of reconstructing the valve from its own reduced tissue of the cusps, and also proposed to exclude the atrialized part of the ventricle [28]. In our country, the first attempt to perform valve plastic surgery was made by E.N. Meshalkin, using a strip of Ayvalon to restore the posterior and septal cusps, but the result was unsatisfactory, and only in 1978 I.K. Okhotkin reconstructed the valve. In 1979, based on the concept of K. Hardy, a team of surgeons from the Mayo Clinic (USA) under the direction of Professor G. Danielson developed the most advanced technique for that time.

The original method consisted of vertical plication of the atrialized part of the RV, "pulling" of the functional fibrous ring to the true fibrous ring using U-shaped sutures on pads, and narrowing of the fibrous ring along the anteroposterior commissure (Fig. 4). This technique is applicable provided there is sufficient mobility of the anterior tricuspid valve cusp (leaflet). As a result, a single-leaflet valve is formed [29]. M. Brown et al. applied the G. Danielson method in 182 patients.

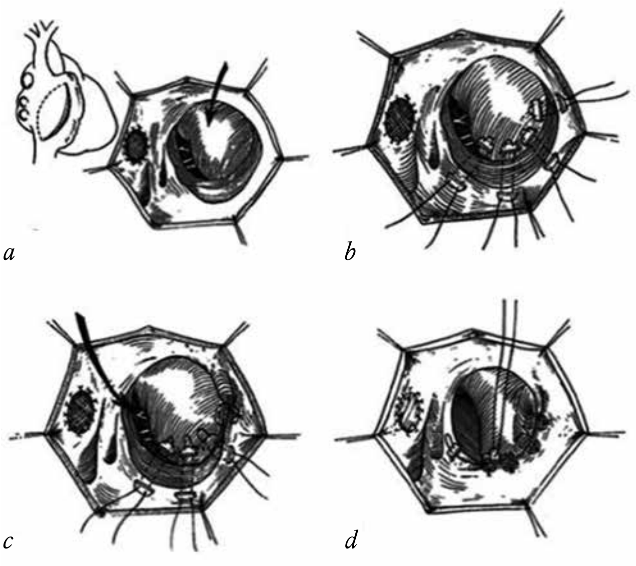


Fig. 4. Surgical correction according to the method of G. Danielson (*a–d*). Image modified from [29]

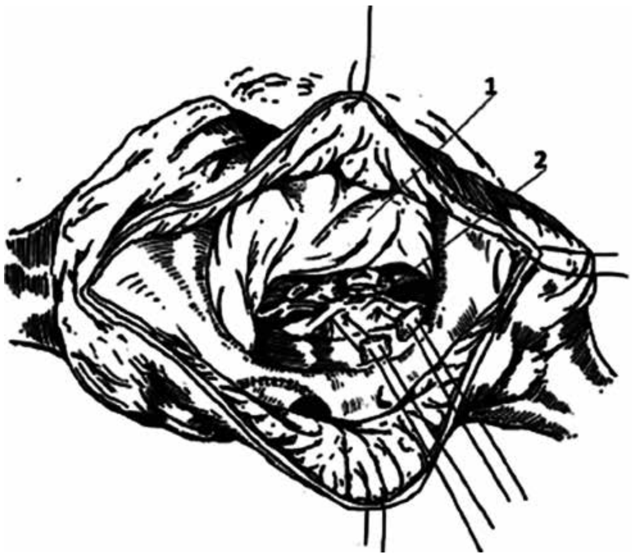


Fig. 5. Surgical correction according to the method F. Sebening stitch. Image modified from [31]: *1* – anterior cusp; *2* – plication of the posterior cusp

Mortality in the early postoperative period was 5%, over the course of 10 years – 12%. Over the next 20-year observation period, the number of reoperations with this method is 36%. This operation for a long time had no analogues for the correction of different types of EA.

The following technique was developed by the German surgeon F. Sebening. The essence of this method is to move the papillary muscle of the anterior cusp of the TV closer to the interventricular septum towards the true tricuspid ring (Fig. 5). The ultimate goal of this method is to create a single-leaflet valve. In this case, the anterior cusp, which is usually mobile, approaches the edge of the true fibrous ring [30].

This method can be combined with other methods of creating a competent TV, and it can be used as an independent method [31].

Takeushi Komoda (2007) used a combination of F. Sebening stitch and Hetzer techniques (see below). The study included 28 patients. In a group of 11 people, a combination of methods was used. Postoperative observation was carried out for 32 months. As a result, there was no postoperative mortality in the group with a combination of methods, and there were also no reoperations [32, 33].

The techniques of G. Danielson and F. Sebening stitch were similar and consisted of creating a valve by plicating the cusp tissue from the ventricle. They had positive aspects, but led to deformation in the area of the interventricular groove, which could lead to compression of the right coronary artery and its branches. This method is not widespread, since it was applicable only in patients with types A and B, when the tissue of the posterior and septal cusps is sufficiently mobile, and the anterior cusp should be of sufficient area and not limited in the movement of the chords.

In 1988, the French heart surgeon A. Carpentier for the first time drew attention to the possibility of creating a valve using its own hypoplastic cusp tissue. In his innovative technique (Fig. 6), he first described the delamination of the anterior and posterior cusps of the TV by dissecting fibrosed chords from the walls of the myocardium, starting from the highest point (functional ring).

Dissection of fibrosed chords gives mobility to cusp tissue for subsequent distribution over the entire surface of the true fibrous ring. However, chords attached to the papillary muscle must remain intact in order to avoid cusp prolapse. Then, the atrialized part is plicated along the posterior wall of the RV and narrowing of the true fibrous ring of the valve. Previously delaminated cusps are sutured onto a true fibrous ring with a clockwise rotation. Thus, a bicuspid valve is created at the level of the true fibrous ring. In adult patients, the fibrous ring is additionally fixed with a stented support ring. S. Chauvaud et al. reported 9% mortality in the early postoperative period and 13% mortality during the 10-year period. The number of reoperations was 11% over the next 20 years [14].

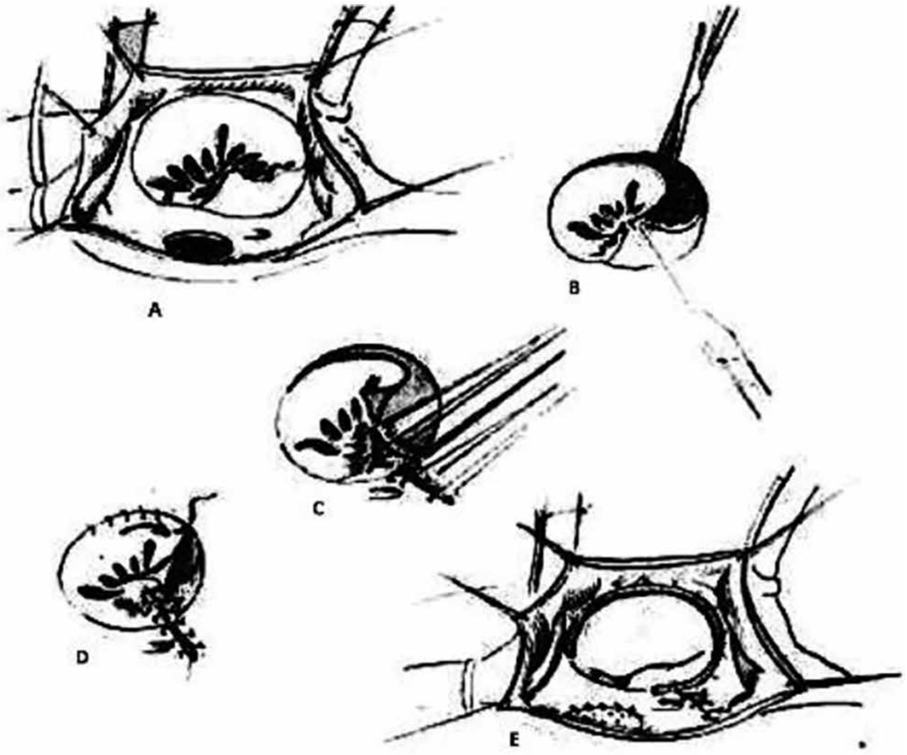


Fig. 6. Surgical correction according to the method of A. Carpentier (*a–e*). Image modified from [14]

The next intervention option was the technique of the German cardiac surgeon R. Hetzer, developed in 1998. The author describes various options for plastic surgery in his works, but the key point of his technique is suture plication of the TV. In this case, the posterior part of the TV is stitched with the septal part. In some cases, R. Hetzer suggested creating a valve of the double orifice type (Fig. 7). In this case, the cusp tissues are not mobilized to create a valve, and the atrialized part is not plicated; failure on the valve is reduced by reducing the surface area of the true fibrous ring [34]. The treatment results were published in 2015: mortality was 2.4% in the early postoperative period and 8.7% during the 10-year period. The number of reoperations left 7.1% over the next 20-year follow-up period [35]. In his works, R. Hetzer described various options for narrowing the fibrous ring, but the method of stitching the anterior and posterior parts of the fibrous ring is taken as a basis.

In 2000, the C.J. Knott-Craig method was published, which is used in the neonatal period by performing two-ventricular correction. C.J. Knott-Craig et al. performed surgical

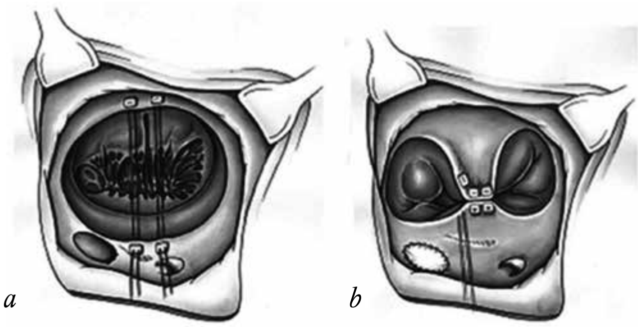
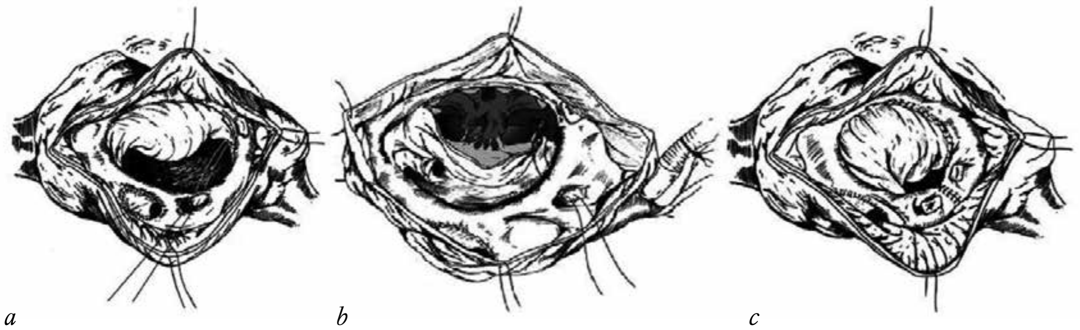


Fig. 7. Surgical technique of the double orifice (*a–b*). Image modified from [34]

treatment at an Oklahoma hospital on 27 children with EA, 22 of whom were newborns. The first step is the delamination of the front edge of the anterior valve cusp to ensure its mobility. Then, a suture is performed through the dominant papillary muscle of the anterior cusp with the bringing of the latter to the interventricular septum on the opposite side. To suture the enlarged fibrous ring, sutures are placed on the anteroposterior commissure of the valve or through the medial wall of the coronary sinus. This maneuver provides pulling the papillary muscle of the anterior cusp to the opposite wall of the ventricle, and also gives greater freedom of the anterior cusp and increases its coaptation.

In the original method, a resection of the wall of the right atrium in the form of an ellipse is performed. When performing it in the lower corner of the incision, one should be careful not to damage the right coronary artery, since in newborns the border of the atrialized part of the ventricle and the right atrium is quite difficult to distinguish. Then, the atrialized part of the RV is sutured. The technique of TV plastic is similar to the technique of G. Danielson, the result of which should be the creation of a single-leaf valve. A prerequisite for this is the mobility of the anterior valve cusp [36].

If the anterior cusp has multiple chordal adhesions, then with an insufficient cusp length, it is possible to increase its surface using an autopericardial patch [37].



a *b* *c*

Fig. 8. Surgical correction according to C.J. Knott-Craig (*a*–*c*). Image modified from [36]

Atrial septal defect closure is usually performed with a defect of 4 mm left. In the neonatal period, the shunt has a discharge function for right-left discharge with right ventricular heart failure and increased pulmonary vascular resistance, which remains in newborns for the first few weeks of life.

In 2001, the arsenal of surgical interventions was supplemented by the technique of the Chinese cardiac surgeon Q. Wu. This technique was aimed at improving valve function due to the tissue of the posterior cusp. Q. Wu describes the technique as follows (Fig. 9): the posterior and septal cusps from the RV walls are mobilized, the cusps are stitched and attached to the level of the true fibrous ring, creating a bicuspid valve. In some cases, plastic on the septal cusp with an autopericardial patch was performed. In this technique, instead of plicating the atrialized part, a resection of the right ventricular triangular flap is performed [38, 39].

a *b* *c*

Fig. 9. Surgical correction according to the Wu method. Image modified from [38]: *a* – initial view of the valve (the delamination boundary is indicated by a dashed line); *b* is the final view of the valve; *c* – suturing of the atrialized part; *1* – autopericardial patch

According to the results of Dr. Q. Wu et al., the early and long-term postoperative period in 34 patients proceeded without complications. TV insufficiency was insignificant [40]. The use of this method has significant limitations, since in extreme forms the tissue of the septal and posterior cusps is practically absent. With resection of the atrialized part and suturing with a twisted suture, the risk of postoperative bleeding and damage to the right coronary artery and its branches increases.

The most progressive method today is considered to be a "cone" reconstruction of the TV. This method was first proposed by the Brazilian cardiac surgeon J. da Silva in 1989. The basis for this reconstruction was the technique of A. Carpentier [41]. To date, the greatest experience in conducting "cone" reconstruction has been accumulated in the Mayo Clinic (Rochester, USA).

Surgical technique (Fig. 10) is as follows. Access to the TV is through the right atriotomy after connecting the heart-lung machine. Separation of the anterior cusp begins at 12.00 with the relative application of the clock-face to the TV, retreating from the fibrous ring a few millimeters. The incision continues clockwise towards the rear valve cusp. Fibrous and muscle adhesions between the anterior valve cusp and the right ventricle are dissected, releasing it. This is the most important surgical technique in the operation, since the maximum amount of tissue of the anterior cusp for subsequent plastic depends on its result. In some cases, longitudinal cuts are made 1/3–1/4 of the length from the edge of the anterior cusp, forming so-called neochords for better valve capacity in diastole.

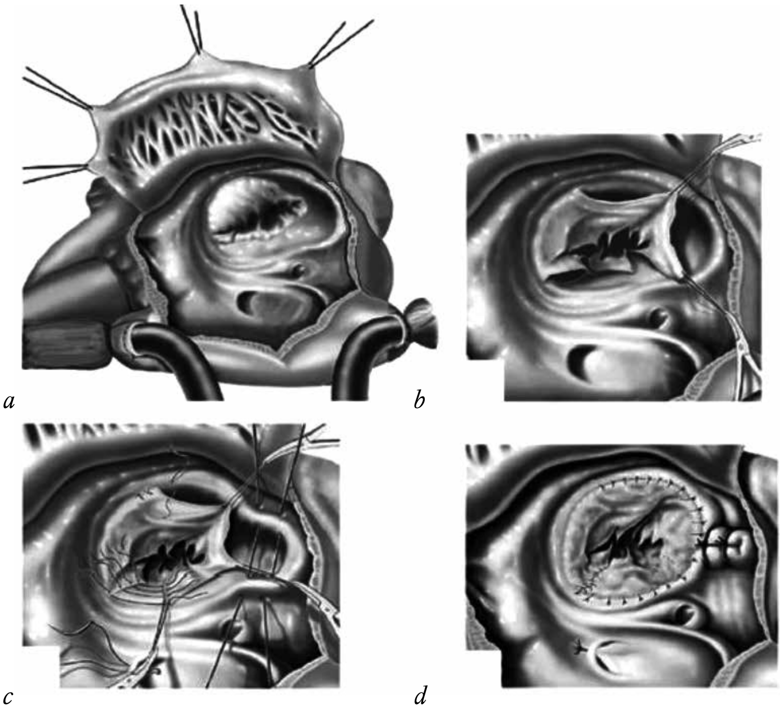


Fig. 10. Cone reconstruction of the tricuspid valve. Image modified from [42]: *a* – view of the valve before reconstruction; *b* – delamination of the anterior and posterior cusps; *c* – stitching of the valve cusps; *d* – final view of the valve in the form of a cone [41]

This technique is used when it is not possible to separate the edges of the cusp. After separation of the anterior cusp, the posterior and septal cusps follow, if possible. Upon completion of the process of delamination of the cusps, longitudinal plication of the atrialized part of the RV is performed with narrowing of the fibrous ring of the right atrioventricular valve. After plication, the mobilized tissue of the cusps is sutured together, forming a single cusp, which is distributed 360° around the circumference and is fixed to the true fibrous ring.

The cone reconstruction described by J. da Silva and colleagues differs from previous valvuloplasty methods in that it is closest to the "anatomical correction". The final result of the "cone" reconstruction includes a 360° distribution of TV tissue at the level of the true fibrous ring. This allows the TV cusps to close in the same way as in a normal valve. In addition, the reconstructed TV is attached to the true fibrous ring, so that the valve attachment point is now in its normal anatomical position. The thinned atrialized part of the RV is plicated, thus eliminating the dyskinetic part of the RV. "Cone" reconstruction restores valve anatomy better than any method described above, and can be applied to a wide variety of valve anatomical variations encountered with Ebstein's anomaly [42].

The results of Dr. J. da Silva et al. described 52 patients operated on by this method. Early postoperative mortality was 3.8%, over the next 7 years – 14%, over the observation period - only 4 reoperations. C. Pizarro et al. reported about the implementation of the "cone" reconstruction on two children in the neonatal period, and one child was operated on before the age of 1 year. There was no postoperative mortality, and observation during the 1st year showed a positive trend according to echocardiography and chest x-ray. All patients retained functional capacity of the I class and TV deficiency under the 1st degree [43].

The article by K. Holst and J. Dearani (2018) presents the results of the treatment of 235 patients with "cone" reconstruction and a subsequent ten-year follow-up period. The study included 134 children and 101 patients over 18 years of age. In the early postoperative period there was one death (0.4%), the number of reoperations was 14 cases (5.9%). Long-term valve insufficiency was within the 1st – 2nd degree, statistically significantly decreased RV dysfunction and decreased apical diastolic RV area [44].

M. Ibrahim et al. on the example of 23 cases of "cone" reconstruction, they showed a decrease in insufficiency on TV and an increase in the end-diastolic volume of the left ventricle [45]. R. Lange et al. using echocardiography and magnetic resonance imaging showed an increase in stroke volume and a decrease in the size of the RV 6 months after this treatment method [46].

In Russia, this technique is actively used in cardiac surgery clinics in Tomsk [47], St. Petersburg [48], and Samara [49]. In the Cardiology Research Institute of Tomsk, "cone" reconstruction has been used since 2011; during this period more than 40 cases of its implementation have been accumulated.

With reduced RV function and inability to adequately provide pulmonary blood flow, a bidirectional cavopulmonary anastomosis is indicated. The method is performed as follows: the superior vena cava is cut off from the right atrium 0.5–1 cm above its entry (to exclude damage to the sinus node), the right atrium is sutured. The right pulmonary artery is dissected along, strictly above the superior vena cava, and sutured to the superior vena cava [50, 51]. The functions of the anastomosis of the superior vena cava and the right pulmonary artery are as follows: reduction in preload on the right ventricle (in childhood by about 1/2 venous return and 1/3 in adulthood); increased preload on the left ventricle.

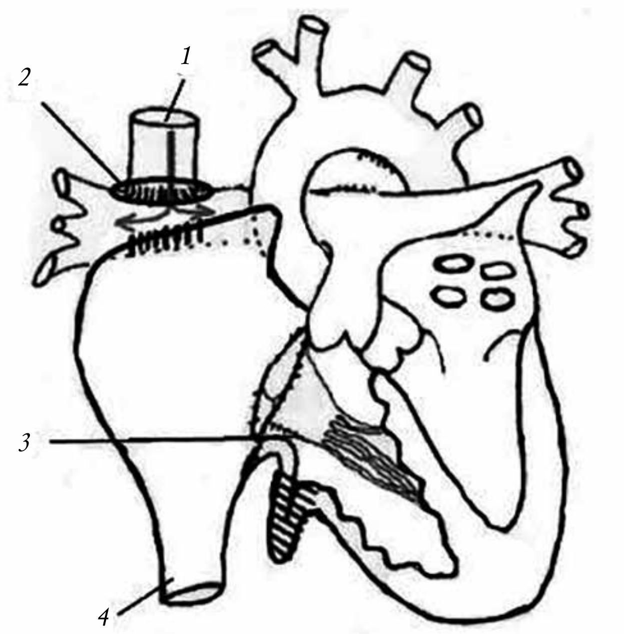


Fig. 11. Bidirectional cavopulmonary anastomosis in combination with the "cone" reconstruction of the tricuspid valve: *1* – superior vena cava; *2* – bidirectional cavapulmonary anastomosis; *3* – cone reconstruction of the tricuspid valve; *4* – inferior vena cava

CONCLUSION

Surgical treatment of Ebstein's anomaly has been a problem for more than half a century. After C. Bernard replaced TV with a prosthesis for the first time, many different TV techniques were developed. The first attempts at valve plastic surgery were made by S. Hunter and W. Lillehei in 1957. Valve prosthetics can be called a "desperate operation" when the surgeon is unable to construct a new valve from its own tissues. There are works on the use of own autopericardial tissue and the use of bone marrow cells - the precursors of $CD133^+$ endotheliocytes in the treatment of EA. In Russia, this technique is being developed at the A.N. Bakulev National Medical Research Center of Cardiovascular Surgery under the supervision of Academician L.A. Bokeriya [52].

The accumulation of experience and the improvement of surgical techniques have led to significant progress in the treatment of this heart disease. To date, according to world literature, the method of choosing the surgical treatment of EA is a "cone" reconstruction, which shows good results both in the near and in the distant postoperative period. However, debatable questions remain about the appropriate anatomy of the defect, the limiting possibilities of mobilizing cusp tissue for correction, and indications for performing a bidirectional cavopulmonary anastomosis. The ability to perform a "cone" reconstruction or other type of valve repair for Ebstein's anomaly also depends on the anatomy of the valve and subvalvular apparatus.

The most important feature for performing valve plastic surgery is the free, non-adhered edge of the anterior valve cusp, from which the largest volume of tissue is obtained. Reconstruction of the valve is difficult if the anterior cusp is strongly displaced to the apex of the RV, and there are extensive fibrous adhesions of the valve cusp with the adjacent myocardium. In his work, J. Stulak et al. indicate that the necessary conditions for successful correction are: delamination of the anterior cusp of more than 50% of its area; the presence of long chords and papillary muscle, which does not cause obstruction of the outflow tract of the right ventricle. The more tissue can be obtained by separating the cusp from the myocardium, the smaller the gradient and insufficiency on the valve can be expected in the end.

Thus, the "cone" reconstruction is considered the most "anatomical" of all existing (today) types of correction, and can be performed for a wide range of anatomical variants of Ebstein's anomaly in both childhood and adulthood.

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Takotsubo syndrome after mitral valve replacement and defibrillation

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ABSTRACT

The article describes the case of Takotsubo syndrome, which arose in a 71-year-old female patient after a mitral heart valve replacement , performed due to its severe (3rd degree) insufficiency of non-rheumatic genesis. This pathology is quite rare. Questions of its etiology and pathogenesis remain controversial. A special feature of this case is the development of reversible left ventricular dysfunction in the early postoperative period after mitral valve replacement. The reduction of the left ventricular ejection fraction to 25% with hyperkinesia of its basal parts with subsequent recovery of the ejection fraction to 56% 3 weeks after the onset of the disease was observed. There were also electrocardiographic changes simulating acute circular myocardial infarction with the absence of hemodynamically significant lesions of the coronary arteries. The patient had risk factors for this syndrome (age, female gender, stress situation, surgical intervention, administration of dobutamine and fluoroquinolones). For the reasons given above, the presence of Takotsubo syndrome was suspected. This case demonstrates the possibility of developing takotsubo syndrome after cardiosurgical interventions and defibrillation.

Key words: takotsubo cardiomyopathy; valvular heart disease; ventricular fibrillation; mitral valve replacement; cardiac surgery; defibrillation.

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Синдром такоцубо после протезирования митрального клапана (клинический случай)

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РЕЗЮМЕ

В статье представлен случай синдрома такоцубо, резвившегося у 71-летней пациентки после протезирования митрального клапана сердца, выполненного в связи с его выраженной (3-я степень) недостаточностью неревматического генеза. Данная нозология является редко встречающейся. В литературе имеются единичные разрозненные сообщения о синдроме такоцубо после кардиохирургических вмешательств и электроимпульсной терапии. Вопросы этиологии и патогенеза остаются дискутабельными. Особенностью данного случая является развитие обратимой дисфункции левого желудочка в раннем послеоперационном периоде после протезирования митрального клапана – снижение фракции выброса левого желудочка до 25% с гиперкинезией его базальных отделов с ее последующим восстановлением до 56% через 3 нед после дебюта заболевания. Также имели место электрокардиографические изменения, имитирующие острый циркулярный инфаркт миокарда, при отсутствии гемодинамически значимых поражений коронарных артерий. У пациентки были выявлены факторы риска развития данного синдрома (возраст, женский пол, стрессовая ситуация, хирургическое вмешательство, дефибрилляция, применение добутамина и фторхинолонов). На основании перечисленного было заподозрено наличие синдрома такоцубо. Данный случай демонстрирует возможность развития синдрома такоцубо после кардиохирургических вмешательств и дефибрилляции.

Ключевые слова: кардиомиопатия такоцубо, приобретенные пороки сердца, фибрилляция желудочков, протезирование клапана сердца, кардиохирургия, дефибрилляция.

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

Источник финансирования. Авторы заявляют об отсутствии финансирования.

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INTRODUCTION

Takotsubo syndrome (TTS), also known as Takotsubo cardiomyopathy, stress cardiomyopathy or broken heart syndrome, is a benign, reversible abnormality, characterized with a fugitive systolic ventricular dysfunction. Its clinical evidence is myocardial infarction with the absence of coronary arteries stenosis [1, 2]. The etiology of ST has not been fully studied, but scientists acknowledge its connection with excess catecholamines from physical or emotional stress. There exist several theories concerning TTS etiology: coronary artery spasm, left ventricular outflow tract obstruction,

increase in catecholamine levels, disorder in calcium regulatory system, oxidative stress and increase in production of transforming growth factor beta [3].

Despite numerous descriptions of TTS in scientific literature, there exist only isolated cases of this disorder in patients after cardiosurgical interventions. Such cases are only represented in foreign sources [4–8].

We present a case of TTS diagnosed in a patient after mitral valve replacement and defibrillation.

CASE REPORT

Female patient aged 71 was admitted to the cardiac surgery department for elective surgery. Patient had a diagnosis of non-rheumatic multiple valve disease: mitral valve prolapse, mitral valve insufficiency, aortic insufficiency, tricuspid insufficiency, permanent atrial fibrillation with normal sinus rhythm, heart failure with preserved ejection fraction, IIA, class IV.

Echocardiography was performed during the preoperative stage with the following results: left atrium – 6.2 cm; left ventricle end-diastolic size – 4.9 cm; left ventricle end-systolic size – 2.9 cm; left ventricle posterior wall – 1.1 cm; ventricular septum – 1,1 cm; ejection fraction (EJ) – 71%; right ventricle – 2.3 cm. The test also showed mitral valve's leaflets thickened into the left atrium (anterior mitral leaflet – >1.0 cm, posterior mitral leaflet – >0.9 cm) with significant mitral valve regurgitation (peak mitral flow velocity – 470 cm per second, vena contracta diameter – 0.8 cm). An angiography of coronary arteries did not reveal any significant lesions. Preoperative electrocardiogram (ECG) shows atrial fibrillation with high systolic blood pressure (Fig. 1)

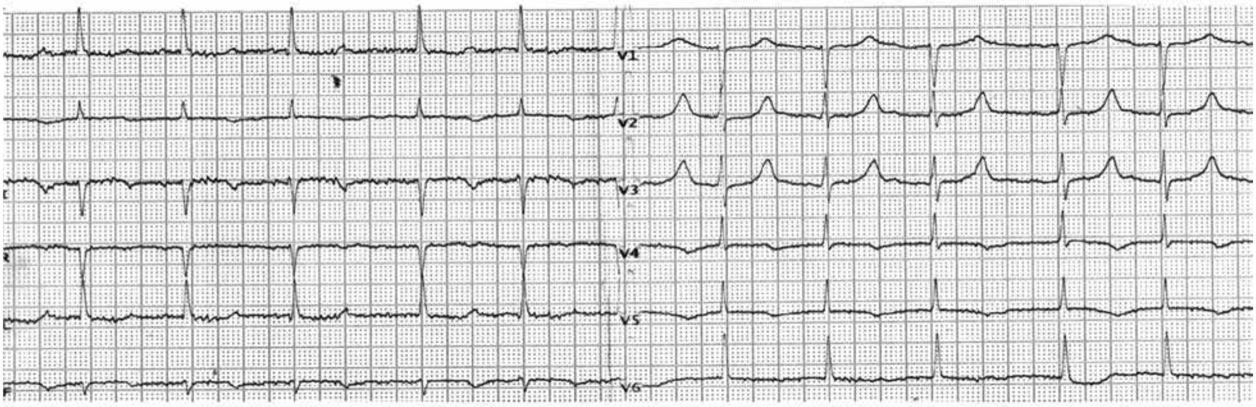

Fig. 1. Patient's preoperative ECG: scanning speed 25 mm/sec; voltage 1 мВ = 10 mm; heart rate 60–75 bpm; QTc 520 ms

The mitral valve replacement through median sternotomy with "Medtronic" HancockIIT510 trileaflet bioprosthesis, CinchSZ – 31 mm; reduction annuloplasty of tricuspid valve and left atrial appendage closure were performed in conditions of normothermic cardiopulmonary bypass and pharmaco-cold cardioplegia. Atrial fibrillation resumed by the end of the operation with a heart rate of 50–60 bpm.

Early postoperative period proceeded with predisposition to atrial fibrillation with heart rate <60 bpm (Fig. 2)

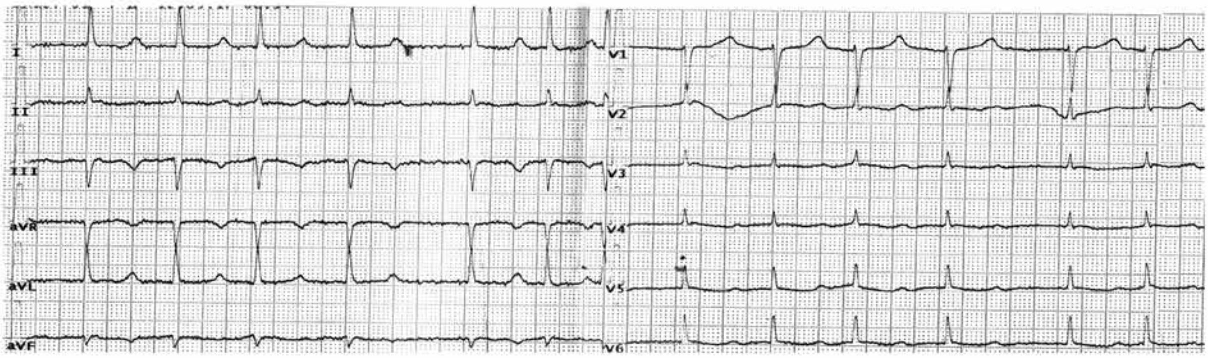

Fig. 2. Patient's ECG, 1st postoperative day: scanning speed 25 mm/sec; voltage 1 мВ = 10 mm; heart rate 58–100 bpm; QTc 557 ms (here and in pictures 2–4)

In the peri-operative period, the patient was receiving ciprofloxacin as a preventive measure for the prevention of infectious complications. Ventricular fibrillation developed in the patient on the third day after operation in the setting of relative well-being (normal general, electrolyte and biochemical blood values and central hemodynamics parameters). Biphasic defibrillation with 150 J was performed immediately. Atrial fibrillation was restored with the heart rate 55–65 bpm, arterial pressure 130/170 mmHg, central venous pressure 40 mm. H_2O. Electrocardiography revealed QT interval prolongation up to 754 ms and T-wave inversion in leads I, II, III, AVF, V_{3-6} (Fig. 3).

Pulmonary edema developed 6 hours after defibrillation. Echo-cardiography showed aneurysmal dilatation of left ventricular apex with ejection fraction reduction to 25% and hyperkinesis in the basal area of the left ventricle.

After patient's transfer to cardiac surgery department on the 9th day after ventricle fibrillation, the ECG was following (Fig. 4).

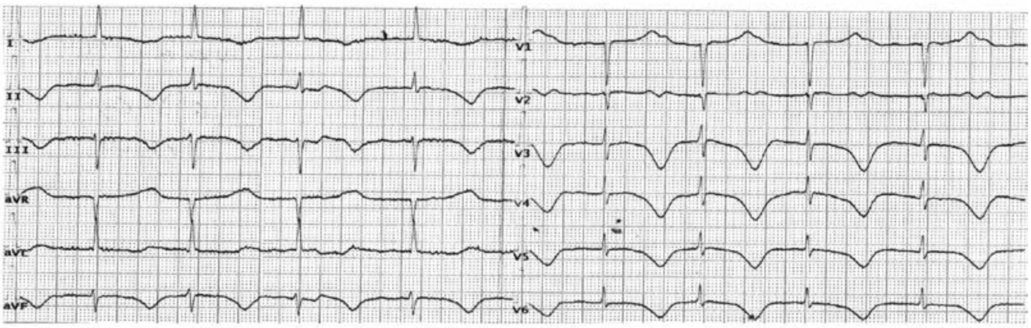

Fig. 3. Patient's ECG, 1st day after defibrillation

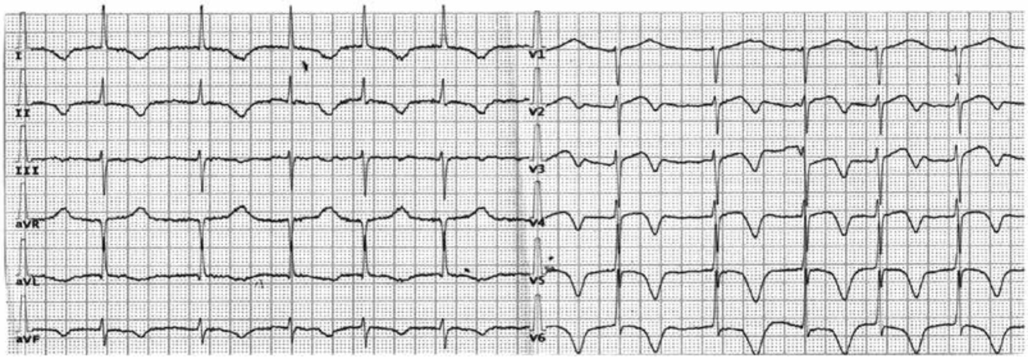

Fig. 4. Patient's ECG, 9th day after defibrillation

With the course of time, the patient stabilized and was discharged from the department in satisfactory condition on the 21st day. Discharge echo-cardiography: left atrium – 5.0 cm; left ventricle end-diastolic size – 5.3 cm; left ventricle end-systolic size – 3.8 cm; left ventricle posterior wall – 1.4–1.6 cm; ventricular septum – 1.5–1.8 cm; ejection fraction (EJ) – 56%; right ventricle – 3.2 cm; prosthetic mitral valve with pressure differential 15 mmHg; significant left ventricular myocardial hypertrophy; fluid traces in the upper part of pericardial cavity; aneurysm is not visible.

DISCUSSION

Clinical manifestations of Takotsubo syndrome are long retrosternal pains, dyspnea, sudden development of acute heart failure, severe ventricular arrhythmia which may lead to heart rapture [9].

Abnormal changes in ECG in acute phase (first 12 hours) include ST segment elevation or depression from the isoelectric period, newly developed left bundle-branch block and sometimes Q-wave formation. T-wave inversion and prolongation of QT interval is also possible in many derivations within 24–48 hours after the development of disease's clinical manifestation or triggering stress factor. QT interval prolongation is often significant (more than 500 ms) and, thus, predisposes to the development of torsades de pointes (TdP) and ventricle fibrillation [1].

Echocardiography reveals an area of regional myocardial contractility lesion that is larger than arterial blood supply area and various complications (left ventricular outflow tract obstruction, mitral valve regurgitation, thrombus formation and heart rapture) [1]. Angiography of coronary arteries shows the absence of hemodynamically relevant stenosis that could have been a reason for the myocardial contractility lesions mentioned above [1].

It may be noted that in the presented clinical case many TTS risk factors are present: age, female sex, stress situation, surgical intervention, dobutamine and ciprofloxacin injections. Since the influence of transforming growth factor beta on the TTS genesis and mitral valve prolapse development is proved, it can be assumed to influence the abnormality genesis in the presented case [10].

Diagnostic criteria, suggested by the specialists of Mayo clinic and further developed by Heart Failure Association (2016), exist nowadays [9]. Five of these criteria can be found in our patient: temporary regional myocardial contractility lesion after surgical intervention; regional myocardial contractility lesion takes place in the parts where blood is supplied by more than one coronary artery; absence of hemodynamically significant lesions of the coronary arteries; abnormal changes in the electrocardiogram (T-wave inversion and prolongation of QT interval up to 754 ms with its gradual shortening during the acute phase of the disease; restoration of the ejection fraction to 565.

Based on the mentioned above, the diagnosis of TTS was suggested in the patient.

CONCLUSION

The clinical case presented in this article demonstrates relevancy and significance of Takotsubo syndrome as a potential complication after surgical interventions on mitral valve or electrical cardioversion.

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ONE MORE SIBMED JOURNAL IS INCLUDED IN SCOPUS

A research and production journal Drug Development and Registration (published by Center of Pharmaceutical Analytics, LLC) successfully passed evaluation by Scopus Content Selection and Advisory Board and was recommended for inclusion in Scopus international abstract and citation database. Experts stressed high level of publications in the journal and its importance for the Russian market.

Founders of the journal:

- Center of Pharmaceutical Analytics, LLC
- I.M.Sechenov First Moscow State Medical University
- Siberian State Medical University

Objectives of the journal

The research and production, peer-reviewed journal Drug Development and Registration is a quarterly up-to-date free publication and an information portal for professionals engaged in drug circulation.

The journal is intended for pharmaceutical production companies and their workers from development, quality control, registration, production and development departments as well as for workers of laboratories, contract research organizations and research institutions. The main objective of the journal is to integrate research and practical advances in the area of drug development and registration and boost research and practical skills of specialists engaged in drug circulation.

Five main sections of the journal encompass the development process of a drug, from its design to obtaining a drug license. The first section is devoted to search and development of new drugs, the second – to pharmaceutical technology and includes steps from design and production of initial pharmaceutical ingredients, technologies and equipment to development of standard and therapeutically effective drugs.

The third section describes analytical methods of quality control. The fourth section is devoted to approaches to assessing drug safety and carrying out pre-clinical and clinical trials. The fifth section considers such issues as validation methods, marketing authorization application and life cycle of a drug in the GxP environment. The journal accepts both reviews and experimental articles by Russian and international researchers in the area of development and registration of drugs.

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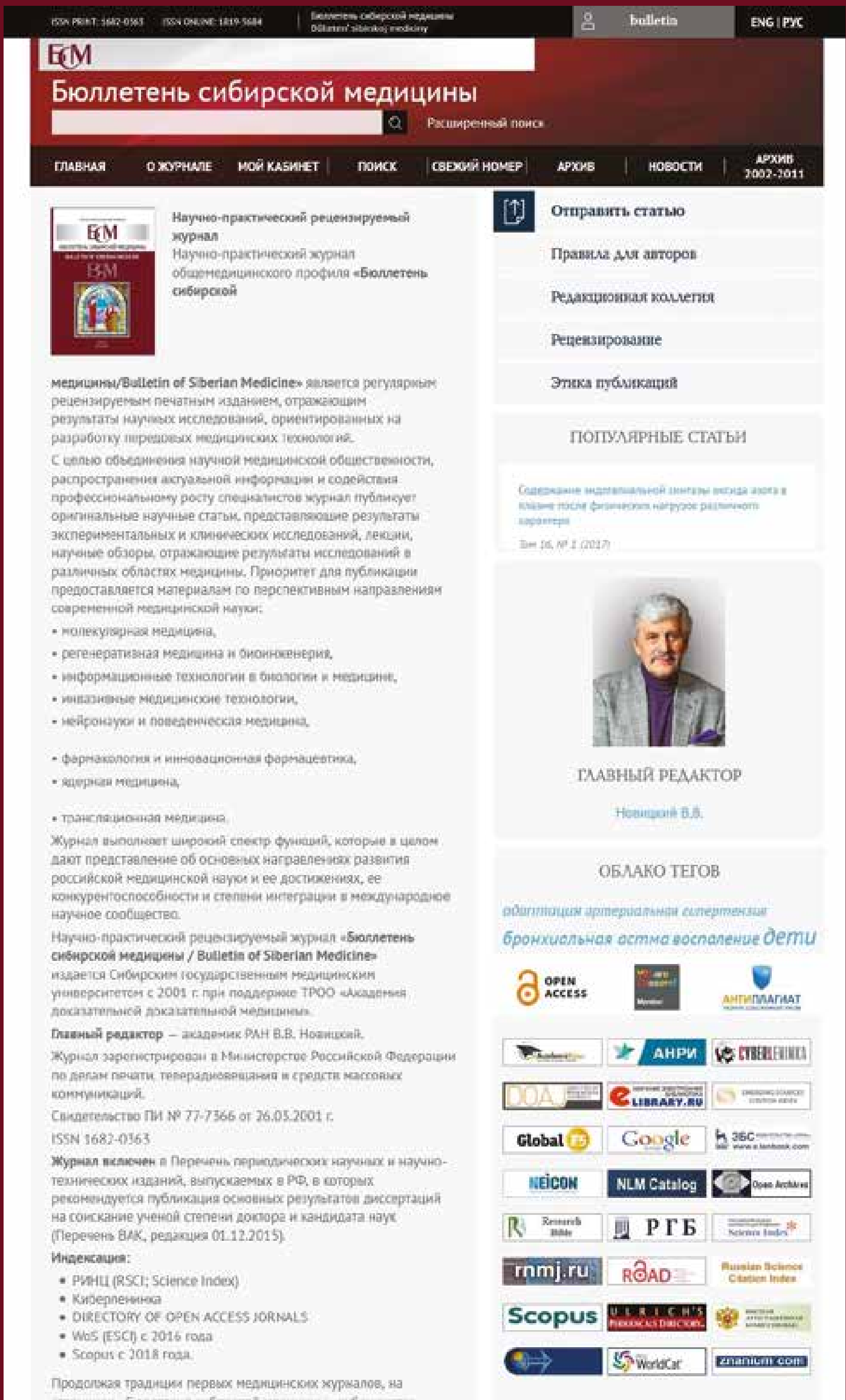

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