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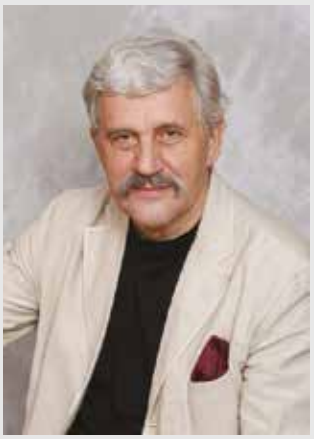
7 января 2021 года ушел из жизни главный редактор журнала НОВИЦКИЙ Вячеслав Викторович (родился 23 августа 1946 г.) – ученый-патофизиолог с мировым именем, академик РАН (РАМН)



Вячеслав Викторович окончил лечебный факультет Томского медицинского института (1969, в н.в. Сибирский государственный медицинский университет – СибГМУ). Свою научную деятельность он начал в студенческие годы (со 2-го курса) под непосредственным руководством заведующего кафедрой патофизиологии, заслуженного деятеля науки РСФСР, профессора Д.И. Гольдберга. Как активный кружковец и общественник, по окончании вуза был оставлен для дальнейшей работы в институте, где прошел все этапы становления как научного сотрудника, педагога и администратора.

Защитив в 1972 г. под научным руководством профессоров Д.И. Гольдберга и Е.Д. Гольдберга кандидатскую диссертацию «Реакции системы крови при введении 5-фторурацила и фторафура в эксперименте», продолжил изучение побочных эффектов различных противоопухолевых препаратов, а также исследования в области радиационной гематологии и экспериментальной онкологии. В 1986 г. защитил докторскую диссертацию «Реактивность системы крови в ранние и отдаленные сроки после действия противоопухолевых антибиотиков антрациклинового ряда» (научный консультант – член-корреспондент АМН СССР, профессор Е.Д. Гольдберг). Ученое звание старшего научного сотрудника по специальности «патологическая физиология» присвоено в 1977 г., ученое звание доцента по кафедре патологической физиологии – в 1985 г., звание профессора – в 1989 г., звание член-корреспондента РАМН – в 1989 г., звание действительного члена (академика) РАМН – в 2005 г., звание действительного члена (академика) РАН – в 2014 г.

В 1988 г. был первым избранным на альтернативной основе проректором по научной работе, в декабре 1997 г. – первым избранным ректором Томского мединститута (СибГМУ). В период 2000-2017 гг. заведовал кафедрой патофизиологии.

Будучи прогрессивным, талантливым администратором и человеком, истинно преданным науке, Вячеслав Викторович большое внимание уделял воспитанию научно-педагогических кадров и вовлечению молодежи в науку. Он создал собственное направление в томской школе патофизиологов – патофизиология системы крови и онкофармакология, в рамках которого объединились опытные и молодые преподаватели-исследователи СибГМУ для изучения вопросов патогенеза цитостатической болезни, фундаментальных механизмов нарушений структурно-метаболических свойств клеток крови при психических расстройствах и соматической патологии, патологической реактивности системы крови при инфекционном процессе. Подготовил 108 кандидатов и 45 докторов наук, большинство из которых защитились в возрасте до 35 лет. Научная школа академика РАН В.В. Новицкого шесть раз подтверждала звание «ведущей научной школы РФ» (2003, 2006, 2008, 2012, 2014, 2016). В 2010 году коллектив научной школы В.В. Новицкого был удостоен одной из наиболее престижных наград академии наук – премии им. И.В. Давыдовского по общей патологии за цикл работ «Молекулярные основы патологии клеток крови при социально значимых заболеваниях».

В.В. Новицкий является автором более 800 научных работ в области теоретической гематологии, патофизиологии, цитологии и клеточной биологии, в том числе 44 монографий, 18 учебных пособий, 2 авторских свидетельств и 17 патентов РФ на изобретения. Создатель, редактор и соавтор уникального атласа по электронной микроскопии эритроцитов и пяти изданий учебника по патологической физиологии для студентов медицинских вузов (1994, 2001, 2006, 2009, 2018 гг.).

Научные достижения Вячеслава Викторовича Новицкого признаны не только в России, но и за рубежом: действительный член Польской Академии медицины (с 1998), действительный член Международной академии медицины Альберта Швейцера (Польша, с 1999), professor honoris causa of philosophy (in humanities) Польской Академии медицины (2006). Кавалер 15 орденов и медалей научных сообществ и академий Польши, Германии, Франции, Великобритании, в том числе трех орденов командорского класса.

Член правления Российского научного общества патофизиологов.

Заслуженный деятель науки РФ (2000). Заслуженный работник культуры РФ (1996). Почетный гражданин г. Томска (2006). Почетный профессор СибГМУ (2016). Депутат Государственной думы Томской области 2-го, 3-го и 4-го созывов (1997–2001, 2001–2006, 2006–2011).

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СОДЕРЖАНИЕ

CONTENTS

Dear readers and authors,
I am delighted to greet you as the Editor-in-Chief of the journal "Bulletin of Siberian Medicine".

It is a great honor for me to chair the editorial board of a scientific journal that was founded and had been led for many years by Vyacheslav Novitskiy, Academician of RAS and my mentor. The editorial board of the journal embraces renowned Russian and foreign scientists, leaders and members of international professional associations and scientific communities. To a large extent, it became possible due to dedication and contribution of Vyacheslav Novitskiy and his colleagues – top experts in the promising areas of modern medical science that the journal is focused on.

Due to fruitful work of the editorial board and you, dear authors, the journal is becoming more interesting and rich in content with every new issue. The level of publications and, consequently, the level of the journal are growing.

Inclusion of the journal in the international citation bases is an essential stage in its evolvement as a high-impact journal that provides access to the latest advances in medicine. Therefore, we pay great attention to the ultimate correctness of published information, expand the pool of authors, and invite globally renowned scientists to publish their advancements in our journal. Accessibility of the published materials for Russian and foreign readers is of utmost importance for us, that is why all articles are open access, free, and available in two languages: Russian and English.

The global pandemic is posing particular difficulties for all of us, including obstacles in organizing and carrying out research. However, we need to join our efforts in studying new medical problems and direct our research potential to their solution.

We are always open for discussion and cooperation and will try to pay due consideration to your comments and feedback.

Best wishes and thank you in advance for your contribution to the journal "Bulletin of Siberian Medicine"!

Dr. Olga I.Urazova
Professor,
Corresponding Member of RAS

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Association of smoking with coronary artery disease depending on other cardiovascular risk factors

Bazdyrev E.D., Maksimov S.A., Galimova N.A., Mulerova T.A., Indukaeva E.V., Artamonova G.V., Barbarash O.L.

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ABSTRACT

Aim. To study the possibility of the presence of coronary artery disease (CAD) depending on the smoking status, as well as to estimate the association of smoking with other traditional risk factors in residents of Kemerovo region aged 25–64 years.

Materials and methods. We analyzed the results of the multicenter epidemiological study "Epidemiology of cardiovascular diseases and their risk factors in the Russian Federation" in Kemerovo region obtained from a random sample of 1,599 subjects aged 25–64 years. Besides the smoking status, the following parameters were analyzed: gender, age, education, diabetes mellitus (DM) and arterial hypertension (AH) history, hypercholesterolemia, hypertriglyceridemia, high level of low-density lipoproteins (LDL), low level of high-density lipoproteins (HDL), hyperglycemia, obesity, alcohol abuse, and depression.

Results. Three groups were formed depending on the smoking status: group 1 included 484 (30.3%) current smokers, group 2 included 317 (19.8%) former smokers and group 3 consisted of 798 (49.9%) individuals who had never smoked. The groups did not differ in the prevalence of CAD. When determining the rank significance of the impact of risk factors on the possibility of CAD development in the overall population, it was revealed that the age affected the risk of CAD the most, while smoking and low HDL had minimal impact. The impact of the smoking factor on CAD was higher in the representatives of the older age group; however, even in the subjects aged ≥ 50 years, the smoking factor was not the leading one and followed DM, hypertriglyceridemia, depression, and obesity. The possibility of CAD development in smokers, as opposed to individuals who had never smoked, increased when smoking was accompanied by hypercholesterolemia, hypertriglyceridemia, male sex, lack of higher education, depression, and age of ≥ 50 years. The possibility of CAD development in former smokers, as opposed to non-smokers increased when smoking was accompanied by hyperglycemia.

Conclusion. Smoking is not the primary risk factor in CAD detection in the studied sample. In the presence of additional risk factors, the impact of smoking on the possibility of CAD detection increases.

Key words: coronary artery disease, smoking, risk factors.

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

Source of financing. The authors state that they received no funding for the study.

Conformity with the principles of ethics. All individuals were notified of the objectives of the study and signed an informed consent to participate in the study. The study was approved by the local Ethics Committee at the Research Institute for Complex Issues of Cardiovascular Diseases (Protocol No. 71 of 02.09.2013).

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Связь курения с ишемической болезнью сердца в зависимости от других факторов сердечно-сосудистого риска

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РЕЗЮМЕ

Цель. Изучение вероятности наличия ишемической болезни сердца (ИБС) в зависимости от статуса курения, а также оценка связи курения с другими традиционными факторами риска у жителей Кузбасского региона в возрасте 25–64 лет.

Материалы и методы. Проведен анализ результатов многоцентрового эпидемиологического исследования «Эпидемиология сердечно-сосудистых заболеваний и их факторов риска в Российской Федерации» в Кемеровской области, полученных на случайной выборке 1 599 человек в возрасте 25–64 лет. Помимо статуса курения анализировались пол, возраст, образование, наличие сахарного диабета (СД) и артериальная гипертензия, гиперхолестеринемия, гипертриглицеридемия, высокий уровень липопротеидов низкой плотности, низкий уровень липопротеидов высокой плотности (ЛПВП), гипергликемия, ожирение, злоупотребление алкоголем и депрессия.

Результаты. В зависимости от статуса курения сформированы три группы. Первая группа – курящие в настоящее время, 484 человека (30,3%), вторая – курившие в прошлом, 317 (19,8%), и третья группа – никогда не курившие, 798 человек (49,9%). Группы не имели различий по распространенности ИБС. При определении ранговой значимости влияния факторов риска на вероятность ИБС в общей популяции выявлено, что возраст является самым сильным фактором, а курение и низкий уровень ЛПВП – минимальными. Степень влияния на ИБС фактора курения выше у представителей старшей возрастной группы, однако даже у лиц в возрасте 50 лет и старше факт курения не занял лидирующие позиции и следовал за СД, гипертриглицеридемией, депрессией и ожирением. Вероятность ИБС у курящих в сравнении с никогда не курившими усиливается при сочетании курения с гиперхолестеринемией, гипертриглицеридемией, мужским полом, отсутствием высшего образования, наличием депрессии и возрастом 50 лет и старше. У куривших в прошлом к не курящим – при сочетании с гипергликемией.

Заключение. Курение является не первостепенным фактором риска при выявлении ИБС в исследуемой выборке, а при наличии дополнительных факторов риска влияние курения на вероятность выявления ИБС усиливается.

Ключевые слова: ишемическая болезнь сердца, курение, факторы риска.

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

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

Соответствие принципам этики. Все пациенты подписали информированное согласие на участие в исследовании. Исследование одобрено локальным этическим комитетом НИИ КПССЗ (протокол № 71 от 02.09.2013).

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INTRODUCTION

Coronary artery disease (CAD) has been a leading cause of death and disability for many years, not only in the Russian Federation, but all over the world. It is generally accepted that risk factors, such as impaired lipid and carbohydrate metabolism, arterial hypertension (AH), excessive alcohol intake, low physical activity, obesity, and smoking, play a crucial role in the development of cardiovascular diseases (CVD). Research in recent years has focused primarily on the analysis of cause-and-effect relationships from the point of view of the isolated influence of these factors on the possibility of developing CAD. Unfortunately, there are not enough studies that demonstrate a comprehensive approach to assessing a possible impact of smoking in combination with other traditional cardiovascular risk factors.

MATERIALS AND METHODS

The analysis was based on the results of the study "Epidemiology of cardiovascular diseases and their risk factors in the Russian Federation" in Kemerovo region, where a random sample of male and female adults aged 25–64 years (1,628 people) was investigated. The final sample size was 1,599 people after exclusion of individuals with incomplete data. Their influence on the final results was not analyzed, since the percentage of missing data was less than 2%.

The study was conducted in accordance with the standards of good clinical practice and the principles of the Helsinki Declaration. The patients signed an informed consent approved by the institution's Ethics Committee before being included in the study.

The presence of CAD was assessed according to the sum of 3 epidemiological criteria: ECG encoded changes later classified according to the Minnesota code and the Rose questionnaire and myocardial infarction (MI) in the medical history.

Depending on smoking habits, the respondents were divided into three groups. Current smokers were those who smoked at least one cigarette a day or quit smoking less than a year ago. "Former" smokers were patients who had quit smoking a year or more before inclusion in the study, non-smokers were those who had never smoked [1].

In addition to smoking, the following cardiovascular risk factors were analyzed: sex, age, education, diabetes mellitus (DM) and AH, hypercholesterolemia, hypertriglyceridemia, high LDL, low HDL, hyperglycemia, obesity, alcohol abuse, and depression.

The smoking status (non-smokers / former smokers / smokers), education (higher education / lack of higher education), diabetes (according to the respondent), peculiarities of alcohol consumption, and the level of depression were assessed according to the survey data. Alcohol consumption was assessed based on the data on the frequency, amount and type of alcoholic beverages consumed. The volume of alcohol consumption for the year was calculated, followed by conversion to average daily values in grams of ethanol. Alcohol abuse was classified with estimated average daily ethanol consumption of more than 72 grams.

The hospital scale of anxiety and depression (HADS), validated in Russia, was used to assess depression. The 75th percentile of depression in this sample was calculated, in case values were above it, the level of depression was considered to be increased. AH was classified at systolic blood pressure (SBP) ≥140 mm Hg and / or diastolic blood pressure (DBP) ≥ 90 mm Hg, regardless of the level of blood pressure during the intake of antihypertensive drugs. The person was considered to be obese if the value of the Body Mass Index was ≥ 30 kg / m^2. Hypercholesterolemia was defined when the concentration of total cholesterol was over 5.0 mmol / l, hypertriglyceridemia was detected if the triglyceride level was more than 1.7 mmol / l, high LDL was registered if values were greater than 3.0 mmol / l, low HDL – if values were less than 1.0 mmol / l, and fasting hyperglycemia was classified at the glucose level of more than 5.6 mmol / l.

The distribution of quantitative indicators was evaluated, taking into account that the distribution did not deviate significantly from the normal value. Descriptive statistics methods were used to calculate the average value (*M*) and standard deviation (*SD*) for quantitative indicators and frequency for qualitative indicators. The Kruskall – Wallis test and the Pearson Chi-square test were used to evaluate differences in quantitative indicators. The ranking of the risk factor influence on the probability of developing CAD was determined using decision trees. The method of discriminant one-dimensional

branching for categorical and ordinal predictors was applied.

Equal cost of misclassification of objects and a priori probabilities proportional to the size of classes of the dependent variable were taken as the forecast accuracy criteria. Pruning according to the classification error initiated a branching stop, the minimum number of misclassified objects was 12, and the value of a standard error was 1.0. The importance of risk factors in the analysis of classification trees was estimated according to a conventional 100-point scale, 100 conventional units (c.u.) were accepted as an important predictor that impacted most on the classified object.

Logistic regression analysis was used to assess a relationship between smoking and CAD; the results were adjusted for sex, age, hypertriglyceridemia, education, depression, obesity, diabetes, and hypertension. Odds ratio (OR), 95% confidence interval (CI), and *p*-level of OR were calculated. At the first stage, the relationship of smoking with CAD was evaluated in the total sample. At the second stage, it was assessed alternately in groups based on the presence / absence of risk factors.

Statistical analysis was performed using Statistica 6.1 software. The critical level of statistical significance was 0.05. At the level of $0.05 < p < 0.10$, the trend was considered to be statistically significant.

RESULTS

In the sample of 1,599 subjects, CAD was verified in 264 (16.5 %) respondents, of which 28 (10.6 %) previously had MI. It should be noted that 801 (50.1 %) respondents were former or current smokers. Comparative analysis (Table 1) shows that groups did not differ in the prevalence of CAD, prior MI, and stroke. Notably, current smokers were males of younger age with no signs of cholesterol or carbohydrate metabolism disorders. At the same time, fewer people among them had higher education and more people among them abused alcohol.

Table 1

Clinical and anamnestic characteristics of groups depending on smoking habits

Parameter	Current smokers 484 (30.3 %)	Former smokers 317 (19.8 %)	Non-smokers 798 (49.9 %)	*p*-level difference in the groups
Age, years, (M ± SD)	44.5±11.0	46.6±11.6	48.3±11.3	< 0.0001
Sex, male, (*n*, %)	312 (64.5)	195 (61.5)	182 (22.8)	< 0.0001
CAD, (*n*, %)	83 (17.1)	50 (15.8)	131 (16.4)	0.87
MI, (*n*, %)	8 (1.6)	10 (3.1)	10 (1.2)	0.090
Chronic bronchitis, (*n*, %)	92 (19.0)	54 (17.0)	108 (13.5)	0.028
Bronchial asthma, (*n*, %)	15 (3.1)	9 (2.8)	36 (4.5)	0.28
Digestive diseases, (*n*, %)	136 (28.1)	103 (32.5)	346 (43.4)	< 0.0001
Kidney diseases, (*n,* %)	74 (15.3)	74 (23.3)	258 (32.3)	< 0.0001
AH, (*n*, %)	185 (38.2)	156 (49.2)	352 (44.1)	0.0074
DM, (*n*, %)	11 (2.3)	19 (6.0)	33 (4.1)	0.028
Stroke, (*n*, %)	7 (1.4)	11 (3.5)	14 (1.7)	0.11
Cardiac arrhythmia, (*n*, %)	77 (15.9)	60 (18.9)	179 (22.4)	0.016
Obesity, (*n*, %)	132 (27.3)	114 (36.0)	312 (39.1)	< 0.0001
Depression, (*n*, %)	100 (20.7)	51 (16.1)	153 (19.2)	0.27
Alcohol abuse, (*n*, %)	146 (30.2)	66 (20.8)	56 (7.0)	< 0.0001
Higher education, (*n*, %)	153 (31.6)	127 (40.1)	349 (43.7)	< 0.0001
Hypercholesterolemia,(*n*, %)	232 (47.9)	176 (55.5)	441 (55.3)	0.024
Hypertriglyceridemia, (*n*, %)	98 (20.2)	76 (24.0)	150 (18.8)	0.15
Low HDL level, (*n*, %)	11 (2.3)	8 (2.5)	6 (0.7)	0.032
High LDL level, (*n*, %)	297 (61.4)	215 (67.8)	542 (67.9)	0.041
Hyperglycemia, (*n*, %)	76 (15.7)	59 (18.6)	141 (17.7)	0.52

The influence of cardiovascular risk factors on the probability of CAD was ranked. In the total sample, with all the considered risk factors taken into account, age criteria had the maximum rank (100 c.u.) of influence on the probability of CAD (Table 2).

Table 2

Ranking of the impact of risk factors on the probability of CAD

Parameter	Risk factor ranking of the probability of CAD (c.u.)		
	Total sample	Persons < 50 years	Persons ≥ 50 years
Sex, male	8	100	32
Age	100	83	45
Smoking	1	31	52
Higher education	15	66	43
DM	14	8	100
AH	13	76	12
Hypercholesterolemia	2	16	15
Hypertriglyceridemia	13	39	96
High LDL level	2	14	25
Low HDL level	1	21	11
Hyperglycemia	13	41	42
Obesity	19	56	67
Alcohol abuse	6	23	30
Depression	27	53	80

Other risk factors had lesser impact (in the descending order): depression – 27 c.u., obesity – 19 c.u., higher education – 15 c.u., etc. Ranking assessment of the risk factor influence on the probability of CAD was performed in two age groups due to the overwhelming influence of age. Sex, age, AH, higher education, and depression had the influence rank of more than 50 c.u. in the < 50-year-old group.

It is worth noting that smoking as one of the leading factors determining the probability of CAD has the lowest rank in the general population. However, with age differentiation, the influence of this risk factor increases. Thus, in people younger than 50 years, the rank influence of smoking was 31 c.u., and in the older age group (50 years and older) it elevated to 52 c.u. Besides smoking, the rank influence of more than 50 c.u. was registered for DM (100 c.u.), hypertriglyceridemia (96 c.u.), depression (80 c.u.), and obesity (67 c.u.).

Age influenced CAD the most, while smoking and low HDL levels had the lowest rank influence in the general population. The degree of influence of smoking on CAD was greater in the older age group, but even in people aged ≥ 50 years it was not the greatest and followed DM, hypertriglyceridemia, depression, and obesity.

Further, the probability of CAD was calculated depending not only on smoking, but also on smoking combined with other risk factors. Due to the large volume of data obtained, Table 3 shows only statistically significant relationships.

Table 3

Probability of CAD depending on smoking in combination with other cardiovascular risk factors

Parameter	OR	95 % CI	*p*-level
Smokers vs. non-smokers			
Smoking	1.53	1.05–2.22	0.025
Hypercholesterolemia	1.74	1.05–2.89	0.031
Hypertriglyceridemia	2.39	1.11–5.12	0.025
Male sex	1.92	1.01–3.68	0.049
Lack of higher education	1.71	1.07–2.72	0.024
Depression	2.36	1.15–4.83	0.018
Age ≥ 50 years	1.82	1.11–2.99	0.028
Former smokers vs. non-smokers			
Smoking	1.31	0.86–1.99	0.20
Hyperglycemia	2.79	1.10–7.11	0.030
Smokers vs. former smokers			
Smoking	1.47	0.96–2.27	0.078
Male sex	2.16	1.18–3.96	0.012
Lack of higher education	1.74	1.02–2.97	0.041
Depression	2.58	1.01–6.61	0.047
Age ≥ 50 years	1.87	1.08–3.24	0.024

Current smokers were 53% more likely to have CAD than non-smokers. In addition, a direct statistically significant association was found between the probability of CAD if smoking was combined with other cardiovascular risk factors, such as hypercholesterolemia, hypertriglyceridemia, male sex, lack of higher education, depression, and age over 50 years. When smoking was combined with other risk factors, the association with CAD became close to statistically significant: AH (OR = 1.63, 95% CI 0.94–2.83, $p = 0.082$), hyperglycemia (OR = 2.63, 95% CI 0.98–7.04, $p = 0.053$), obesity (OR = 1.83, 95% CI 0.99–3.38, $p = 0.054$).

There was also a direct association of smoking with CAD (OR higher than 1.00) in former smokers and non-smokers who did not have other risk factors, but it was statistically insignificant. In this regard, it is appropriate to say that the influence of smoking is more pronounced in people with comorbidities and additional cardiovascular risk factors.

Next, respondents who had quit smoking for more than a year before the study and non-smokers were analyzed. In former smokers, the association of smoking with CAD was direct, but statistically insignificant. Of all the factors considered, a combination of the former smoker status only with hyperglycemia was significantly associated with CAD.

Additionally, a combination of the former smoker status with disorders of lipid metabolism, such as hypercholesterolemia (OR = 1.68, 95% CI 0.96–2.94, p = 0.071), hypertriglyceridemia (OR = 2.31, 95% CI 0.96–5.58, p = 0.060), and obesity (OR = 1.82, 95% CI 0.95–3.52, p = 0.072), was approaching the statistical significance level. Therefore, lower probability of CAD is not strongly associated with smoking cessation in case these risk factors are present.

Statistically insignificant trends in smoking cessation among men and women are worth noting. In the "smokers vs. non-smokers" analysis, prominent association of CAD with sex was registered among men (men OR = 1.92, 95% CI 1.01–3.68, p = 0.049 compared to women OR = 1.20, 95% CI 0.74–1.94, p = 0.45). In the "former smokers vs. non-smokers" analysis, the probability of CAD was higher among women (women OR = 1.33, 95% CI 0.79–2.25, p = 0.8 compared to men OR = 0.94, 95% CI 0.45–1.98, p = 0.87), although no statistical significance was shown. This may indirectly indicate that smoking cessation and reduced probability of CAD are less likely associated in women than in men.

Changes in the association with CAD during smoking cessation are more evident when associations with CAD are compared in current smokers and former smokers. In the total sample, the decrease in the strength of the association with CAD in former smokers, is reflected in high values of the OR of current smokers, as opposed to former smokers (the differences are close to statistically significant values, OR = 1.47, 95 % CI 0.96–2.27, p = 0.078).

According to the conducted logistic regression analysis, low probability of CAD in combination with smoking cessation is associated with male sex, lack of higher education, age of over 50 years, and depression. Additionally, during smoking cessation, a number of factors become almost statistically significant in relation to reduced probability of CAD: AH (OR = 1.47, 95% CI 0.96–2.27, p = 0 .078), absence of hyperglycemia (OR 1.52, 95% CI 0.93–2.48, p = 0.095), and absence of obesity (OR = 1.68, 95% CI 0.94–2.98, p = 0.077). The presence or absence of such factors as hypercholesterolemia and hypertriglyceridemia does not affect reduction of CAD probability even during smoking cessation.

DISCUSSION

Smoking is the leading risk factor for CVD. According to the study "Epidemiology of cardiovascular diseases and their risk factors in the Russian Federation", 27.7% of people smoke in Russia. The prevalence of this risk factor in Kemerovo region was higher than in other regions, both among men (49.8 %) and women (22.9 %) [1]. Similar data were demonstrated in 2016, in the global survey of adult population of the Russian Federation. It showed that in Russia 36.4 million (30.5 %) people use tobacco products on a regular basis (14.5% of women and 49.8% of men) [2]. The use of tobacco products is the cause of death for 5.4 million people annually and accounts for 1 in 10 deaths among adults worldwide [3]. Smoking plays a leading role in the development of atherosclerosis and, thereafter, CAD. Smoking has been shown to have a negative effect on the endothelial function, stimulate thrombosis, potentiate oxidative stress and inflammation, and cause impairment of lipid metabolism [4].

The study found that smoking is associated with the probability of CAD. However, in the general population, smoking was not classified as a highly significant factor, giving the leading role instead to age, depression, and obesity. Taking into account division of the sample by age, smoking association became more important in people over 50 years. It was demonstrated that the probability of CAD was 53% higher in smokers compared to those who had never smoked. There were no differences in the probability of CAD when comparing former smokers and current smokers. In addition, there were no differences in the probability of CAD in former smokers and non-smokers. However, the study was limited by the absence of analysis on the time frames of smoking cessation. It is known that CVD risk is lowered after 10–15 years of smoking cessation (the values approach those of non-smokers) [4]. This may explain the results of the study.

This study demonstrated that the influence of smoking on the probability of CAD increased if other risk factors were present. Thus, smoking more than doubled the probability of CAD (in comparison to values in non–smokers) in case the examined individuals had depression and hypertriglyceridemia. Besides, it 1.5 times increased the probability of CAD in males aged ≥ 50, with hypercholesterolemia and lack of higher education. Former smokers were almost 3 times more likely to have CAD than non-smokers, even with hyperglycemia. An important conclusion of this study was that smoking cessation is associated with a lower probability of CAD mainly in men aged ≥ 50, with a lack of higher education and depression. This is a one-stage study, which does not allow for a possibility to discuss similar effect of the interaction between these factors and smoking on the risk of CAD development. In the meantime, the results are consistent with other studies that have shown that age, depression, glucose and cholesterol levels, excessive alcohol consumption, etc. are independent adverse factors in terms of CVD risk [1, 5–7].

The study "The Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries" (INTERHEART) showed that psychosocial factors account for 32% of the overall risk of developing MI, which is comparable to smoking [8]. Based on data from 53 separate studies and 4 meta-analyses, the American Heart Association concluded that depression is a predictive adverse risk factor for both general and cardiovascular mortality, as well as non-fatal cardiovascular events in both men and women [8]. A prospective study (Cardiovascular Health Study) involving 4,493 patients with a 6-year follow-up demonstrated the association between the increase in depression and the risk of developing CAD.

Thus, the risk of developing CAD was 1.15 per every 5 units of increase in the average depression score [7]. In addition, there are studies that indicate that CAD may potentiate depression. An observational study conducted by the Women`s Health Initiative found that postmenopausal women with established CAD risk factors (smoking, obesity, and DM) had a higher risk for concomitant depression after adjustment for age, race, education, and income [9].

There are several mechanisms that can possibly link depression and CAD, such as hypothalamic-pituitary-adrenocortical system dysfunction and increased cortisol levels, platelet activation; proinflammatory cytokines, and genetics [10]. In addition, bad habits and lifestyle associated with depression, such as smoking, excessive alcohol consumption, lack of physical activity, unhealthy diet, lack of social support, and poor compliance are associated with the risk of developing CAD [6].

According to available data, the effect of glucose levels on the risk of developing CVD among women and men is not the same. In the publication by P.W.F. Wilson et al. [11], the analysis of the Framingham study concluded that the frequency of CVD was associated with blood glucose levels in women without diabetes, while no such association was observed in men. S.V. Ahn et al. [12] in the longitudinal 11-year follow-up study of 159,702 individuals demonstrated that the relative risk of CAD in women increased in the pre-diabetes glucose range, whereas in men the risk increased solely with diabetes glucose level. The reason why hyperglycemia in women leads to a higher risk of CAD is unclear. Several mechanisms can explain this phenomenon. Hyperglycemia may have a stronger additive or synergistic effect if coupled with obesity, AH, hypercholesterolemia, and smoking in women [12].

Additionally, a high concentration of glucose increases oxidative stress, causing overproduction of the superoxide radical in the mitochondria [13]. The role of oxidative stress in production in the mitochondria causes further deterioration of endothelial function (endothelial dysfunction) and subsequent changes in vascular wall morphology [14]. Fluctuations in the glucose level can have a big impact on the activation of neutrophils, platelets, and cytokines. Abnormal activation of neutrophils and platelets is the main determinant of vascular catastrophes in patients with diabetes, contributing to high inflammatory reactions and high frequency of thrombotic events [15]. A recent study has also shown that sudden fluctuations in glucose levels can increase levels of interleukin-6, tumor necrosis factor-α, and intercellular adhesion molecule-1, leading to cardiovascular damage [16].

CONCLUSION

Thus, this study demonstrated high frequency of smoking as one of the leading risk factors for developing CVD. Smoking alone is not the primary

risk factor of CAD in the study sample, however, in the presence of additional risk factors, the impact of smoking increases. In this regard, criteria, such as male sex, lack of higher education, age, depression, and carbohydrate and lipid metabolism disorders, are unfavorable.

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Authors contribution

Bazdyrev E.D. – conception and design, analysis and interpretation of data, justification of the manuscript and critical revision of the manuscript for important intellectual content, final approval of the manuscript for publication. Maksimov S.A. – conception and design, analysis and interpretation of data. Galimova N.A. – conception and design, analysis and interpretation of data. Mulerova T.A. – justification of the manuscript and critical revision of the manuscript for important intellectual content. Indukaeva E.V. – justification of the manuscript and critical revision of the manuscript for important intellectual content, final approval of the manuscript for publication. Artamonova G.V . – critical revision of the manuscript for important intellectual content, final approval of the manuscript for publication. Barbarash O.L. – critical revision of the manuscript for important intellectual content, final approval of the manuscript for publication.

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New possibility of application of bacteriophages to prevent infectious complications in free skin grafting (bacteriophages in skin grafting)

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ABSTRACT

Aim. To prevent infectious processes in the area of a recipient wound in free skin grafting with a split-graft.

Materials and methods. A method was developed for immobilizing bacteriophages in the area of split-thickness skin grafts through transferring a solution containing bacteriophages into a gel form. Microbiological and clinical studies of the effectiveness of the proposed method were performed.

Results. The viability of bacteriophages in a gel dressing for up to 4 days was confirmed, as well as the reduced likelihood of local infectious complications in skin grafting.

Conclusion. The gel composition containing bacteriophages allows for a quick response to changes in current hospital microflora to effectively counteract the dangers of nosocomial infection.

Key words: bacteriophages, free skin grafting, split-thickness skin grafts, wound complications, microflora.

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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 Privolzhsky Research Medical University (Protocol No. 1 of 27.02.2018).

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Новая возможность применения бактериофагов для профилактики инфекционных осложнений при свободной кожной пластике (бактериофаги при кожной пластике)

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РЕЗЮМЕ

Цель. Профилактика инфекционного процесса в области реципиентной раны при свободной кожной пластике расщепленным трансплантатом.

Материалы и методы. Разработан способ иммобилизации бактериофагов в области аутодермотрансплантата путем перевода раствора, содержащего бактериофаги, в гелевую форму. Выполнены микробиологические и клинические исследования эффективности предложенного способа.

Результаты. Подтверждена жизнеспособность бактериофагов в гелевой повязке в сроки до 4 сут и снижение вероятности развития местных инфекционных осложнений при кожной пластике.

Заключение. Гелевая композиция, содержащая бактериофаги, позволяет оперативно реагировать на изменения актуальной госпитальной микрофлоры и эффективно противодействовать опасности нозокомиального инфицирования.

Ключевые слова: бактериофаги, свободная кожная пластика, расщепленный трансплантат, раневые осложнения, микрофлора.

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

Источник финансирования. Авторы заявляют об отсутствии финансирования.

Соответствие принципам этики. Все пациенты подписали информированное согласие на участие в исследовании. Исследование одобрено локальным этическим комитетом Приволжского исследовательского медицинского университета (протокол № 1 от 27.02.2018).

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INTRODUCTION

The number of patients who need plastic surgery to close soft tissue defects is steadily increasing. The relevance of the problem of closing soft tissue defects is explained both by an increase in frequency of military, domestic and man-made injuries, and by the growing level of technology in the field of vascular surgery, which ensures limb preservation in obliterating vascular diseases, including the presence of trophic ulcers of soft tissues [1].

Plastic closure of a skin defect with a split-thickness graft is one of the most common operations because of its relative safety and technical simplicity. However, one of the limiting factors is high sensitivity of the graft to the development of infection. This is determined by high prevalence of hospital flora with high virulence and antibiotic resistance, as well as by the technical peculiarities of these operations. The graft on recipient wounds is fixed by a multilayer bandage for a period of up to 4–7 days. Lack of light, nutrient availability, liquids, and favorable temperature regime create ideal conditions for the growth and proliferation of microorganisms under the bandage. During the first 4–5 days, the graft feeds only due to the diffusion of oxygen and nutrients from the vessels of the recipient wound and does not have its own vascular network, which makes it nearly defenseless against microorganisms [2].

The aim of the study was to prevent an infectious process in the area of a recipient wound in free skin grafting with a split graft, which is one of the fundamental conditions for an uncomplicated course of the early postoperative period.

To achieve this goal, it is necessary to solve two problems: to choose an agent capable of overcoming antibiotic resistance of the hospital flora and to maintain its concentration under the dressing, taking into account the need for minimal mechanical and chemical effects on the graft. Currently, to overcome the phenomenon of antibiotic resistance,

the possibility of using bacteriophages is being actively studied [3].

The basic principle of the treatment for infectious processes using bacteriophages was formulated by F. D'Hérelle, the discoverer of this type of viruses, as follows: "The bacteriophage should be introduced into the body in such a way as to realize the fastest and most intimate contact of it with bacteria to be destroyed" [4]. To create and maintain a high concentration of bacteriophages around the graft, we used modern wound dressings developed by the Russian company "Novye Perevyazochnye Materialy" LLC (Moscow).

MATERIALS AND METHODS

To protect the graft from hospital infection, a method of local application of bacteriophages has been developed [5]. Previously, the method of retrospective analysis identified the actual hospital microflora, which is the cause of local wound complications in skin grafting. Using commercially available specimens of bacteriophages, a set to which the identified hospital pathogens are sensitive was prepared. Then a dressing with the selected bacteriophages was created for the recipient wound. To immobilize the bacteriophages, the solution in which they are located was transferred into a gel state, which was then applied to the graft.

The blank for the dressing is a film made of polyvinyl alcohol, a hydrophilic biocompatible polymer, which is a suitable matrix to immobilize bioactive substances [6]. The polymer film contains a phosphate buffer to create an acid-base medium (pH 6.6 ÷ 7.8) optimal for bacteriophages. The thickness of the film is 40 microns, but when adding a solution with bacteriophages, the film absorbs it, swells within 30–60 seconds and transforms into gel with formation of a gel plate.

The method was carried out in the following way. Split skin grafting was performed. After fixing the graft on the recipient wound, a bandage was prepared intraoperatively. To achieve this, 0.05–0.2 ml/cm^2 solution of bacteriophages, to which the identified hospital pathogens were sensitive, was added to the film of polyvinyl alcohol. As a result, the film and solution transformed *into a gel plate*. Then, the graft and recipient wound were covered with the resulting gel plate.

To control the viability and efficiency of bacteriophages immobilized in a gel dressing, bacteriological studies were performed *in vitro* and *in vivo*. In the *in vitro* study, a gel plate containing bacteriophages was obtained by the proposed method. The inability and bioavailability (release) of bacteriophages from the gel dressing were determined on the lawns of the *Staphylococcus aureus* test strain.

In the Petri dish with a lawn of the of *Staphylococcus aureus* test strain, as a matter of control, a drop of a solution containing bacteriophages was applied (control 1), as well as a sample of the wound coating produced by applying a physiological solution onto the film (control 2), and a prototype wound coverage of 1 cm^2, obtained by applying a bacteriophage solution onto the film. It was then incubated at 37 °C, the results were assessed visually after 24 hours and by the presence or absence of lysis zones. To determine the duration of the viability period of bacteriophages in the gel, its samples of 1 cm^2 were applied to the lawns of test cultures 48, 72, and 96 hours after the formation of the gel plate.

In clinical practice, the proposed method was used after performing split skin grafting in 25 patients with chronic soft tissue wounds. All patients participating in the clinical study signed an informed consent to do this, and the study was carried out in accordance with the requirements of the Declaration of Helsinki of the World Medical Association (as revised in 2013).

The criterion for the inclusion of patients in the study was the state of the recipient wound surface, estimated at 16–17 points on the scale of wound readiness for free split skin grafting [7]. The control group consisted of 108 patients with chronic soft tissue wounds who underwent split skin grafting in the period 2014–2017. The viability and bioavailability (release) of bacteriophages from the gel dressing *in vivo* was determined in 4 patients the experimental group. In these patients, bacteriophages with sensitive test strains of *Staphylococcus aureus* were used as an active antibacterial agent. At the first dressing, the gel covering the graft was collected with a sterile spatula and applied to a Petri dish with the lawn of the *Staphylococcus aureus* test strain. The criterion for the effective

prevention of infectious processes in the graft area was considered to be a decrease in the frequency of local inflammatory complications.

Statistical analysis of the obtained data was carried out by Statistica 10.0 software. Fisher's exact method was used to assess the statistical significance of differences when comparing qualitative effects in pairs of distributions. The critical value of the significance level was equal to 5% ($p \leq 0.05$).

RESULTS

In a retrospective analysis of the microbiological research data, the control group of 108 patients demonstrated local purulent-inflammatory complications in 24 cases (22%) after free split skin grafting. These complications were represented by lysis and purulent fusion of the graft and were associated with the presence of *Streptococcus pyogenes* in the wound (5 cases) and non-fermenting gram-negative bacteria, such as *Pseudomonas aeruginosa* (6 cases), *Acinetobacter* spp. (4 cases). According to the manufacturer ("Microgen" of the Ministry of Health of Russia), the ability to lyse strains of *Staphylococcus aureus*, *Pseudomonas aeruginosa, Streptococcus, Escherichia coli, Proteus mirabilis, and Klebsiella pneumoniae* is possessed by the commercial official drug "Polyvalent pyobacteriophage", which was chosen as an anti-infective agent to protect the split skin graft. The treatment group had 1 (4%) case of purulent fusion in a part of the graft ($p = 0.045$).

When analyzing the results of the bacteriological studies *in vitro*, a "negative colony" appeared in the place of a phage drop (control 1), that is, a lysis zone (complete suppression of the visible growth of a microorganism) (Fig. 1). The same lysis zone was discovered in the gel containing the phage. In the gel region containing the saline solution, the lysis zones were not detected. The lytic properties of bacteriophages were retained 48, 72, and 96 hours after the formation of a gel plate from the bacteriophage solution (Fig. 2).

A clinical example of using the proposed method is described below.

Patient K., born in 1962, was admitted to the department of purulent surgery of the City Clinical Hospital No. 30 in Nizhny Novgorod on January 16, 2019 with the following diagnosis:

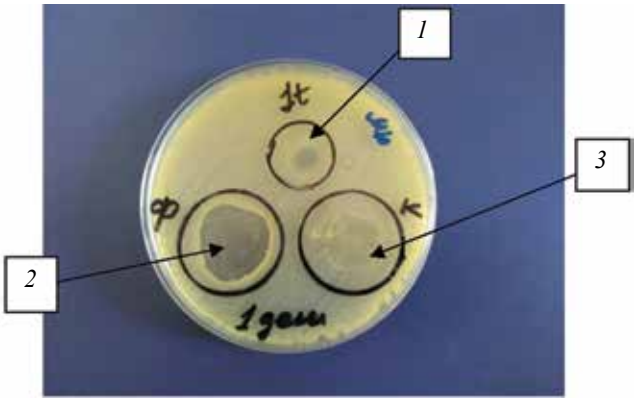

Fig. 1. Petri dish with negative colonies (lysis zones) on tthe lawns of *Staphylococcus aureus* test strains: *1* – in the places where the bacteriophage solution was applied (control); *2* – in the places where the gel obtained from the *in vitro* bacteriop hage solution was applied (gel exposure – 24 hours); *3* – the absence of lysis zones in the area where the gel obtained from sterile saline solution was applied

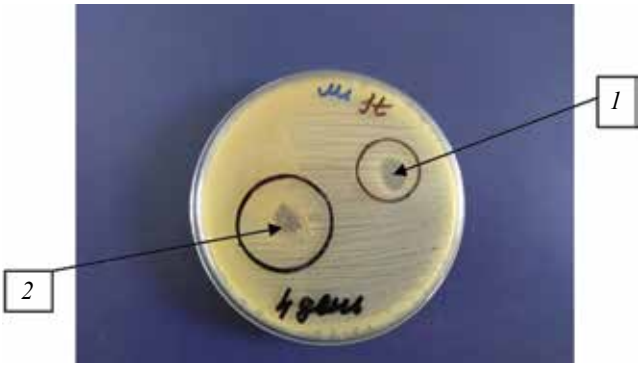

Fig. 2. Petri dish with negative colonies (lysis zones) on the lawns of the Staphylococcus aureus test strain: *1* – in the places where the bacteriophage solution was applied (control); *2* – in the places where the gel obtained from the bacteriophage solution was applied (gel exposure – 96 hours), *in vitro*

Decompensated type 2 diabetes mellitus (target HbA1 level < 7.5%). Diabetic polyneuropathy, sensorimotor form. Diabetic foot syndrome, neuroischemic form, Wagner II, condition after amputation of IV–V toes of the left foot dated 12.06.2018. Ischemic heart disease: atherosclerotic cardiosclerosis. hronic heart failure II A (FC II). Stage II arterial hypertension, 2nd degree. Risk 3. Dyslipidemia. Grade 2 obesity.

On the lateral surface of the left foot, a 3 × 4 cm wound was formed, about 0.4 cm deep. Microbiological investigations revealed the presence of wound exudates, therein an association of *Pseudomonas aeruginosa* and *Proteus mirabilis* 10^7 CFU/ml.

After the transition of the wound process to phase II and the elimination of microorganisms from the wound, it was decided to close the chronic wound of the lateral surface of the left foot with a free split skin graft. A bacteriophage solution was prepared ("Polyvalent pyobacteriophage"), which, according to the bacteriological analysis, hospital pathogens were sensitive to. Bacteriophages were kept in a liquid medium in 20 ml vials.

On 19.01.2019 free split skin grafting was performed with a 0.3 mm thick split flap, which was taken from the antero-lateral surface of the left thigh. After fixing the split skin graft, a bandage for the recipient wound was prepared intraoperatively. To achieve this, 10 ml of the "Polyvalent pyobacteriophage" solution was applied onto a 10 × 10 cm film made of polyvinyl alcohol and containing a phosphate buffer with a pH (6.6 ÷7.8) in the amount of (1÷3) × 10^{-5} mol/g. As a result, a gel plate was formed. Then, the graft and the recipient wound were covered with the obtained gel plate (Fig. 3). Aseptic dressings were applied onto the gel plate and then removed after four days (Fig. 4).

At a visual examination, the graft was viable, fixed to the recipient wound, and covered with a thin layer of gel. There were no signs of an infectious process. The gel was collected with a sterile spatula and applied to a Petri dish with a test culture. After a day of exposure, transparent lysis zones of the test culture were revealed in the area of gel application (Fig. 5), which indicates the presence of a bacteriophage with lytic activity ++++ (4 plus points) in the gel.

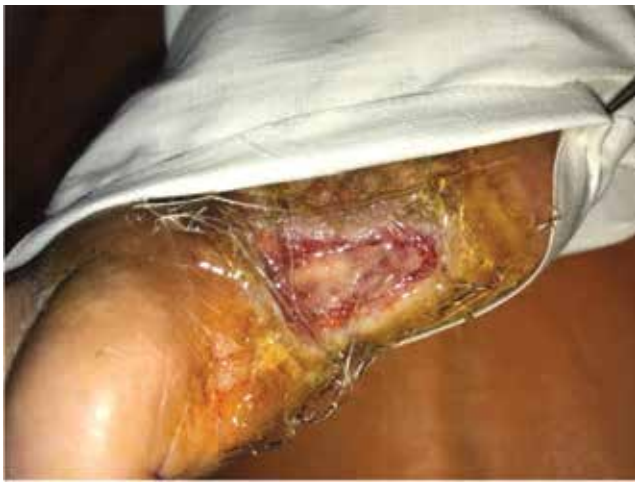

Fig. 3. Gel containing a bacteriophage is applied to the graft

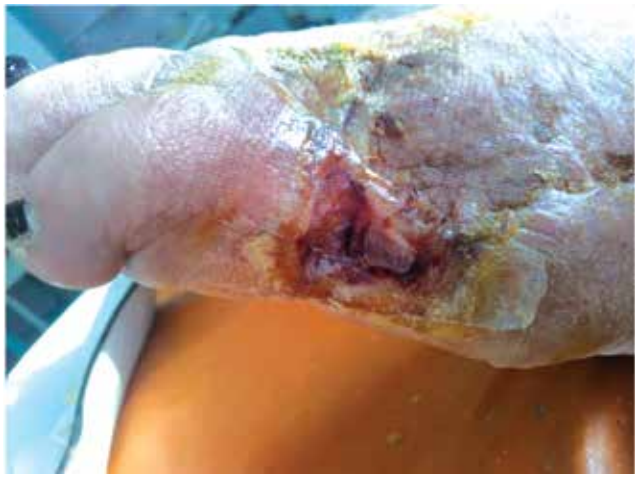

Fig. 4. The 4th day after free split-thickness skin grafting. The graft is viable and coated with gel

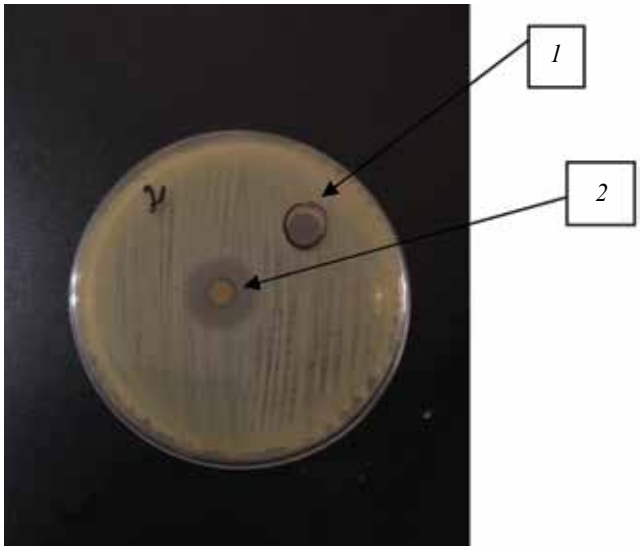

Fig. 5. Petri dish with negative colonies (lysis zones) on the lawns of the Staphylococcus aureus test strain: *1* – in the places of applying a fresh solution containing bacteriophag e (control); *2* – in the places where the gel obtained from a bacteriophage solution and located on the wound in the form of a bandage for 4 days was applied

DISCUSSION

Currently, the following basic bacteriophage properties determining feasibility of their use in the prevention and treatment of surgical infection can be distinguished [3]:

– lack of influence on the physiological microflora;

– stimulation of specific and non-specific immunity factors;

– possibility of being used in patients with allergic reactions to antibiotics;

– full compatibility with any drugs;
– lack of toxic and teratogenic effects.

Traditional topical application of bacteriophages is maintaining a moist environment around the wound by irrigating the wound and dressing with a solution containing bacteriophages [4]. Taking into account that as gauze dressings become dry, bacteriophage activity decreases sharply, it is necessary to periodically and abundantly moisten dressings with a bacteriophage solution. There is also a need to change dressings frequently, which leads to the misuse of resources and does not provide graft rest.

In order to simplify the application technology and lengthen the period of bacteriophage activity on the wound surface, foreign and domestic researchers are actively searching for technologies that allow bacteriophage immobilization in the structure of polymer carriers. Modern technologies [8] propose a method of covalent bacteriophage immobilization on a nanostructured support in the form of nonwoven nanofibrous material of polycaprolactone. In this case, bacteriophages are located in a given position: the capsid is firmly bound to the carrier, and the tail remains free, which allows them to actively influence bacteria. In another study [9], with the aim of industrial production of wound dressings with bacteriophages, the effect of the type of polymer matrix on the activity of bacteriophages immobilized in the structure of coatings by introducing a polymer into a solution and subsequent drying by different methods was investigated.

The best results were obtained by the authors when staphylococcal and Pseudomonas phages in the structure of the polymeric biodegradable dressings of polyester bromide were immobilized using freeze-drying. However, a fundamental disadvantage inherent in all methods of industrial immobilization of bacteriophages on a bandage is that it is impossible for a surgeon in an operating room to select a bacteriophage for the pathogen that is relevant in a given medical organization, taking into account the sensitivity of a particular strain of a microorganism.

In addition, it is necessary to solve the technically difficult problem of preserving the bacteriophages viability during creation, transport, and storage of the dressing. In the following study [10], it is proposed to apply a solution containing bacteriophages onto a collagen hemostatic sponge and then cover the graft with it. However, it is known that a collagen sponge contains boric and acetic acid, and in an acidic medium, bacteriophages are inactivated, because their maximum activity is manifested at pH from 6.6 to 7.8 [4].

Thus, to date, the search for an effective and inexpensive way to counter the danger of nosocomial infection has not led to success. Modern microbiological laboratories provide the surgeon with accurate information about the actual hospital microflora and its sensitivity to antibacterial drugs and have methods for determining the sensitivity of microflora to bacteriophages and selecting an effective bacteriophage for the conditions of a particular medical organization. The possibility of prophylactic use of bacteriophages under these conditions to prevent an infectious process caused by nosocomial microorganisms becomes real.

CONCLUSION

Thus, the problem of bacteriophage immobilization with the preservation of their function in the skin grafting area for a period of up to 4–5 days has been solved. The gel composition created ex tempore and containing bacteriophages makes it possible to quickly respond to changes in the actual hospital microflora and effectively counteract the danger of nosocomial infection.

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Authors contribution

Beschastnov V.V. – conception and design of the study, justification of the manuscript and critical revision of the manuscript for important intellectual content. Ryabkov M.D., Pavlenko I.V., Leont'ev A.E., Tulupov A.A., Kichin V.V. – analysis and interpretation of data. Yudanova T.N. – final approval of the manuscript for publication.

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Possibilities of radionuclide diagnostics of Her2-positive breast cancer using technetium-99m-labeled target molecules: the first experience of clinical use

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ABSTRACT

Background. The main purpose of the Her2/neu status determination in clinical practice is to determine the indications for the appointment of targeted therapy. The main methods for detecting the Her2/neu status are the immunohistochemical method (IHC) and the fluorescence *in situ* hybridization (FISH); however, despite their widespread use, they have a number of significant disadvantages. Over the past few years, radionuclide diagnostics using a new class of alternative scaffold proteins that meet all the requirements for optimal delivery of radionuclides to tumor cells has become widespread.

Aim. To study the possibility of clinical use of a radiopharmaceutical based on technetium-99m-labeled target molecules for the diagnosis of breast cancer with the Her2/neu overexpression in humans.

Materials and methods. The study included 11 patients with breast cancer ($T_{1-4}N_{0-2}M_0$) before systemic therapy: 5 patients with Her2/neu overexpression; expression of the marker was not detected in 6 patients. In all cases, morphological and immunohistochemical studies were performed. In case of Her2/neu 2+, FISH analysis was performed. The radiopharmaceutical was prepared immediately before administration, after which it was slowly injected intravenously into the patient. Scintigraphic studies in the "WholeBody" mode and SPECT of the chest organs were performed 2, 4, 6 and 24 hours after injection.

Results. Radiochemical yield, radiochemical purity and activity before administration were (80 ± 4)%, (98 ± 1)%, and (434 ± 19.5) MBq, respectively. The greatest uptake by normal organs was observed at a time interval of 6 hours in the kidneys and at a moderate activity in the liver and lungs at the same time interval. The organ with the highest absorbed dose was the kidneys; significant accumulation was also detected in the adrenal glands, gallbladder, liver, pancreas, and spleen. The smallest accumulation of the studied drug was observed in the brain (0.001 ± 0.000) mGy and skin (0.001 ± 0.000) mGy. The effective dose was (0.009 ± 0.002) mGy. The difference between tumors with positive and negative Her2-neu expression was found at all time points. In this case, the best indicator was determined after 2 hours of drug injection ($p < 0.05$).

Conclusion. Based on the results obtained, it can be indicated that the investigated radiopharmaceutical can be considered as a new additional method for the diagnosis of Her2-positive breast tumors.

Key words: alternative scaffold proteins, radionuclide diagnostics, Her2/neu, breast cancer, targeted therapy.

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

Source of financing. The work was carried out within the framework of the Ministry of Science and Higher Education grant on the topic "Development of targeted molecules based on scaffold proteins for the diagnosis and

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therapy of malignant neoplasms: theranostic approach" (Agreement No. 075-15-2019-1925).

Conformity with the principles of ethics. The patients signed an informed consent to participate in the study. The study was approved by the Bioethical Committee of the Cancer Research Institute of the TNRMC (Protocol No. 9 of 01.09.2020).

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Возможности радионуклидной диагностики Her2-позитивного рака молочной железы с использованием меченных технецием-99m таргетных молекул: первый опыт клинического применения

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РЕЗЮМЕ

Актуальность. Главной целью определения статуса Her2/neu в клинической практике прежде всего является измерение показаний для назначения таргетной терапии. Основными методами выявления статуса Her2/neu являются иммуногистохимический метод и флуоресцентная гибридизация *in situ* (FISH). Несмотря на распространенность, они имеют ряд существенных недостатков. В течение последних нескольких лет большое распространение приобретает радионуклидная диагностика с использованием нового класса альтернативных каркасных белков, отвечающих всем требованиям для оптимальной доставки радионуклида к опухолевым клеткам.

Цель. Изучение возможности клинического использования радиофармацевтического препарата на основе меченных технецием-99m адресных молекул для диагностики рака молочной железы с гиперэкспрессией Her2/neu у человека.

Материалы и методы. В исследование включены 11 больных раком молочной железы ($T_{1–4}N_{0–2}M_0$) до проведения системной терапии: пять человек с гиперэкспрессией Her2/neu; у шестерых экспрессия маркера выявлена не была. Во всех случаях выполнялось морфологическое и иммуногистохимическое исследование. При наличии значения Her2/neu 2+ проводился FISH-анализ. Препарат готовился непосредственно перед манипуляцией, после чего медленно внутривенно вводился пациенту. Сцинтиграфические исследования в режиме WholeBody и однофотонная эмиссионная компьютерная томография органов грудной клетки выполнялись через 2, 4, 6 и 24 ч после введения.

Результаты. Показатели радиохимического выхода, радиохимической чистоты и активности препарата непосредственно перед введением составили (80 ± 4)%, (98 ± 1)% и (434 ± 19,5) МБк соответственно. Наибольший захват нормальными органами отмечался на временном отрезке в 6 ч в почках; умеренная активность – в печени и легких на том же временном промежутке. Изучение доз абсорбции препарата показало, что органом с наибольшей абсорбцией изучаемого соединения являлись почки; значительная аккумуляция определялась также в надпочечниках, желчном пузыре, печени, поджелудочной железе и селезенке. Наименьшее накопление изучаемого препарата отмечалось в головном мозге (0,001 ± 0,000) мГр и коже (0,001 ± 0,000) мГр. Эффективная доза при этом составила (0,009 ± 0,002) мГр. Различие между опухолями

с положительной и отрицательной экспрессией Her2-neu было выявлено на всех временных точках. Наилучший показатель при этом определялся через 2 ч инъекции препарата ($p < 0,05$).

Заключение. На основании полученных результатов можно свидетельствовать о том, что исследуемый радиофармацевтический препарат возможно рассматривать в качестве нового дополнительного метода диагностики Her2-позитивных опухолей молочной железы.

Ключевые слова**:** альтернативные каркасные белки, радионуклидная диагностика, Her2/neu, рак молочной железы, таргетная терапия.

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

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Соответствие принципам этики. Все пациенты подписали информированное согласие на участие в исследовании. Исследование одобрено биоэтическим комитетом НИИ онкологии Томского НИМЦ (протокол № 9 от 01.09.2020).

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INTRODUCTION

Receptors of the epidermal growth factor family or EGFR (ErbB1/HER1, ErbB2/HER2, ErbB3/HER3, ErbB4/HER4) play an important role in the functioning of normal and tumor cells, responsible for the processes of cell division, differentiation, proliferation, migration, and apoptosis [1, 2]. The main attention of researchers is paid to the study of one member of the EGF family – the receptor for epidermal growth factor 2 (Her2/neu), the overexpression of which is detected in 15–20% of invasive breast cancer cases and is characterized by a poor prognosis and aggressive course of the tumor process [3, 4].

Establishing the Her2/neu status in clinical practice is primarily necessary to determine the indications for the appointment of targeted therapy using drugs, such as trastuzumab, pertuzumab, and trastuzumab emtamzine in combination with chemotherapy or in mono-mode, which significantly improves survival rates in patients with overexpression of this marker [5, 6]. The main methods for diagnosing the Her2/neu status include immunohistochemical method and fluorescence *in situ* hybridization (FISH). Significant disadvantages of these techniques are the following: the impossibility of performing an *in vivo* study to determine the spread of a tumor process; the evaluation of cases associated with heterogeneity of the Her2/neu receptor expression in tumor tissue; the need to perform invasive procedures (biopsy and (or) surgery), as well as possible differences in expression of the marker in the primary tumor and metastatic foci [7, 8].

The ongoing search for new effective agents has contributed to the development of molecular constructs that are alternative to the binding domains of antibodies and possess such characteristics as specific binding exclusively to the “target” antigen, lack of immunogenicity, stability, and the possibility of rapid chemical modification during research [9]. Over the past few years, a new class of alternative scaffold proteins (scaffolds) that meet all the requirements for optimal delivery of a radionuclide to tumor cells has become widespread [10]. The undoubted advantages of these constructs include significantly smaller sizes in comparison with the standard antibody, stable structure, additional functionalization and expression in the bacterial system, high thermal stability, and the possibility of direct chemical synthesis [11].

Currently, targeted radionuclide methods with high specificity for various molecular targets located on the surface of tumor cell membranes and making it possible to visualize foci of various sizes (the primary tumor node and metastatic foci) are becoming more widespread for the diagnosis of malignant tumors [12]. Until recently, monoclonal

antibodies (mAbs) were used as the main component of a radioimmunoconjugate [13].

However, the results of studies using mAbs did not meet the expectations assigned to them and revealed a number of features that significantly limit their use in clinical practice. Upon careful study, it turned out that mAbs have significantly reduced efficiency of interaction with the antigen, suboptimal pharmacological properties, slow distribution in the body, poor tissue penetration, and excretion by the kidneys (due to the high molecular weight of 150 kDa) [14]. The most significant disadvantage is the high immunogenicity of the used murine mAbs, which in response to their administration leads to the formation of neutralizing antibodies and, accordingly, to the occurrence of hyperimmune reactions and a decrease in the treatment effectiveness [15]. It became obvious that clinical use requires a radical modification of mAbs, including the correction of size, affinity, valence, etc. [16, 17].

One of the representatives of alternative scaffolds is ADAPT6, the albumin-binding domains of streptococcal protein G which are small in size (46–59 amino acid residues, molecular weight of 5–7 kDa). At the stage of preclinical studies, it was demonstrated that ADAPT6 labeled with various radionuclides shows a high contrast image of Her2-positive tumors in mouse xenografts just a few hours after injection [18, 19].

The aim of the present study was to investigate the possibility of clinical use of the ^{99m}**Tc**-ADAPT6 radiopharmaceutical for the diagnosis of breast cancer with overexpression of Her2/neu in humans.

MATERIALS AND METHODS

The clinical study was registered (ClinicalTrials.gov Identifier: NCT03991260) and approved by the Bioethical Committee of the Cancer Research Institute of TNRMC. It included 11 patients with breast cancer ($T_{1–4}N_{0–2}M_0$) before systemic chemotherapy or targeted therapy: in five people, Her2/neu overexpression was detected, and in six individuals, the expression of the marker was not detected. The average age of the patients was 50.7 ± 2.3 years. All patients signed a voluntary informed consent with information on the disclosure of the data received (clause 3 of article 13 of the Federal Law of the Russian Federation No. 323-FZ dated 21.11.2011).

The criteria for inclusion in the study were the following: newly diagnosed and morphologically verified breast cancer ($T_{1–4}N_{0–3}M_{0–1}$); the general condition of patients with 0–2 score according to the ECOG/WHO system; signed informed consent of the patient to participate in the research. Exclusion criteria were the presence of severe anemia, leukopenia, thrombocytopenia, sepsis, cachexia, severe concomitant pathology, claustrophobia, and refusal from treatment.

All patients were under dynamic observation for 48 hours after the administration of the radiopharmaceutical (RP) with an assessment of complaints, heart rate (HR), blood pressure (BP), and body temperature at various time intervals (before the administration of the drug, after 2, 4, 6, 24, and 48 hours after the injection). Additionally, all patients underwent laboratory tests (complete blood count and biochemistry and general urine analysis before the administration of the test compound, after 48 hours and 7 days) in the laboratory of the Cancer Research Institute of TNRMC.

Morphological research methods. In all cases, a morphological and immunohistochemical study of the biopsy material of the primary tumor was performed according to standard methods in the Laboratory of General and Molecular Pathology of the Cancer Research Institute, TNRMC. Diagnosis of breast cancer was established according to the "Histological classification of breast tumors" (World Health Organization, 2019). Immunohistochemical study of the biopsy material was carried out using antibodies from Dako (USA) to the oncoprotein c-erbB-2 (working dilution 1 : 500, rabbit). When evaluating the results, cases with no staining or with weak, intermittent membrane staining (categories 0 and 1+) were considered negative, and cases with strong staining of the entire cytoplasmic membrane of more than 10% of tumor cells (categories 3+) were positive. In the presence of weak to moderate staining of the entire cytoplasmic membrane of more than 10% of tumor cells (category 2+), all patients underwent FISH analysis.

FISH analysis was performed using an ERBB2(17q12)/SE17 DNA probe (Kreatech, USA); the reaction result was evaluated using an Axiostar PLUS fluorescent microscope (Carl Zeiss, Germany). The test results were considered positive when the ratio of the average copy number of the

Her2/neu gene and the average number of chromosome 17 centromeres in the cell was more than 2.2.

Radionuclide research methods. The drug was prepared immediately before administration in the Department of Radionuclide Diagnostics of the Cancer Research Institute of TNRMC according to the tricarbonyl method using the CRS Isolink kit (Center for Radiopharmaceutical Science, Paul Scherrer Institute, Villigen, Switzerland) [20].

To achieve the goals under aseptic conditions, the CRS Isolink kit (2.9 mg sodium tetraborate decahydrate, 7.8 mg sodium carbonate, 4.5 mg disodium boranocarbonate, and 9.0 mg potassium sodium tartrate tetrahydrate) was added with 500 μl (2 GBq) of eluate $^{99m}TcO^{4-}$ and incubated for 30 minutes at 100° C. Then, 500 μl of tricarbonyl technetium was added to 500 μg of ADAPT6 and incubated for 60 minutes at 50° C. Purification of the obtained compound from protein impurities and ADAPT6 molecules not bound to technetium was carried out using NAP-5 purification columns (GE Healthcare, Sweden). Radiochemical yield and purity were determined using thin-layer radiochromatography. Chromatogram analysis was performed using a Hitachi Chromaster HPLC systems chromatograph with a radioactive detector. The drug obtained after purification was diluted in 10 ml of sterile 0.9% NaCl solution, taken through a sterilizing filter and, after measuring the activity, was slowly injected into the patient intravenously.

Scintigraphy in WholeBody mode. Scintigraphic studies were performed on an E.CAM 180 gamma camera (Siemens, Germany) in the WholeBody mode using parallel high-resolution collimators for the energy of 140 keV in the supine position 2, 4, 6, and 24 hours after injection at a scanning speed of 12 cm / min.

Single-photon emission computed tomography was also performed in patients in the supine position 2, 4, 6, and 24 hours after drug administration. The field of view included the neck, axillary region, and chest to the level of tracheal bifurcation. 32 projections (each projection was 30 seconds long) were recorded into a 64 × 64 pixel matrix without hardware magnification.

Data processing and indicators used. During the study, the obtained data were subjected to post-processing using the specialized E. Soft software package (Siemens, Germany), while the level of drug accumulation in the main organs and tissues was studied by tracing the region of interest (ROI) on the WholeBody images in front and rear projections. The RP biodistribution was presented as a percentage of its accumulation in the regions of interest to the total score in both projections.

The nature of the RP accumulation in the study area was also assessed: symmetry, intensity, uniformity of accumulation; the presence and number of focal inclusions of the indicator in the studied organ, regional lymph nodes (asymmetric areas of RP hyperfixation were considered pathological); the presence of other foci of pathological RP inclu- sion within the study area. In addition, in the study groups, a tumor/background quantitative indicator was calculated, reflecting the degree of drug accumulation in the pathological focus in comparison with intact tissues. The indicator was assessed by tracing the ROI of the tumor on axial slices with the best visualization of the latter; symmetric regions of interest of the contralateral mammary gland were used as a "background".

Statistical processing of the results was carried out using the Statistica 10.0 for Windows software package and the nonparametric Mann – Whitney method. The difference between the two compared values was considered significant if the probability of their identity was less than 5% ($p < 0.05$). To calculate the RP absorption dose, the OLINDA/EXM 1.1 software was used with the application of an "adult woman" model.

RESULT AND DISCUSSION

According to the analysis, the indicators of radiochemical yield, radiochemical purity, and activity of the drug immediately before administration were (80 ± 4)%, (98 ± 1)%, and (434 ± 19.5) MBq, respectively. During the study, as well as during the observation period of the patients, no complaints, changes in HR, BP or temperature were detected. Changes in blood and urine parameters were also not detected.

The greatest uptake by normal organs was observed at a time interval of 6 hours in the kidneys ((32 ± 9)% / ID / organ). Moderate activity of the compound was observed in the liver ((2.6 ± 0.8)% / ID / organ) and lungs ((2.0 ± 0.6)% / ID / organ) at the same time interval; the smallest uptake was in the small intestine at two hours after administration ((0.9 ± 0.3)% / ID / organ). The results are shown in Fig. 1 and in Table 1.

Table 1

The greatest uptake of ^{99m}Tc by normal organs on planar scintigraphy after ^{99m}Tc-ADAPT6 injection, % / ID / organ

Organ	Time period after injection			
	2 h	4 h	6 h	24 h
Kidneys	27 ± 10	31± 12	32 ± 9	29 ± 10
Lungs	3.3 ± 0.8	2.5 ± 0.8	2.0 ± 0.6	1.4 ± 0.8
Liver	3.2 ± 1.1	2.2 ± 1.1	2.6 ± 0.8	2.4 ± 1.0
Small intestine	0.8 ± 0.3	0.9 ± 0.3	0.8 ± 0.3	0.6 ± 0.2

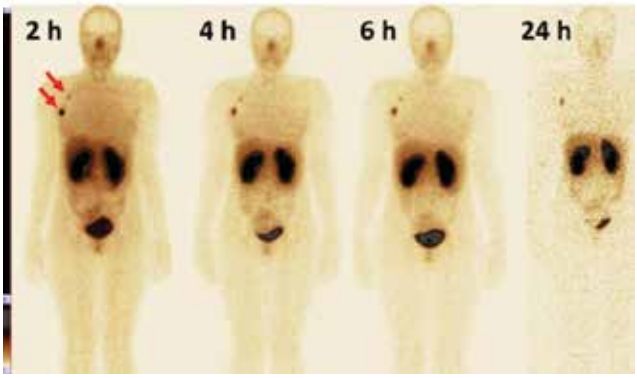

Fig. 1. Distribution of the ^{99m}Tc-ADAPT6 drug in organs and tissues of a breast cancer patient with positive Her2/neu expression 2, 4, 6, and 24 hours after injection: anterior and posterior views (arrows indicate the tumor)

The study of the absorption doses of the ^{99m}Tc-ADAPT6 drug showed that the organ with the highest absorption of the studied compound was the kidneys, (0.135 ± 0.42) mGy. Significant accumulation was also determined in the adrenal glands (0.023 ± 0.005) mGy, gall bladder (0.013 ± 0.008) mGy, liver (0.011 ± 0.008) mGy, pancreas (0.011 ± 0.008) mGy, and spleen (0.011 ± 0.008) mGy. The smallest accumulation of the studied drug was observed in the brain (0.001 ± 0.000) mGy and skin (0.001 ± 0.000) mGy. The effective dose was (0.009 ± 0.002) mGy (Table 2).

Table 2

Distribution of ^{99m}Tc-ADAPT6 in organs and tissues after administration in breast cancer patients

Index	Absorption dose, mGy
Adrenal glands	0.023 ± 0.005
Brain	0.001 ± 0.000
Breast	0.007 ± 0.002
Gall bladder	0.013 ± 0.008
Lower colon wall	0.005 ± 0.001

Table 2 (continued)

Index	Absorption dose, mGy
Small intestine	0.006 ± 0.001
Stomach	0.006 ± 0.001
Upper colon wall	0.007 ± 0.001
Heart	0.004 ± 0.001
Kidneys	0.135 ± 0.42
Liver	0.011 ± 0.008
Lungs	0.005 ± 0.001
Ovaries	0.008 ± 0.002
Pancreas	0.011 ± 0.002
Muscle	0.003 ± 0.000
Red bone marrow	0.004 ± 0.001
Osteogenic cells	0.006 ± 0.001
Skin	0.001 ± 0.000
Spleen	0.011 ± 0.003
Thymus	0.005 ± 0.002
Thyroid	0.009 ± 0.004
Bladder	0.012 ± 0.007
Uterus	0.005 ± 0.001
Whole body	0.004 ± 0.001
Equivalent effective dose, mSv / MBq	0.017 ± 0.004
Effective dose, mSv / MBq	0.009 ± 0.002

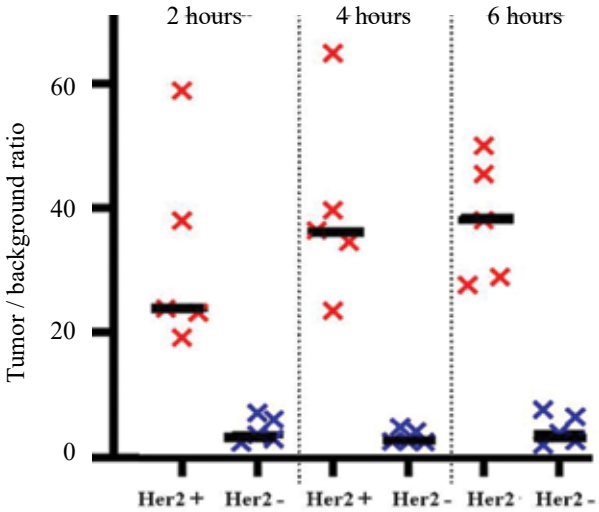

Fig. 2. Tumor / background ratio in patients with positive and negative expression of Her2</neu at different time points after drug administration

The difference between tumors with positive and negative expression of Her2-neu (comparison with the "background" site in the contralateral breast) after intravenous administration of ^{99m}Tc-ADAPT6 was detected at all time points. In this case, the best

indicator was determined 2 hours after the drug injection ($p < 0.05$) (Fig. 2).

CONCLUSION

This study has demonstrated the safety of clinical use, as well as good tolerance of ^{99m}Tc-ADAPT6 in breast cancer patients. The most significant results were found on drug accumulation in tumor tissue in patients with different Her2/neu expression. Thus, the revealed differences between Her2-positive and Her2-negative tumors ($p < 0.001$) will contribute to the optimization of approaches to the prescription of targeted drugs in oncological practice in the future. The accumulation of ^{99m}Tc-ADAPT6 in tumors with negative expression of the Her2/neu receptor is explained by the presence of at least 500 thousand receptors on the surface of the tumor cell, while with a positive status of this molecular parameter, its expression is up to 10 million receptors per cell. Similar phenomena are also observed when using radiopharmaceuticals, the targeting modules of which are representatives of other classes of target molecules [21].

Thus, based on the results obtained, it was concluded that the ^{99m}Tc-ADAPT6 radiopharmaceutical can be considered as a new additional method for diagnosing Her2-positive breast tumors.

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Bragina O.D., Chernov V.I., Tolmachev V.M. – conception and design, analysis and interpretation of data, justification of the manuscript and critical revision for important intellectual content, final approval of the manuscript for publication. Garbukov E.Yu., Doroshenko A.V. – collection of clinical material. Vorobyeva A.G., Orlova A.M. – justification of the manuscript and critical revision for important intellectual content.

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Anatomic grounds for the transposition of the thoracodorsal nerve in case of neurotization of brachial plexus nerve damage

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ABSTRACT

The aim. To identify topographic, anatomic, and constitutional features of thoracodorsal and musculocutaneous nerves of brachial plexus.

Materials and methods. Anthropometry of 45 corpses was carried out to determine height; length of the trunk and upper extremities; circumference of the neck, thoracic cage, shoulder and forearm; lateral dimensions of shoulders, thoracic cage and pelvis; anteroposterior size of the thoracic cage; and neck size. Morphometry of all brachial plexus components (length, thickness of nerves and angles of their origin) was performed after its anatomical preparation.

Results. The cephalic type of brachial plexus with participation of C4 spinal nerve was found in 7% of cases. The caudal type with inclusion of Th2 spinal nerve was found in 4% of cases. In 4% of cases, there was no musculocutaneous nerve, at the same time the shoulder biceps innervates the median nerve. In 93% of cases, the thoracodorsal nerve originates from posterior secondary bundle along lower posterior surface, in 7% of cases, it is an axillary nerve branch. Neck circumference is directly correlated with thoracodorsal nerve length: the larger the neck circumference is, the greater the nerve length is. In females, linear regression equations were derived, which allow to estimate thoracodorsal nerve length knowing the thoracic cage width.

Conclusion. The length of the thoracodorsal nerve determines the possibility of its transplantation into the musculocutaneous position. Neck circumference and, in females, the width of the thoracic cage, for reliability, should be used as external size biomarkers for donor and recipient nerves.

Key words: brachial plexus, morphometry, thoracodorsal nerve, musculocutaneous nerve, transposition.

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Анатомическое обоснование транспозиции грудоспинного нерва при невротизации поврежденных нервов плечевого сплетения

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РЕЗЮМЕ

Цель. Выявление топографо-анатомических и конституциональных особенностей грудоспинного и мышечно-кожного нервов плечевого сплетения.

Материалы и методы. Проведена антропометрия 45 трупов с определением роста, длины туловища и верхней конечности, окружности шеи, грудной клетки, плеча и предплечья, поперечных размеров плеч, грудной клетки и таза, переднезаднего размера грудной клетки, обхвата шеи. После анатомического препарирования плечевого сплетения проведена морфометрия всех его компонентов (длины, толщины нервов и углов их отхождения).

Результаты. Цефалический тип плечевого сплетения с участием спинального нерва C4 установлен в 7%, каудальный тип с включением спинального нерва Th2 – в 4% случаев. В 4% случаев отсутствует мышечно-кожный нерв, двуглавую мышцу плеча при этом иннервирует срединный нерв. В 93% случаев грудоспинной нерв отходит от заднего вторичного пучка по задненижней поверхности, в 7% это ветвь подмышечного нерва. Обхват шеи имеет прямые значимые корреляции с длиной грудоспинного нерва – чем больше обхват шеи, тем больше длина нерва. У женщин выведены уравнения линейной регрессии, на основании которых можно вычислить предположительную длину грудоспинного нерва при известном значении ширины грудной клетки.

Заключение. Длина грудоспинного нерва является определяющей детерминантой возможности его пересадки в позицию мышечно-кожного. В качестве внешнего биомаркера размеров нерва-донора и нерва-реципиента целесообразно использовать размеры обхвата шеи и для достоверности у женщин – ширину грудной клетки.

Ключевые слова: плечевое сплетение, морфометрия, грудоспинной нерв, мышечно-кожный нерв, транспозиция.

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

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INTRODUCTION

Currently, microsurgical treatment of brachial plexus injuries is guided by the modern concept “from distal to proximal”, which implies the restoration of nervous regulation as close to paralyzed muscle as possible [1–3]. In this regard, the development and implementation of neurotization of damaged nerves using nerve transfer technology is underway, which requires a detailed study of anatomic features of donor and recipient nerves [4–6].

The most common type of brachial plexus injury is avulsion from the upper trunk. It is known that the upper trunk is formed by the fusion of C5 and C6

spinal nerves, further suprascapular and subclavian nerves branch from it in a distal direction. The lateral secondary bundle (C5, C6, C7) is then formed taking some of the nerve fibers from C7, with musculocutaneous nerve and lateral root of the median nerve as final branches [7]. In case of upper trunk avulsion, significant violation of upper extremity function occurs in which innervation is lost by suprascapular and musculocutaneous nerves, as a result of which there is shoulder abduction and external rotation, shoulder adduction and flexion, and forearm flexion, respectively. It appears useless to restore integrity at the level of the upper extremity, since degenerative processes in distal parts of nerves advance regeneration in the proximal segment of the upper trunk, which leads to irreversible muscle atrophy. Evidently, effective treatment of brachial plexus injuries is impossible without the use of distal nerve transfers. In this regard, there is increased practical interest in the thoracodorsal-nerve, which is used not only in reconstructive surgery, but also as a donor nerve in case of brachial plexus peripheral nerve damage. This is the reason for conducting numerous studies revealing various anatomic features of this nerve.

Transplantation of thoracodorsal (TDN) and intercostal nerves to the damaged musculocutaneous nerve is being performed successfully [8, 9]. However, intraoperative access and the tunnel during the transposition of the thoracodorsal nerve are traumatic and useless, if the parameters of donor and recipient nerve do not suit [10, 11]. In this regard, the need for preoperative diagnosis of thoracodorsal (donor) and musculocutaneous (recipient) nerve size is quite obvious [12]. However, published studies do not include information about the possibility of determining true sizes of the musculocutaneous and thoracodorsal nerve at the preoperative stage, as well as sizes of other nerves in a particular patient to select the optimal transplantation method [13, 14]. Nevertheless, it has been proved that constitutional features of the human body structure determine its anatomical varieties that influence body and system functioning, which is important for clinical practice [15].

Thus, the aim of this study was to identify topographic, anatomical, and constitutional features of thoracodorsal and musculocutaneous nerves of the brachial plexus.

MATERIALS AND METHODS

The study was conducted on 45 female and male corpses of the second period of adulthood (36–60 years). Most of the studied corpses were male (n = 31 (69%)). There were 14 (31%) female corpses. The cause of death in all cases was somatic diseases without upper extremity, chest, neck or head damage.

The first stage included anthropometric corpse examinations: determination of height, length of the trunk and upper extremities; neck, thoracic cage, shoulder and forearm circumferences; lateral dimensions of shoulders, thoracic cage and pelvis; the anteroposterior size of the thoracic cage.

Anatomical preparation of the brachial plexus with subsequent instrumental measurements was the next step. Sequential measurement of the length, thickness and angles of all elements of the brachial plexus was carried out, as well as measurement with reference to the coordinate point, the clavicle center. The measurement of thoracodorsal nerve length was carried out from the place of its origin (posterior cord) to its entry into the latissimus dorsi muscle. In case there was a nerve split into branches prior to the muscle, the nerve aggregate length before and after branching was taken into account. Measurement of musculocutaneous nerve length was carried out from the place of formation (lateral cord) to coracobrachialis muscle perforation. After isolation and fixation in 10% neutral formalin solution, brachial plexus elements were measured under the MBS-10 stereoscopic magnifier.

Based on the obtained indicators, a database was made in MS Excel 9.0 program. The statistical processing of the results was carried out using Statistica for Windows 6.0. The article includes only indicators that follow normal distribution according to Shapiro – Wilk test. This allowed to use parametric statistical methods, including descriptive statistics, correlation (r – correlation coefficient, p – achieved significance level) and regression analyses, variant analyses carried out by means of the sigma deviation method. When describing the studied indicators, the following values were used: mean value (M) and standard deviation (σ), which are presented in the form of $M \pm \sigma$. Based on sigma deviations of neck circumference, thoracodorsal and musculocutaneous nerves length, as well as on formation level

of the latter, all the corpses were divided into three types. When testing static hypotheses, differences were considered significant at $p < 0.05$.

RESULTS

After anatomical preparation, it was found that C5, C6, C7, C8, and Th1 roots of spinal cord segments are involved in the formation of the brachial plexus in 89% (40/45) of cases. The cephalic type of the brachial plexus with the participation of C4 spinal nerve was found in 7% (3/45), and the caudal type with the inclusion of Th2 spinal nerve was found in 4% (2/45) of cases. There was one case when C4 and C5 spinal nerves were combined into the primary superior trunk, while C6 and C7 nerves were combined into the primary middle trunk. Variability of brachial plexus long branch formation was determined. There is no musculocutaneous nerve in 4% (2/45) of cases, while the median nerve innervates biceps brachii. In 93% (42/45) of cases, the thoracodorsal nerve originates from the posterior cord along the lower posterior surface, in 7% (3/45) of cases, it is a branch of the axillary nerve, located on the front surface.

To assess the possibility of transposition of healthy nerves to damaged ones, it is necessary to know their size. The length of the thoracodorsal nerve before entering the broadest dorsum muscle ranges from 7.0 cm to 18.9 cm; the average value is 13 cm, $n = 45$. Based on sigma deviations (13.0 ± 2.6) of length, the thoracodorsal nerve is divided into three types before entering the latissimus dorsi muscle. The types are the following: short nerve < 10.4 cm – 11% (5/45), medium-length nerve 10.4–15.6 cm – 74% (33/45), long nerve > 15.6 cm – 15% (7/45). The length of the musculocutaneous nerve up to coracobrachialis muscle perforation varies from 2.0 cm to 17 cm, and the average value is 6.8 cm, $n = 45$.

Taking into account the risks of invasive interventions, the inefficiency of transposition due to lack of length and, consequently, nerve tension with such complications as vellication and causalgia, it is necessary to develop non-invasive and reliable methods of detecting thoracodorsal nerve size. In this regard, the conducted correlation analysis revealed direct significant interconnection ($r = 0.317$, $p = 0.033$) of thoracodorsal nerve length and neck circumference. The length of the thoracodorsal nerve significantly increases with the length of neck circumference.

The anthropometric study revealed neck circumference varying from 26 cm to 39 cm, the average values are 32.8 cm, $n = 45$. Based on sigma deviations (32.8 ± 3.2) of neck circumference, all the corpses were divided into three groups: neck circumference < 29.6 cm – 15.5% (7/45), neck circumference 29.6–36.1 cm – 69% (31/45), and neck circumference > 36.1 cm – 15.5% (7/45). It was found that in individuals with neck circumference > 36.1 cm in 71% (5/7) of cases, the thoracodorsal nerve is long, and in 29% (2/7) of cases it is of average length. Whe the neck circumference varies between 29.6–36.1 cm, medium-length thoracodorsal nerve occurs in 78% (24/31) of cases, short nerve – in 16% (5/31) and long nerve – in 6% (2/31) of cases. In individuals with neck circumference < 29.6 cm, short (50%) and medium-length (50%) thoracodorsal nerves are found in equal proportion (Fig. 1).

Table 1 shows that there are significant differences in thoracodorsal nerve length in individuals with different neck circumference. The larger the neck circumference is, the longer the thoracodorsal nerve is and vice versa.

Table 1

The thoracodorsal nerve length (in cm) in individuals with different neck circumference ($n = 45$)

Parameter	Neck circumference > 36.1 cm	Neck circumference 29.6–36.1 cm	Neck circumference < 29.6 cm	p
Thoracodorsal nerve length	14.2	13.4	12.3	< 0.05

Based on sigma deviations (6.8 ± 3.2) of muscular-cutaneous nerve length to coracobrachial muscle perforation, three following types were determined: short nerve < 3.6 cm – 13% (6/45), medium-length nerve 3.6–10.0 cm – 65% (29/45), and long nerve > 10.0 cm – 22% (10/45). Three levels are marked according to the distance from the clavicle center to the place of musculocutaneous nerve formation taking into account sigma deviations (7.4 ± 2.1): high level < 5.3 cm – 7% (3/45), medium level – from 5.3 to 12.7 cm – 80% (36/45), low level > 12.7 cm – 13% (6/45). There is direct correlation ($r = 0.30$, $p = 0.049$) between the length of thoracodorsal and musculocutaneous nerves.

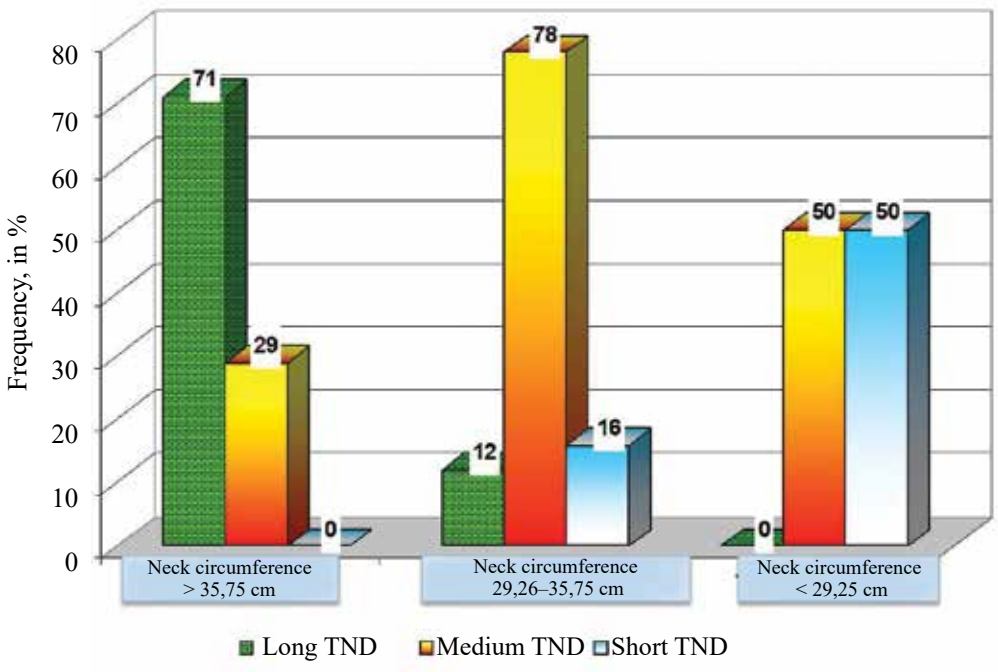

Fig. 1. Constitutional features of the thoracodorsal nerve.

Furthermore, in individuals with short musculocutaneous nerve, in five cases, medium-length pectoral-spinal nerve is observed, and in one case, short nerve is identified. In people with long musculocutaneous nerve, in seven cases there is medium-length thoracic back nerve, and in three people, there is a long one.

In the case of paired comparison of thoracodorsal and musculocutaneous nerve lengths, it was found that in 80% (36/45) of the studied corpses, the nerve length allows for successful transplantation of the thoracodorsal nerve to the position of the musculocutaneous nerve. In 20% (9/45) of cases, transposition of the thoracodorsal nerve to the position of the musculocutaneous nerve for neurotization of the damaged musculocutaneous nerve is not possible due to the lack of length of the former and low level of formation of the latter.

When comparing corpses by gender, males did not reveal any significant correlation between the length of the thoracodorsal nerve and anthropometric data. A significant inverse correlation between the length of the thoracodorsal nerve and the width of the thoracic cage ($r = –0.6$, $p = 0.03$) is revealed in females. The more the width of the thoracic cage is, the shorter the length of the thoracodorsal nerve is. Regression analysis revealed linear features (Fig. 2) and the equation of the relationship between the length of the thoracodorsal nerve and the width of thoracic cage (thoracodorsal nerve length, in cm = 20.1536–0.2846 × thoracic cage width, in cm). Knowing thoracic cage width by means of the revealed equation, one can determine the length of the thoracodorsal nerve can be determined.

Furthermore, there was a direct correlation between musculocutaneous nerve length and thoracodorsal nerve length ($r = 0.52$, $p = 0.05$) in females. Regression analysis revealed linear features and equation of correlation between musculocutaneous nerve length and thoracodorsal nerve length (musculocutaneous nerve length, in cm = –1.6129 + + 0.6496 × thoracodorsal nerve length, in cm) (Fig. 3). These equations make it possible to determine the length of the donor nerve and the recipient nerve without invasive interventions in females.

To assess the accuracy of the equation for determining the length of the thoracodorsal nerve with the known width of the thoracic cage, a comparative analysis was performed (Table 2). It was revealed that the length of the thoracodorsal nerve, measured after anatomical preparation, does not significantly differ from the indicators obtained using the proposed equation ($p = 0.07$). Furthermore, the spread in values from the regression equation to the actual length of the thoracodorsal nerve is 1.5 cm.

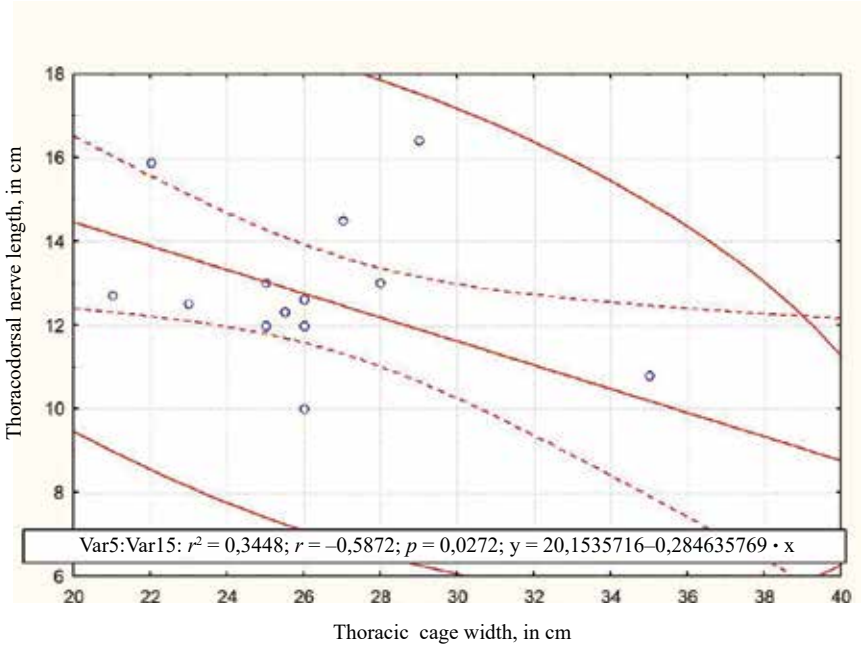

Fig. 2. Features and equation of relationship of the thoracic cage width and the thoracodorsal nerve length in females

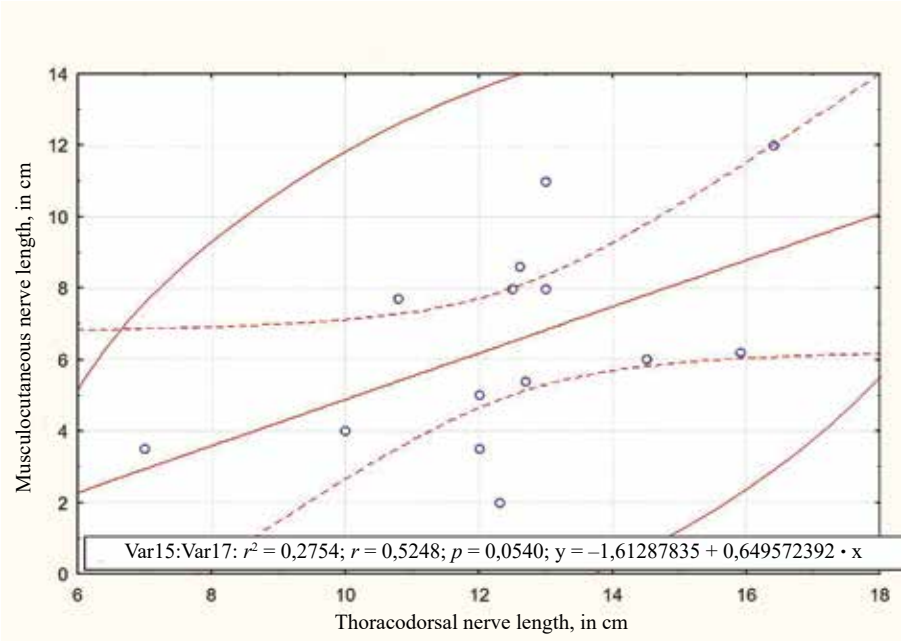

Fig. 3. Features and equation of correlation between the length of thoracodorsal and musculocutaneous nerves in females

Table 2

Comparison of the results of the experimental study in females		
Parameter	Thoracodorsal nerve length, cm (after preparation)	Thoracodorsal nerve length, cm (according to the equation)
Mean value	13.6	12.6
Standard deviation	2.57	1.20
Achieved significance level (p)	0.07	

DISCUSSION

It is known that when the trunk or cord of the brachial plexus is damaged, it is impossible to restore nervous regulation at the place of its rupture. The conduction of nerve impulses from these structures of the brachial plexus to forming nerves is violated. In this regard, transplantation of donor nerves to damaged recipient nerves is widely used

for the absolute restoration of extremity functions. The present study has shown that in 20% of cases it is impossible to carry out the transfer of the thoracodorsal nerve to the position of the musculocutaneous nerve due to large diastasis. Therefore, other sources of neurotization are necessary in such cases. According to the data by a number of other authors, such cases are rare and, despite the potential of modern diagnostics, the mismatch of nerve length during transposition is found during the operation, which is accompanied by nerve tension with subsequent complications, increase in operation time, and sometimes useless and traumatic interventions [12]. It is worth noting that in surgical practice while suturing nerves, the maximum permissible tension is the one at which the nerve segments can be pulled together by simultaneous tying of two 8/0 sutures, which corresponds to diastasis up to 2.0–2.5 cm [16].

In previous studies, anatomical features of the thoracodorsal nerve were examined from the point of its use as distal nerve transfer [17]. In most cases, the thoracodorsal nerve is formed from the posterior cord (C7, C8), which coincides with our results. In 93% of cases, it departs from the posterior cord, and in 7% of cases, it is a branch of the axillary nerve. Therefore, its transfer is effective in case of nerve damage that is formed from spinal nerves C5 and C6, which, according to statistics, are more likely to rupture during injuries of the brachial plexus [11]. The thoracodorsal nerve is located under the lateral edge of the latissimus dorsi muscle, superficially from the vascular pedicle. This makes it possible to isolate the nerve along its way to the entrance to the muscle. Our data showed that the average length of the thoracodorsal nerve before entering the broadest back muscle is 13.0 cm. According to the data of other studies, this value does not exceed 12.3 cm [7]. It is worth noting that when transplanting the distal end of the thoracodorsal nerve, the latissimus dorsi muscle does not lose its function, since it has an additional source of innervation, which is the subscapular nerve.

According to the results of the study, transposition of donor nerve to recipient nerve depends on the nerve length correspondence. According to M. Samardzić [9], nerve lengths and their cross-sectional area correspondence are of great importance for transposition, but nerve length correspondence is crucial.

It was found in the present study that neck circumference and, in women, chest width are required to determine the reliable true size of thoracodorsal and musculocutaneous nerves.

Thus, anatomical and topographic features of the thoracodorsal nerve make it most suitable for transposition in case of peripheral upper limb nerve damage, while the ability to determine the true size of the thoracodorsal and musculocutaneous nerves before surgery will allow to choose an optimal transplantation method for a particular patient and improve treatment results significantly.

CONCLUSION

Based on the study, the following conclusions have been made.

1. There is a significant direct correlation between the length of the thoracodorsal nerve and neck circumference in humans ($r = 0.317$, $p = 0.033$).

2. In 20% of cases in humans, nerve size does not allow for transplantation of the thoracodorsal nerve to the position of the musculocutaneous nerve.

3. In females, there is a significant inverse correlation between the thoracodorsal nerve length and the thoracic cage width ($r = 0.6$, $p = 0.03$). Equation of relationship between the width of the thoracic cage and the length of the thoracodorsal nerve (length of the thoracodorsal nerve, in cm = 20.1536 – 0.2846 × width of the thoracic cage, in cm) was revealed.

The length of the thoracodorsal nerve determines the possibility of its transplantation into the musculocutaneous position. It is reasonable to use neck circumference and, in females, the width of the thoracic cage as external size biomarkers for the donor and recipient nerves.

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Segmentation of focal liver lesions and virtual resection based on computed tomography data

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ABSTRACT

The aim of the study was to test the work of plugins for segmentation and virtual resection of focal liver lesions based on CT data.

Materials and methods. Analysis of CT data of the abdominal organs with bolus contrast enhancement in 80 patients with focal liver lesions was carried out. Segmentation and 3D-modeling of the CT data was carried out by radiologists and the surgeon in the 'Autoplan' system.

Results. The nosological structure of the liver in patients was determined (the most common were hemangiomas in 21.25% of 80 patients, cysts in 20%, parasitic cysts in 20%, etc.), according to the computed tomography results. The segmentation of the liver, its focal lesions, arteries, and veins was carried out using the 'Autoplan' system. The surgeon determined the volume of the parenchyma and focal liver formations using the standard function 'volume of segmentation', chose the optimal treatment tactics, and performed a virtual liver resection. In some cases, the use of segmentation and preoperative planning made it possible to avoid an inefficient surgery. The effectiveness of modeling changed the treatment tactics of 42 patients.

Conclusion. The obtained results indicate that the use of the 'Autoplan' system plugins for planning an abdominal surgery allows doctors: 1) to carry out segmentation of the liver, focal lesions, and blood vessels; 2) to determine the location of a focal formation in a particular segment, their combinations; 3) to perform a virtual resection, evaluate the structures passing through it; 4) to choose the optimal tactics of intervention or abandon it due to objective anatomical reasons.

Key words: computed tomography, preoperative 3D-modeling, segmentation, liver resection, 'Autoplan' system.

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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 Samara State Medical University (Protocol No. 205 of 19.02.2020).

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Сегментация очаговых образований печени и виртуальная резекция на основе данных компьютерной томографии

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РЕЗЮМЕ

Цель работы – протестировать работу плагинов по сегментации и виртуальной резекции очаговых образований печени на основе данных компьютерной томографии.

Материалы и методы. Проведен анализ данных компьютерной томографии органов брюшной полости с болюсным контрастированием 80 пациентов с очаговыми образованиями печени. Сегментация и 3D-моделирование томограмм проводилось в системе «Автоплан» врачами-рентгенологами при непосредственном участии врача-хирурга.

Результаты. Определена структура нозологий печени у пациентов (наиболее часто встречались гемангиомы у 21,25% из 80 пациентов, кисты у 20% обследуемых, паразитарные кисты у 20% больных и т.д.) по данным компьютерной томографии. Затем проводилась сегментация печени, ее очаговых образований, артерий и вен с помощью системы «Автоплан». Хирург определял объем паренхимы и очаговых образований печени с помощью стандартной функции «объем сегментации», выбирал оптимальную тактику лечения и проводил виртуальную резекцию. В ряде случаев применение сегментации и предоперационного планирования позволило отказаться от заведомо неэффективной операции. В результате результативность моделирования в информировании хирурга изменила тактику ведения 42 пациентов.

Заключение. Полученные результаты свидетельствуют о том, что использование плагинов системы «Автоплан» для планирования абдоминальной хирургии позволяет: 1) провести сегментацию печени, очаговых образований и сосудов; 2) определить расположение очагового образования в том или ином сегменте, их комбинации; 3) провести виртуальную плоскость резекции, оценить структуры, проходящие через нее; 4) выбрать оптимальную тактику вмешательства или отказаться от него вследствие объективных анатомических причин.

Ключевые слова: компьютерная томография, предоперационное 3D-моделирование, сегментация, резекция печени, система «Автоплан».

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

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

Соответствие принципам этики. Все лица, участвующие в исследовании, подписали информированное согласие на участие в исследовании. Исследование одобрено локальным этическим комитетом Самарского государственного медицинского университета (протокол № 205 от 19.02.2020).

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INTRODUCTION

Surgery planning is an important and integral step, especially in abdominal surgery. This process determines features of a clinical case and identifies possible problems during the operation. Planning includes standard physical examination of a patient, laboratory data analysis, and imaging techniques. For a comprehensive assessment of computed and magnetic resonance tomography imaging, modeling with constructing a polygonal model is used. This technique makes it possible not only to supplement a standard study with visual three-dimensional images with pathology mapping, but also to obtain important numerical parameters. Modeling is based on the segmentation process [1, 2]. Segmentation is

division of an image into areas for which a certain criterion of uniformity is met [3].

Segmentation tasks can be divided into two classes: search and selection of areas in the image, the characteristics of which are reliably known, and automatic search for areas with some similar char- acteristics. Segmentation, preoperative planning and preparing for intraoperative navigation are possible with the help of the 'Autoplan' system, which includes a workstation for a radiologist. This system was developed at the Center for Breakthrough Research of SamSMU 'Information Technologies in Medicine'.

The aim of the research is to test the work of plugins for segmentation and virtual resection of liver lesions based on the computed tomography data.

MATERIALS AND METHODS

The data of abdominal CT with contrast enhancement of 80 patients with focal liver lesions were analyzed. Studies were carried out on Toshiba Aqullion 32 CT (Japan). Then images in DICOM format were loaded into the 'Autoplan' hardware and software complex (registration certificate 2019/8153 of 27.02.2019) [4]. Segmentation and 3D-modeling were carried out by radiologists with the direct participation of the surgeon. The surgeon drew a virtual line of resection and set points of interest (relationship with branches of the portal vein, sufficiency of blood flow in the preserved part, etc.). The processing of one study took from 15 to 30 minutes, depending on the complexity of pathology and tasks.

RESULTS AND DISCUSSION

The following types of automatic segmentation are known: 1) thresholding; 2) region growing; 3) border detection; 4) texture segmentation methods.

The 'Autoplan' system uses the first three types of automatic segmentation in the segmentation system. Thresholding is the simplest segmentation method. It implies selection of areas homogeneous in brightness according to a threshold, which is determined automatically. Growing regions is a method that is based on joining regions closest in brightness. The idea behind the method is to analyze a pixel and grow the area to which it belongs based on most of its neighbors. Border detection is a method that is used to detect abrupt changes in image brightness and find borders and contours [5]. To solve the most frequent tasks, plugins for automatic segmentation based on "average models" have been developed in the 'Autoplan' system: 1) automatic segmentation of the body surface; 2) automatic segmentation of organs (liver, kidneys, spleen, lungs and trachea).

For vessels, an automatic segmentation plugin is also implemented in the presence of images of the corresponding phase of contrast enhancement: arterial for segmentation of arteries, venous for segmentation of veins. This plugin software module is used to visualize individual anatomy of the organ blood supply system, aortic pathologies (aneurysms, wall dissection, occlusion), and sites of tumor invasion into vascular structures in abdominal surgery. Automatic vessel segmentation is performed by establishing a point within the lumen. In this case, complex Fast Marching and Geodesic Active Contours algorithms are used [6].

Two approaches are used to highlight the structure within the created segmentation, for example, a focal lesion within the liver parenchyma. The first one is manual contouring on several sections with further modeling based on the boundary interpolation on raw sections. The second (which is more efficient) one is using the incremental segmentation plugin.

The 'Incremental segmentation' plugin is designed for segmentation by gradual region growing from a given point, based on density and contours of the surrounding tissues. This type of segmentation is the fastest and most convenient of all semi-automatic tools due to the 'spread' of segmentation simultaneously in all three coordinates and due to passing around contrasted tissues.

As a result of computer segmentation, the doctor receives a polygonal model of internal organs with marked areas of pathology.

The structure of liver nosologies identified by CT data is presented in Table. 1.

Segmentation of the liver, focal lesions, arteries, and veins was performed. Further, the function 'dividing liver into segments' was used. In order to do this, standard points were identified on the surface of the liver, after which planes were automatically drawn (Fig. 1), dividing the liver into 8 segments according to the Couinaud classification [7].

Table 1

The structure of liver lesions identified in patients according to the computed tomography

Nosology	Number of patients
Hemangioma	17
Cyst	16
Parasitic cyst	16
Hepatocellular cancer	11
Abscess	10
Metastasis	8
Adenoma	2

The distribution of focal lesions by liver segments is shown in Table. 2.

Afterwards, if it was necessary to perform a surgical intervention, the surgeon determined the volume of focal lesions and liver parenchyma using the standard function 'Segmentation volume'. When the lesion was located in one segment, segmentectomy was chosen as the tactics of treatment; when it was located in several segments, hemihepatectomy or atypical resection was selected.

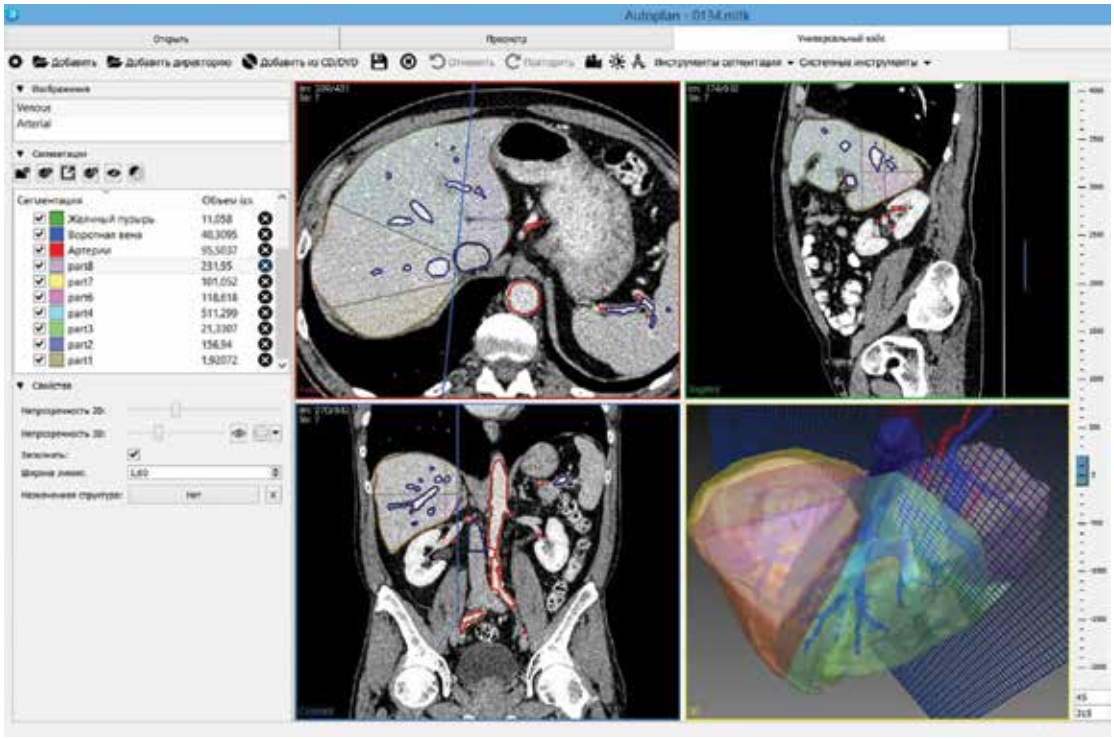

Fig. 1. Segmentation of the liver, arteries, and veins. Dividing the liver into segments using the 'Autoplan' system

Table 2

Distribution of focal lesions by liver segments

Segment	S1	S2	S3	S4	S5	S6	S7	S8	Multiple segments
Number of patients	5	7	8	7	9	7	11	8	18

A virtual resection was carried out, which consisted in constructing a line of any shape, cutting off the volume that the surgeon plans to remove from the bulk of the resulting segmentation (in this case, an organ model). The advantage of performing virtual resection is preoperative informing of the surgeon about possible complications, as the branches of portal vein and hepatic veins are visible in the resection plane [8]. It gives the surgeon the opportunity to choose an optimal resection method based as well on the residual parenchyma volume. The volume of any segmentation, both removed and remaining part of the liver, is displayed automatically [9]. Planning of a right-sided hemi-

hepatectomy in a patient with multiple hydatid cysts is shown in Fig. 2.

In some cases, the use of segmentation and preoperative planning made it possible to abandon the obviously ineffective operation. Here is a clinical example of patient I., 32 years old. The patient went to the hospital with complaints of pain and heaviness in the right hypochondrium, general weakness, loss of appetite, and stool disorder. Computed tomography of the abdominal organs with bolus contrast enhancement was performed according to the standard protocol. The results are shown in Fig. 3.

In the right lobe of the liver, a large lesion with an inhomogeneous structure was found, and in the central parts, a formation with pronounced calcification and hypovascular periphery was detected. Compression of the inferior vena cava and signs of invasion in the branches of the portal and right hepatic veins were observed. Impression: liver alveococcosis.

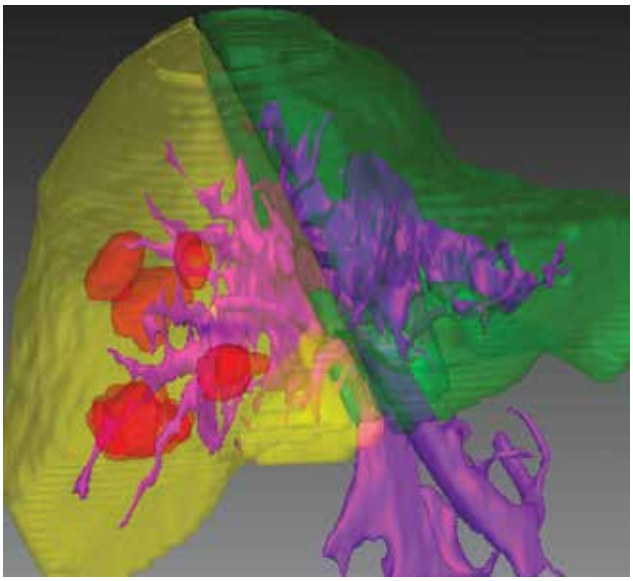

Fig. 2. 3D-model of the liver of the patient K. Green color indicates the preserved part, yellow – the resected part. Hydatid cysts are highlighted with red color, branches of the portal and hepatic veins are highlighted with purple color

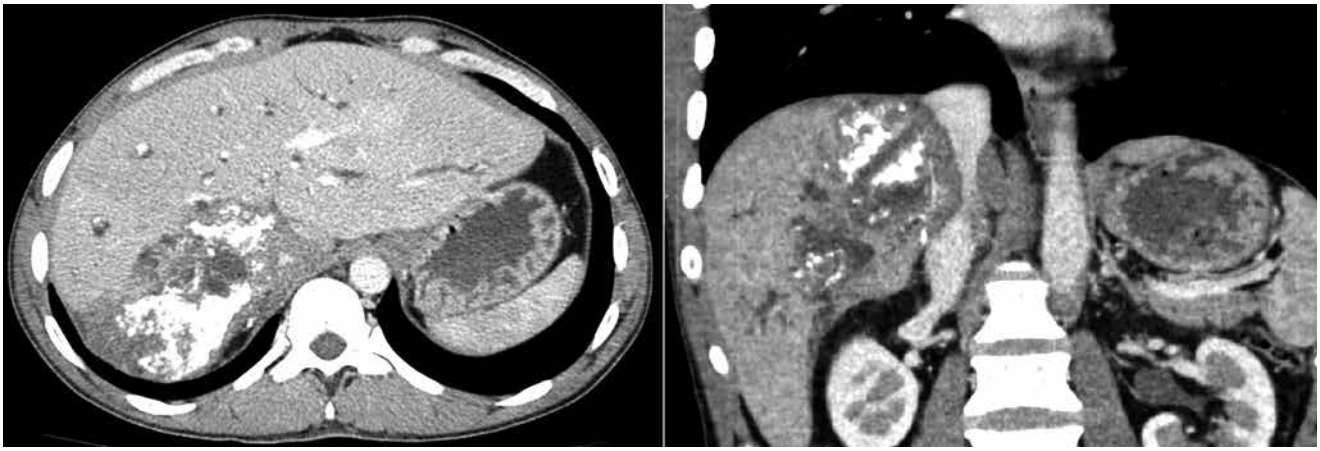

Fig. 3. Computed tomography of the abdominal organs of patient I., axial and coronal reconstruction, venous phase. In the right lobe of the liver, there is a massive heterogeneous zone with calcification

To assess the resectability of the lesion, reconstruction in the 'Autoplan' system was performed. The result is shown in Fig. 4.

Modeling made it possible to detect an extensive arterial and venous invasion of the alveococcus, which did not allow its resection as it could lead to massive intraoperative blood loss. The patient was consulted by a transplant doctor and his data were added to the waiting list for a liver transplant [10].

The effectiveness of modeling in informing the surgeon and changing the tactics in 42 patients is shown in Table 3.

Table 3

The effectiveness of modeling in informing the surgeon and changing the tactics

Performance indicator	Number of patients
The choice of tactics in the form of segmentectomy	9
The choice of tactics in the form of atypical resection	6
The choice of tactics in the form of hemihepatectomy	8
Changing the initial tactics after analyzing planar images	8
Refusal from resection due to the small volume of preserved parenchyma	5
Refusal from intervention due to vascular anatomy	3
Refusal from intervention due to vascular invasion	3

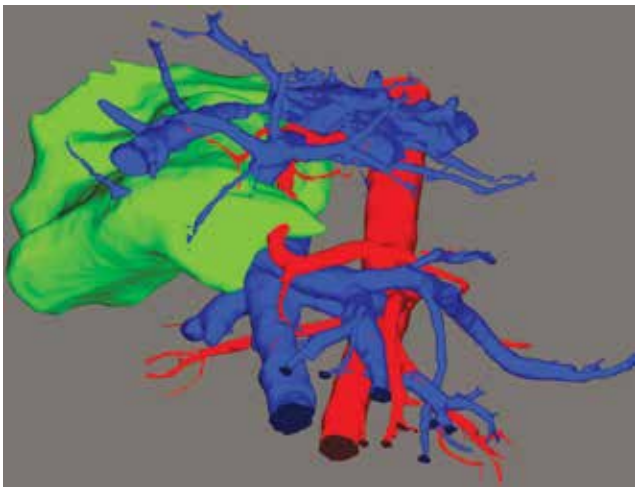

Fig. 4. 3D-model of liver alveococcosis. The zone of alveococcosis is highlighted with green color, invasion of the portal vein, inferior vena cava, and common hepatic artery are clearly shown

CONCLUSION

Thus, the use of the 'Autoplan' system plugins for planning an abdominal surgery allows doctors: 1) to carry out the segmentation of liver, focal lesions and blood vessels; 2) to determine the location of a focal formation in a particular segment, their combination; 3) to perform a virtual resection, evaluate the structures passing through it; 4) to choose the optimal tactics of intervention or abandon it due to objective anatomical reasons.

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Effect of physical load on the concentration of endothelial NO-synthase and platelet-activation factor in plasma of athletes

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ABSTRACT

Aim. To assess the effect of one-time physical load on the concentration of endothelial NO-synthase and platelet-activating factor in blood plasma of athletes training in cyclic and strength sports, as well as in untrained volunteers.

Materials and methods. The study involved 28 men aged 18–25 years, who were relatively healthy and had no disorders of the cardiovascular system. Three groups were formed according to the sports classification. Group 1 (TFG) included highly qualified athletes (Candidates for Master of Sports (CMS), Master of Sports (MS)) of cyclic sports – track and field athletics (middle-distance running, 800–1500 m), n = 10. Group 2 (WG) consisted of highly qualified athletes (CMS, MS) of strength sports – weightlifting, n = 8. Group 3 (CG) was a control group and included untrained men with no sports category, n = 10. All volunteers were examined in the morning on an empty stomach. One day before the study, the athletes were advised to stop the training process. The blood from the cubital vein was taken from all the individuals three times: before exercise (test A), immediately after performing the standard PWC_{170} test on a bicycle ergometer (test B), and 60 minutes after performing the stress test (test C). Determination of the concentration of endothelial NO-synthase (eNOS) and platelet-activating factor (PAF) in plasma was performed by enzyme immunoassay.

Results. It has been shown that the features of endothelial reactivity in athletes of various specializations in comparison with untrained volunteers are significantly associated with the level of eNOS production both at rest and in response to short-term physical exertion. Platelet-activating factor can also affect endothelial reactivity, but to a lesser extent, and is involved only in the mechanisms of adaptation to repetitive high-intensity physical loads.

Key words: endothelium, athletics, weightlifting, training.

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

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Conformity with the principles of ethics. All study participants signed an informed consent to participate in the study. The study was approved by the local Ethics Committee of the Biological Institute of NR TSU (Protocol No. 33 of 02.12.2019).

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Влияние физической нагрузки на концентрацию эндотелиальной NO-синтазы и фактора активации тромбоцитов в плазме у спортсменов

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РЕЗЮМЕ

Цель. Оценить влияние однократной физической нагрузки на концентрацию эндотелиальной NO-синтазы и фактора активации тромбоцитов в плазме крови у спортсменов, тренирующихся в циклических и силовых видах спорта, а также у нетренированных волонтеров.

Материалы и методы. В исследовании участвовали 28 мужчин в возрасте 18–25 лет, условно здоровые, без нарушений со стороны сердечно-сосудистой системы. В соответствии со спортивной классификацией было сформировано три группы. Группа 1 (ЛА): высококвалифицированные спортсмены (кандидаты в мастера спорта (КМС), мастера спорта (МС)) циклических видов спорта – легкая атлетика (бег на средние дистанции 800–1500 м), $n = 10$. Группа 2 (ТА): высококвалифицированные спортсмены (КМС, МС) силовых видов спорта – тяжелая атлетика, $n = 8$. Группа 3 (КГ): контрольная группа – нетренированные мужчины, не имеющие спортивного разряд, $n = 10$. Все волонтеры проходили обследование утром натощак. За 1 сут до исследования спортсменам было рекомендовано прекратить тренировочный процесс. У всех испытуемых трижды бралась кровь из локтевой вены: до нагрузки (проба А), сразу после выполнения стандартной пробы PWC_{170} на велоэргометре (проба В) и через 60 мин после выполнения нагрузочной пробы (проба С). Определение концентрации eNOS и PAF в плазме производилось методом иммуноферментного анализа.

Результаты. Показано, что особенности эндотелиальной реактивности у спортсменов различных специализаций в сравнении с нетренированными волонтерами в значительной степени связаны с уровнем продукции eNOS как в покое, так и в ответ на кратковременные физические нагрузки. Фактор активации тромбоцитов также может оказывать влияние на эндотелиальную реактивность, но в меньшей степени, и вовлекается только в механизмы адаптации к регулярным нагрузкам высокой интенсивности.

Ключевые слова: эндотелий, легкая атлетика, тяжелая атлетика, тренировки.

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INTRODUCTION

Systematic physical activity of various natures has a modulating effect on the cardiovascular system and induces the adaptation processes of all its components, including the vascular endothelium [1, 2]. These changes can have a multidirectional effect on the risk of developing hemodynamic disorders. The effect can be either positive, accompanied by the potentiation of endothelium-dependent reactions, or negative [3]. At the same time, the mechanisms of the described adaptations remain largely unclear. In our previous publications [4, 5], it was

shown that athletes of high qualification have suppression of endothelial functional activity, and its degree is determined by the intensity and type of physical activity.

The mechanisms of endothelium-dependent vascular relaxation are traditionally associated with production of nitric oxide (NO). With the participation of endothelial NO-synthase (eNOS), NO production in the endothelium is enhanced. The content of eNOS in plasma differs depending on the type of physical activity [6]. This may be one of the reasons for the opposite reactions in the cuff test in athletes training in various sports. However, information on the eNOS concentration in the blood of people during different physical activity is often contradictory. Thus, after cyclic exercise, the concentration of eNOS in plasma increases by 36%, whereas after prolonged endurance training, the content of eNOS increases by only 14% [7]. There is also evidence that acyclic loads of submaximal power are not accompanied by increases in the blood eNOS content, in contrast to long-term and intense cyclic loads [8].

Simultaneously with the influence of endothelial factors, the hemostatic system, especially its platelet link, can also participate in the modification of blood flow during physical exercise. With acute and prolonged intense physical activity, there is a tendency to hypercoagulability, especially in untrained individuals. Acute physical activity of maximum intensity induces a transient platelet increase. Platelet activation depends on the intensity of physical activity [9–11]. Platelet-activating factor (PAF) has an important role in this process [12]. Some researchers consider PAF as a compensation mechanism that protects athletes from the risk of developing thrombosis and cardiovascular diseases [13].

The aim of this study was to assess the effect of one-time physical load on the concentration of endothelial NO-synthase and platelet-activating factor in blood plasma in athletes training in cyclic and strength sports, as well as in untrained volunteers.

MATERIALS AND METHODS

The study involved 28 men aged 18–25 years, who were relatively healthy and had no disorders of the cardiovascular system. Three groups were formed according to the sports classification. Group 1 (TFG) included highly qualified athletes (Candidates for Master of Sports (CMS), Master of Sports (MS)) of cyclic sports – track and field athletics (middle-distance running, 800–1500 m), n = 10. Group 2 (WG) consisted of highly qualified athletes (CMS, MS) of strength sports – weightlifting, n = 8. Group 3 (CG) was a control group and included untrained men with no sports category, n = 10. The athletes of WG and TFG groups had been engaged in sports for more than 6 years. All athletes took part in Russian competitions and won prizes.

All volunteers were examined in the morning on an empty stomach. One day before the study, athletes were advised to stop the training process. The blood from the cubital vein was taken from all the participants three times: before exercise (test A), immediately after performing the standard PWC_{170} test on a bicycle ergometer (test B), and 60 minutes after performing the stress test (test C).

BD Vacutainer 5-ml vacuum system and 5 ml Vacuette® Premium tubes with heparin separation gel (Greiner Bio-One, Austria) were used. The heparin concentration in the tubes was 20 U / ml. After 30 min of blood collection, erythrocytes and white blood cells were sedimented for 11 minutes at 2,000 rpm using an LMC 3000 laboratory centrifuge (Biosan, Latvia). The plasma was frozen and stored in a freezer at –20 °C for no more than 30 days.

Measurement of the eNOS and PAF concentrations in plasma was performed by enzyme-linked immunosorbent assay (ELISA) using RayBio Human eNOS ELISA Kit (RayBio®, USA) and Enzyme-linked Immunosorbent Assay Kit for Platelet Activating Factor (PAF) (Cloud-Clone Corporation, USA). All samples were taken in duplicate. Microwell test strips with flat-bottomed wells (12 × 8 wells) were used for the analysis. The microwell strips were incubated on a PST-60HL microplate shaker (Biosan, Latvia) and washed using an Anthos Fluido 2 washer (Biochrom, Great Britain). The sample optical density was measured using an Anthos 2010 spectrophotometer with filters (400–750 nm) and ADAP+ software (Biochrom, Great Britain) at 450 nm and 620 nm as primary and reference wavelengths, respectively.

Statistical data processing was carried out using STATISTICA 8.0. The level of significance in testing the hypothesis that two samples belong to the same general population was assessed using the Kruskal – Wallis test (ANOVA test). Data are pre-

sented as Mean ± Standard Deviation *(M ± m)*. All the participants signed an informed consent to participate in the study and consent to blood sampling. Permission was obtained from the Ethical Committee of Tomsk State University (Protocol No. 33 of 02.12.2019).

RESULTS AND DISCUSSION

The results are shown in Table 1.

Table 1

eNOS and PAF concentrations in blood plasma of healthy volunteers (control group), weightlifters, and track and field athletes before, immediately after, and 60 minutes after the exercisee (data are presented as *M* ± *m*)

Groups	Parameter	Baseline level (sample A)	0 min post exercise (sample B)	60 min post exercise (sample C)
CG (control group) $n = 10$	eNOS (ng/ml)	4.96 ± 0.72	22.5 ± 4.11 $p_4 < 0.001$	22.25 ± 3.82 $p_4 < 0.001$
	PAF (pg/ml)	47.93 ± 3.25	52.04 ± 4.25	53.98 ± 5.37
TFG (track and field group) $n = 10$	eNOS (ng/ml)	2.23 ± 0.42 $p_3 < 0.05$	6.13 ± 0.74 $p_3 < 0.001$ $p_4 < 0.05$	4.66 ± 0.55 $p_3 < 0.001$ $p_4 < 0.05$
	PAF (pg/ml)	40.42 ± 3.27 $p_3 < 0.05$	39.25 ± 4.28 $p_3 < 0.01$	38.16 ± 4.57 $p_3 < 0.01$
WG (weightlifting group) $n = 8$	eNOS (ng/ml)	1.35 ± 0.47 $p_1 < 0.01$ $p_2 < 0.001$	1.05 ± 0.21 $p_1 < 0.001$ $p_2 < 0.001$	1.65 ± 0.07 $p_1 < 0.001$ $p_2 < 0.001$ $p_5 < 0.05$
	PAF (pg/ml)	39.67 ± 4.04 $p_2 < 0.05$	38.62 ± 3.55 $p_2 < 0.01$	45.15 ± 3.24 $p_1 < 0.001$

Note: p_1 – differences between WG and TFG; p_2 – differences between WG and CG; p_3 – differences between TFG and CG; p_4 – as opposed to sample A; p_5 – as opposed to sample B.

The control group had the eNOS concentration of 4.96 ± 0.72 ng / ml, which is significantly higher than this parameter in both groups of athletes. At the same time, in this group, the maximum increase in this parameter immediately after physical exercise and its maintenance at a constant level for 1 hour were recorded. In the athletes of both groups, the baseline level of eNOS was significantly lower: two times lower in the track and field athletes and 4 times lower in the weightlifters. In the TFG athletes, immediately after exercise, an increase in the eNOS concentration by 2.5 times was identified. After 1 hour, it decreased by about 30%. The weightlifters, on the other hand, showed a tendency to a decrease in plasma eNOS concentration immediately after exercise, which returned to the baseline level in an hour.

The results obtained show a high correlation with the previously published data on endothelial reactivity in athletes of various specializations [4, 5]. It has been shown that highly qualified athletes have signs of endothelial dysfunction, which are more significant in the group engaged in strength sports. In the track and field athletes, endothelium-dependent vasodilation is much less pronounced, and in weightlifters, it was not recorded at all, while in untrained persons, it is the most pronounced.

The PAF concentration values in the groups did not differ so significantly (Table 1). In the control group, the baseline level was 47.93 ± 3.25 pg / ml. In the athletes of both groups it was 15% lower. In all the groups, this parameter almost did not change after physical exertion. Only in the weightlifters, 1 hour after exercise, its slight increase was noted. Apparently, there is a decrease in plasma PAF concentration in athletes as a result of regular exercise. This may be a factor suppressing endothelium-dependent vasodilation. Some researchers believe that platelets can stimulate endothelial reactions due to mechanical interaction with the endothelial surface. At the same time, this mechanism is more inertial in comparison with eNOS and is not involved in short-term effects after a single exercise. In weightlifters, an increase in PAF concentration may be associated with blood stagnation, injury to muscle tissue, and microvascular injury.

Physical activity is accompanied by an increase in blood pressure [14], which may influence the growth of endothelial cells [15]. In addition, mechanical stimuli associated with changes in the shear stress and stretching of the vascular wall activate intracellular signaling cascades in the endothelium, which is accompanied by the activation of transcription factors [4].

CONCLUSION

It is obvious that the features of endothelial reactivity in athletes of various sports in comparison with untrained volunteers are significantly associated with the level of eNOS production both at rest and in response to short-term physical exertion. The platelet-activating factor can also affect endothelial reactivity, but to a lesser extent, and is involved

only in the mechanisms of adaptation to repetitive intense physical activity. The revealed patterns can serve both as a mechanism of adaptation to regular training loads and as a risk factor for acute hemodynamic dysfunctions in athletes.

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Biomarkers of clinical and radiological severity of a new coronavirus infection caused by SARS-CoV-2 virus, and their association with a severe variant of its course

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ABSRACT

Aim. To establish biomarkers for clinical, radiological, and laboratory severity of COVID-19 infection and to identify their relationships.

Materials and methods. A retrospective study was carried out which included 155 patients undergoing treatment at the Hospital for War Veterans No. 3 with a confirmed diagnosis of novel coronavirus infection caused by nCoV from April 6 to June 10, 2020. All patients underwent clinical and laboratory examination. An intergroup statistical analysis of clinical and laboratory parameters was carried out depending on the criteria of clinical severity and severity of radiological signs of chest organ pathology according to computed tomography (CT).

Results. Patients with mild COVID-19 showed a lower level of leukocytes, urea, creatinine, bilirubin, and aspartate dehydrogenase (AsAT), as opposed to the corresponding levels in patients with an extremely severe course of the disease. A lower level of calcium in the peripheral blood was found in patients with severe COVID-19, along with an increase in blood glucose.

Patients from the CT1 group as well as patients with a clinically mild course of the novel coronavirus infection had significantly lower levels of neutrophils, urea, creatinine, AsAT, and blood glucose and a higher level of blood calcium in comparison with patients with various CT patterns. In the group of patients with a lethal outcome, cardiovascular diseases were significantly more often detected, as opposed to the discharged patients.

Conclusion. A number of biomarkers characterizing the severity of the novel coronavirus infection caused by the SARS-CoV-2 virus have been identified. However, the revealed differences in the laboratory markers of the clinical and radiological severity of the disease do not currently allow to accurately characterize the nature of the relationship between the clinical severity of the disease, CT findings, and laboratory indicators of COVID-19 severity.

Key words: SARS-CoV-2, COVID-19, lymphocytes, D-dimer, glucose, calcium, CT scan, clinical severity.

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

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Conformity with the principles of ethics. Every participant of the study signed an informed consent. The study was approved by the I.M. Sechenov First Moscow State Medical University (Protocol No. 19-20 of 02.07.2020).

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Биомаркеры клинической и рентгенологической тяжести новой коронавирусной инфекции, вызванной вирусом SARS-CoV-2, и их ассоциация с тяжелым вариантом ее течения

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РЕЗЮМЕ

Цель. Установление биомаркеров клинической, рентгенологической и лабораторной тяжести инфекции COVID-19 и выявление их взаимосвязи.

Материалы и методы. Выполнено ретроспективное исследование, в которое включены 155 пациентов, проходивших лечение в Госпитале для ветеранов войн № 3 с подтвержденным диагнозом «новая коронавирусная инфекция, вызванная SARS-CoV-2» с 6 апреля по 10 июня 2020 г. Пациентам выполнено клинико-лабораторное обследование. Проведен межгрупповой статистический анализ клинико-лабораторных показателей в зависимости от критериев клинической тяжести и выраженности рентгенологических признаков патологии органов грудной клетки по данным компьютерной томографии (КТ).

Результаты. У больных с легким течением COVID-19 отмечался более низкий уровень лейкоцитов, мочевины, креатинина, билирубина и аспартатдегидрогеназы (АсАТ) по сравнению с соответствующим уровнем показателей у пациентов с крайне тяжелым течением заболевания. Выявлен более низкий уровень кальция в периферической крови у пациентов с тяжелым течением COVID-19 и повышение в этой группе уровня глюкозы крови. Пациенты из группы КТ1, как и больные с клинически легким течением новой коронавирусной инфекции, имели достоверно более низкое содержание нейтрофилов, мочевины, креатинина, АсАТ и глюкозы крови и более высокий уровень кальция крови в сравнении с пациентами с различными КТ-паттернами. В группе пациентов с летальным исходом значимо чаще выявлялись сердечно-сосудистые заболевания по сравнению с выписанными больными.

Заключение. Установлено наличие ряда биомаркеров, характеризующих тяжесть течения новой коронавирусной инфекции, вызванной вирусом SARS-CoV-2. Однако выявленные различия в лабораторных маркерах клинической и рентгенологической тяжести заболевания не позволяют в настоящий момент дать однозначный ответ на вопрос о характере взаимосвязи между клинической тяжестью течения, КТ-картиной и лабораторными показателями тяжести COVID-19.

Ключевые слова: SARS-CoV-2, COVID-19, лимфоциты, D-димер, глюкоза, кальций, компьютерная томография, клиническая тяжесть.

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

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

Соответствие принципам этики. От каждого из включенных в исследование участников было получено информированное добровольное согласие. Исследование одобрено Первым МГМУ им. И.М. Сеченова (протокол № 19-20 от 02.07.2020).

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INTRODUCTION

In March 2020, the World Health Organization announced the beginning of a pandemic of a novel coronavirus infection (COVID-19) caused by the SARS-CoV-2 virus, predominantly affecting the respiratory tract [1]. Pathological exposure to SARS-CoV-2 leads to the formation of a hyperergic response of the immune system in some patients [2]. This entails the development of extensive damage to the pulmonary parenchyma, multiple organ failure, clinically reported as a septic shock, and cytokine storm [3–5]. Furthermore, in severely ill patients, viremia causes damage to the heart, liver, pancreas, and kidneys [5–7].

According to various studies, the severity of the clinical condition most significantly correlates with lymphopenia, increased number of neutrophils, C-reactive protein (CRP), ferritin, interleukins-1,6, and elevated D-dimer leading to intravascular hypercoagulation [6–10]. Several studies have recently been published demonstrating the relationship between hypocalcemia and hyperglycemia and a severe course of the disease [3–8].

Cases with multiple organ lesions and hyperactivation of the immune system demonstrate extremely high mortality. Current mortality rate from the novel coronavirus infection ranges from 3 to 10% [1, 2, 11–13]. Despite the large number of published studies investigating markers of a severe COVID-19 course, there is still a lack of reliable early clinical and radiological predictors of such a fatal condition. In addition, several cases reveal clinicoradiological dissociation, when there is a mismatch between the volume of the lesion on the computed tomography of the lungs (CT) and clinical data. Thus, studying the features of the severe and extremely severe clinical course of COVID-19 and searching for its early biological markers should be a priority direction in the current studies.

The aim of the study was to reveal biomarkers of clinical, radiological, and laboratory severity of COVID-19 and to analyze their relationships.

MATERIALS AND METHODS

A retrospective cross-sectional study was conducted. From April 6 to June 10, 2020, by a simple random selection method, 155 patients were included in the study: 82 (52.9%) men and 73 (47.1%) women, who underwent treatment at the Hospital for War Veterans No. 3 with a confirmed diagnosis of the novel coronavirus infection caused by SARS CoV-2. The average age of the patients was 64.0 (59.5–81.0) years.

The diagnosis of the novel coronavirus infection was confirmed by a positive polymerase chain reaction carried out upon admission of patients to the hospital and characteristic CT symptoms of the infection.

The study was carried out according to the principles of Declaration of Helsinki (2013), national guidelines for good clinical practice of the Russian Federation, and other corresponding ethical standards and approved by the local Ethics Committee at I.M. Sechenov First Moscow State Medical University (Protocol No. 19–20 of 02.07.2020).

After admission, all patients underwent clinical and laboratory examination (collection of anamnestic data, physical examination, clinical and biochemical blood tests, and a coagulation test) and chest CT. A follow-up CT scan was performed on the 10th day of the hospitalization, if no indications for earlier investigation were revealed. Chest

CT was performed with multidetector CT scanners (Toshiba Aquilion One 160 (Japan) and Toshiba CXL 64 (Japan)) using a standard CT protocol. The patients were treated according to the current national guidelines for preventing, diagnosing, and treating the new coronavirus infection (COVID-19) [1]. All patients were over 18 years, had no mental disorders, did not suffer from alcohol or drug abuse, and signed an informed consent.

To achieve the established aim, we carried out an intergroup statistical analysis of clinical and laboratory parameters depending on the criteria of clinical severity and the severity of the disease according to CT findings. As clinical criteria of COVID-19 severity, we used the criteria recommended in the methodological guidelines for preventing, diagnosing, and treating the novel coronavirus infection (COVID-19) [1]. As CT criteria of the disease severity, we used the X-ray criteria for diagnosing inflammatory changes in the chest organs in COVID-19 [2].

Statistical analysis was performed using Statistica 10.0 software (StatSoft Inc, USA). Comparisons among the groups were performed using the Kruskal – Wallis H test. Assessment of the differences in the mean values in pairwise independent samples was performed using the Mann – Whitney U test. Qualitative data are presented as absolute or relative (%) values. Quantitative variables are presented as $M \pm SD$, where M is the arithmetic mean, and SD is the standard deviation. The difference in values was considered statistically significant at $p < 0.05$. Correlation analysis with Spearman's correlation coefficient and one-way regression analysis were carried out to analyze the dependencies. The correlation was considered strong if the correlation coefficient was from ± 0.7 to ± 1; it was considered average if the coefficient was from ± 0.3 to ± 0.699; and weak if it ranged from 0 to ± 0.299.

RESULTS

The obtained data indicate a significantly lower level of neutrophils in the peripheral blood in patients with mild COVID-19 in comparison with patients with a severe and extremely severe course of the disease (Table 1).

Table 1

Comparative analysis of laboratory parameters in patients upon admission to the hospital, depending on the severity of COVID-19

Parameter	Clinical severity 1	Clinical severity 2	Clinical severity 3	Clinical severity 4	*p**
Leukocytes	6.3 ± 3.6	7.67 ± 3.9	8.52 ± 4.7	9.47 ± 4.8	0.007
Neutrophils	4.46 ± 3.2	5.55 ± 3.7	6.90 ± 4.5	8.10 ± 4.8	0.02
Total protein	65.3 ± 6.8	64.3 ± 5.2	63.2 ± 5.1	58.4 ± 4.6	0.02
Albumin	36.4 ± 4.1	33.9 ± 5.6	32.6 ± 5.4	29.1 ± 2.6	0.001
Urea	7.3 ± 2.4	6.9 ± 2.2	10.7 ± 3.8	15.3 ± 4.1	0.002
Creatinine	126.5 ± 31.2	114.8 ± 30.1	116.5 ± 29.6	176.1 ± 32.6	0.005
Bilirubin	13.5 ± 4.1	11.3 ± 3.9	13.4 ± 3.6	21.9 ± 5.2	0.007
AST	59.2 ± 26.2	51.9 ± 23.3	91.0 ± 59.3	102.8 ± 65.5	0.01
Calcium	0.98 ± 0.35	0.93 ± 0.24	0.85 ± 0.29	0.49 ± 0.16	0.002
Glucose	6.99 ± 2.7	7.46 ± 2.8	8.28 ± 3.2	11.9 ± 3.8	0.02

* Kruskal – Wallis test (here and in Table 2)

Further, some other statistically significant differences were found in a number of laboratory parameters among patients with a mild course and patients with an extremely severe course of the disease. So, in patients with mild COVID-19, there were lower levels of leukocytes, urea, creatinine, bilirubin, and aspartate aminotransferase (AST), as opposed to the group of patients with extremely severe disease. In addition, patients with a mild course of COVID-19 had significantly higher concentration of albumin than patients with a more severe disease course.

Patients with a severe course of COVID-19 had a significantly lower level of calcium and increased glucose concentration in blood (Table 1). No data on the significant differences in the levels of other electrolytes (sodium, potassium, chlorine, iron) between the groups were obtained.

In order to identify the differences in clinical and laboratory parameters in patients stratified according to the severity of COVID-19 following CT findings, an intergroup comparative analysis was carried out (Table 2).

As it is shown in Table 2, patients from the CT1 group, similarly to patients with a clinically mild course of the disease, had significantly lower levels of neutrophils, urea, creatinine, AST, and blood glucose and a higher level of blood calcium than other participants. Additional laboratory markers were established (lymphocytes, eosinophils, lactate dehydrogenase (LDH), D-dimer, and C-reactive protein (CRP)), according to which the patients in the groups differed and patients with different clinical severity of the disease did not differ. From the data presented in Table 2, it can be seen that patients from the group with less pronounced changes in the lung tissue had a higher level of lymphocytes in the peripheral blood and lower levels of LDH, D-dimer, and CRP.

Table 2

Comparative analysis of laboratory parameters in patients with COVID-19 upon admission to the hospital, depending on the degree of changes detected by CT of the chest organs (CT0–CT4), *M* ± *SD*

Parameter	CT1	CT2	CT3	CT4	*p**
Neutrophils	4.78 ± 3.3	5.98 ± 3.7	5.73 ± 3.6	7.46 ± 3.9	0.01
Leukocytes	1.66 ± 0.62	1.18 ± 0.71	1.29 ± 0.9	0.94 ± 0.32	0.001
Albumin	36.9 ± 4.6	33.3 ± 3.7	33.6 ± 5.2	30.4 ± 2.8	0.001
Urea	6.3 ± 5.2	8.1 ± 3.6	8.5 ± 4.7	12.1 ± 8.3	0.001
Creatinine	123.4 ± 78.1	130.3 ± 65.4	103.5 ± 56.9	152.1 ± 90.0	0.01
AST	43.6 ± 12.5	62.3 ± 24.9	75.2 ± 28.7	77.1 ± 30.8	0.02
LDH	563.4 ± 102.5	826.2 ± 259.4	866.9 ± 234.9	1,103.0 ± 522.4	0.003
Calcium	1.06 ± 0.46	0.94 ± 0.21	0.84 ± 0.32	0.703 ± 0.18	0.01
Glucose	6.49 ± 2.3	7.41 ± 2.7	8.12 ± 2.5	10.0 ± 4.2	0.001
D-dimer	583.2 ± 132.4	1,780.9 ± 1,446.9	1,663.6 ± 1,165.4	1,750.3 ± 1,240.8	0.001
CRP	48.4 ± 23.7	125.8 ± 82.2	127.4 ± 73.5	171.0 ± 90.4	0.001

* Kruskal – Wallis test

The next stage of the statistical analysis included a comparative analysis of the clinical and laboratory parameters in patients with deterioration according to CT, who were divided into groups depending on the outcomes (discharge from the hospital / death). Out of 104 patients with negative CT dynamics, the outcome was fatal in 82 patients. Upon admission, the condition of the patients with subsequent lethal outcome was assessed as severe in 61 (73.8%) cases and as extremely severe in 21 (26.2%) individuals. However, CT patterns upon admission were distributed as follows: CT2 was detected in 19 (23%) patients, CT3 – in 44 (54%) patients, and CT4 – in 19 (23%) patients. Pairwise comparison (Mann – Whitney test) revealed significant differences in the levels of lymphocytes, eosinophils, LDH, D-dimer, and CRP between the CT2 and CT3–4 groups ($p < 0.01$). However, between patients of CT3 and CT4 groups, only insignificant differences ($p < 0.05$) in the concentrations of lymphocytes and urea were revealed.

We also obtained data indicating that a part of the participants (15 (9.6%) patients) with CT3–4 lesions had a mild clinical course of the disease and / or had no pathological changes in the clinical laboratory data at the time of the discharge.

At the next stage of the work, we looked closer at the patients with lethal outcome. All these participants underwent a postmortem examination, so it was possible to establish particular complications of COVID-19 and causes of death with certainty. The postmortem examination data analysis confirmed acute respiratory distress syndrome (ARDS) as a complication of the disease in 80 (97.5%) patients who had died. Pulmonary embolism (PE) was detected in 8 (9.8%) patients, sepsis – in 2 (2.4%) cases, and ischemic stroke – also in 2 (2.4%) patients. Concomitant bacterial infection was found in 58 (70.7%) people, which was confirmed by intravital tracheobronchial aspiration and the presence of areas of neutrophil infiltration in the lung parenchyma upon the postmortem exa – mination.

We studied the distribution of concomitant pathologies in patients with different outcomes of the disease (discharge from the hospital / death). The data between the groups are presented in Table 3.

Table 3

Distribution of comorbidities in patients with different outcomes of COVID-19

Parameter	Discharged patients, *n* = 73	Patients with the lethal outcome, *n* = 82
Arterial hypertension	38 (52%)	67 (81.7%)
Ischemic heart disease	33 (45%)	52 (63.4%)
Chronic heart insufficiency	9 (12%)	44 (54%)
Diabetes	29 (40%)	29 (35.4%)
Malignancy (in the medical history)	44 (60%)	13 (16%)
Chronic lung diseases	47 (64%)	26 (32%)
No comorbidity	26 (36%)	12 (14.6%)

In the group of patients with the lethal outcome, cardiovascular diseases were significantly more often detected in comparison with discharged patients. At the same time, a concomitant pathology in this group was absent only in 14.6% of patients, in contrast to the group of individuals discharged from the hospital, where the concomitant pathology was absent in almost 1/3 of observations. This may indicate that the concomitant cardiovascular pathology to a greater extent is associated with an unfavorable outcome of the disease.

Analysis of the relationships between the data of clinical and laboratory studies and the clinical and radiological severity of COVID-19 showed a strong positive correlation with the levels of neutrophils, albumin, creatinine, urea, and calcium. A strong negative correlation was found between lymphocyte counts and the radiological severity. A moderate positive correlation was found between the level of D-dimer, glucose, and CRP and the radiological severity. These data confirm the value of the comprehensive analysis of laboratory parameters, clinical examination data, and a CT pattern of the disease.

DISCUSSION

The results of our study of clinical biomarkers of severe and extremely severe courses of COVID-19 do not contradict with the data published in the literature. We demonstrated that serum levels of neutrophils, lymphocytes, albumin, urea, creatinine, D-dimer, calcium, glucose, and CRP are associated with the severity and outcome of the disease in patients with COVID-19. Some researchers also revealed the influence of concomitant bacterial infection (leukocytosis, neutrophilia, and increased CRP levels) on aggravation of the course of COVID-19 [5, 6, 9]. According to the same researchers, elevated levels of CRP, AST, LDH, D-dimer, and lymphocytopenia were almost not registered in patients with mild COVID-19, in contrast to patients with a severe course of infection [6, 7]. These findings emphasize rare concomitant bacterial infection and development of multiple organ failure in patients with a mild course of the novel coronavirus infection.

In some other studies, hypocalcemia was used as a prognostic marker of the severe and extremely severe course of COVID-19 [7–9]. Low serum calcium concentration can result from decreased absorption of electrolyte in the intestine, dysregulation of calcium metabolism against the background of hyperparathyroidism and a decrease in vitamin D level, and direct viral exposure. It was shown that in various viral infections calcium is required for penetration of the virus in the host cells, its maturation, and replication. For example, with similar infection caused by a virus of the same SARS-CoV family, intracellular impairment of calcium homeostasis promoted the activation of pro-inflammatory mechanisms and an increase in the levels of IL-1, IL-6, and tumor necrosis factor α. This pathological state resulted in damage to the lung tissue [13].

Hypoalbuminemia and increased serum creatinine and urea may be considered as biomarkers of the development of multiple organ failure [7, 9]. What is more, several studies demonstrated the

connection between hypoproteinemia and hypocalcemia in the acute phase of COVID-19 and their association with the lethal outcome of the disease [5, 10].

Our analysis revealed an increased blood glucose level in patients in the acute phase of COVID-19. Glucose values were significantly higher in the severe and extremely severe course of the disease. Diabetes mellitus is considered one of the most frequent concomitant pathologies that aggravate the course of COVID-19 [14, 15]. In our group of patients, 58 (37.4%) people had had the diagnosis of diabetes mellitus before admission to the hospital. At the same time, hyperglycemia during hospitalization was registered in 72 (46.5%) patients. Since in the majority of patients who had no history of diabetes mellitus, hyperglycemia was transient and resolved at the time of discharge from the hospital, the diagnosis of diabetes mellitus was not made upon discharge.

Such an increase in blood glucose levels detected in our patients is consistent with the results of other studies [8, 16]. Hyperglycemia in COVID-19 patients is likely associated with damage to the pancreas. Pancreatic damage, with increased levels of amylase and lipase in blood, was also reported in the study by D.J. Drucker (2020). According to this author, a moderate increase in glucose levels was found in more than 2/3 of patients [16]. Impairment of carbohydrate metabolism may develop due to tropism of SARS-CoV-2 to two receptors (ACE2 (angiotensin-converting enzyme-2) and DPP-4 (dipeptidylpeptidase-4)), which are involved in various stages of carbohydrate metabolism. In addition, these receptors can be involved in the regulation of inflammatory reactions and the functional state of the cardiovascular system and kidneys. Thus, the expression of ACE2 in both endocrine and exocrine pancreatic tissue can cause the development of pancreatitis in a number of patients against the background of COVID-19, which contributes to the exacerbation or *de novo* emergence of diabetes mellitus in the severe course of this infection [16].

Since the assessment of the clinical severity of the course of COVID-19 is based not only on the results of clinical and laboratory research methods, but also on the radiological characteristics of the pulmonary lesion, several researchers attempted to use CT as a prognostic criterion for the clinical disease severity [2].

In the course of the study, no significant relationship between the clinical and radiological characteristics of COVID-19 and its outcomes was found. Thus, we identified statistically significant differences in the markers of the infectious and inflammatory process, lymphocytes, urea, creatinine, LDH, AST, calcium, glucose, and D-dimer between the groups of patients with different radiological severity at initial CT scanning. These differences were not found in the groups of patients divided according to the same classification upon discharge; even though upon discharge, a lot of patients retained radiological changes corresponding to both CT 1–2 and CT 3–4 grades. There were only minor differences in the lymphocyte and urea levels between the patients from the CT3 and CT4 groups.

In May 2020, an article was published, where the authors point out that older age and high LDH are independent risk factors for a severe course of COVID-19 even in patients with a mild course of the disease [17]. These data also correlate with our results.

CONCLUSION

Our results indicate several biomarkers of the severity of the course of the novel coronavirus infection caused by the SARS-CoV-2 virus. However, the revealed differences in laboratory markers of the clinical and radiological severity of the disease do not currently allow to accurately characterize the nature of the relationship between the clinical severity of the disease course, CT changes, and laboratory parameters of COVID-19 severity.

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Authors contribution

Karnaushkina M.A. – conception and design, drafting of the article, final approval of the manuscript, integrity of all sections of the article. Topolyanskaya S.V. – critical revision of the manuscript for important intellectual content. Antonova E.V. – collection and analysis of the material, drafting of the manuscript. Matsyuk N.V. – analysis and interpretation of data, statistical processing of the results. Vasilyeva I.S. – collection and analysis of the material, drafting of the manuscript. Strutynskaya A.D. – statistical processing of the results, drafting of the manuscript. Tyurin I.E. – conception and design of the study, drafting of the manuscript, editing.

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Results of the microbiota assessment in experimental ulcerative colitis

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ABSTRACT

Background. The increased incidence of inflammatory bowel disease (IBD) in the world and the lack of consensus on the causes and development mechanisms of IBD are the key factors that determine the relevance of the study. According to some authors, in the pathogenesis of the development and occurrence of ulcerative colitis, one of the leading causes is a change in the composition of the colon microflora and the impact of products of microbial metabolism on the enteric nervous system and intestinal motility.

The aim was to study the qualitative and quantitative changes in the colon microbiota in rats when modeling ulcerative lesions.

Materials and methods. The experimental study was carried out using male Wistar rats (n = 24). An original model of ulcerative colitis was used.The quantitative and qualitative composition of the parietal microflora of the distal colon was determined.

Results. In the ulcerative colitis model, changes in the qualitative and quantitative composition of the parietal microflora of the colon were revealed. On the 3rd day, there was a decrease in *Lactobacillus* ssp. and *Escherichia coli*, as well as growth of fungal microflora and appearance of opportunistic bacteria. The changes were progressive in nature, and by the 7th day of the study, marked reduction of the total parietal concentration of the normal flora bacteria and an increased percentage and absolute number of opportunistic microorganisms. By the 10th day of the experiment, with a small increase in the total number of parietal bacteria, the predominant microorganisms were *Bacteroides* ssp. (26.8%) and *Peptococcus* ssp. (27.6%).

Key words: ulcerative colitis, bacteriology, microflora, experiment.

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Результаты оценки микробиоты в условиях экспериментального язвенного поражения толстой кишки

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РЕЗЮМЕ

Введение. Язвенный колит – хроническое рецидивирующее системное воспалительное заболевание с преимущественным поражением слизистой оболочки толстой кишки. Каждый год регистрируется до 20 новых случаев заболевания на 100 тыс. населения, в основном среди лиц трудоспособного возраста. По мнению ряда авторов, в патогенезе развития и возникновения заболевания одну из ведущих причин играет изменение в составе микрофлоры толстой кишки, а также продукты их метаболизма, воздействующие на энтериновую систему и моторику кишечника.

Цель. Изучить показатели микробиоты толстой кишки у самцов крыс линии Вистар при моделировании язвенного поражения.

Материалы и методы. Экспериментальное исследование выполнено с использованием самцов крыс линии Вистар (n = 24). Предложен оригинальный способ модели язвенного колита. Определен количественный и качественный состав пристеночной микрофлоры дистального отдела толстой кишки.

Результаты. Выявлены изменения качественного и количественного состава пристеночной микрофлоры толстой кишки: на 3-и сут отмечали снижение концентрации *Lactobacillus* ssp. и *Escherichia coli*, а также рост грибковой микрофлоры, появление представителей условно-патогенной микрофлоры. Изменения носили прогрессирующий характер, и уже к 7-м сут выявляли выраженное снижение общей пристеночной концентрации бактерий нормофлоры и повышение процентного и абсолютного числа представителей условно-патогенной микрофлоры. К 10-м сут эксперимента при малом увеличении общей численности пристеночных бактерий преобладающей микрофлорой являются *Bacteroides* ssp. (26,8%) и *Peptococcus* ssp. (27,6%).

Ключевые слова: язвенный колит, бактериология, микрофлора, эксперимент.

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

Источник финансирования. Авторы заявляют об отсутствии финансирования.

Соответствие принципам этики. Исследование одобрено комитетом по биомедицинской этике ИНЦХТ (протокол № 7 от 26.06.2017).

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INTRODUCTION

The incidence rate of inflammatory bowel dis- ease (IBD) in the world and the absence of a consensus concerning the reasons and mechanisms of the IBD development are the key factors determining the study relevance. One of the pathogenetic mechanisms of the IBD development is considered to be the abnormality in the intestine microflora composition [1]. In normal conditions, the microflora of the colon contains over 500 species of different microorganisms, 92–95% of which is the so-called obligate microflora represented by *Bifidobacterium, Lactobacillus*, and nonpathogenic *Escherichia coli*

(*E. coli*). Normal microflora executes such functions as regulation of water and salt metabolism, gas composition, metabolism of all nutrients, colonization resistance, detoxication of exogenetic and endogenic substrates, morphokinetic action, mutagenic and immunogenic functions, and energy production for host cells, and, therefore, is an essential "organ" of our body [2–4].

It is known that disruption of the normal intestinal microbiota balance results in occurrence of pathologic processes in mucous membranes. Quantitative and qualitative composition of the colon microbiota depends on a set of both endo- and exogenous factors. Thus, quantitative and qualitative composition of microflora, even for representatives of one species, can vary greatly, within wide limits, which is determined both by the place of residence and diet. Microflora of all patients with IBD is characterized by development of dysbacteriosis, in which a decrease in the normally prevailing *Firmicutes* and *Bacteroidetes* types and an increase in the *Proteobacteria* E21 type are observed. However, no uniform changes in the composition of intestinal microflora characterizing the specific features of this pathology were detected [1].

Changes in the composition of intestinal microflora in patients with irritable bowel syndrome mainly consist in reduction of *Lactobacilli* and *Bifidobacteria* and prevalence of aerobic organisms over anaerobic ones.. Moreover, the parietal composition of microflora in patients with irritable bowel syndrome includes a greater variety of microorganisms than in healthy people [5, 6]. During the development of dysbacteriosis caused by a certain etiological agent, a specific change is the detection of the etiological agent in both the luminal and parietal microflora.

The aim of the work was to study the qualitative and quantitative changes in the composition of the parietal microflora of the colon under conditions of the ulcerative colitis modeling.

MATERIALS AND METHODS

The experimental study was performed at the vivarium of Irkutsk Scientific Center of Surgery and Traumatology (vivarium of the I category, Veterinary Certificate 238 No. 0000023 of 28.11.2015, veterinary service of Irkutsk Region) on male Wistar rats with the weight of 280–350 g (n = 24) and aged over 6 months. Among the experimental animals, 6 rats made up a control group of healthy animals required to obtain reference values. In the group of rats with modeled ulcerative colitis, 6 animals were bred for each microbiota examination term. The animals were kept in the vivarium with free access to water and food in accordance with the national standard "Keeping Experimental Animals in Nurseries of Research Institutes".

The experiments were performed according to the standards of humane animal care, which are regulated by the "Rules of Work Performance with Use of Experimental Animals" (Appendix to the Order of the Ministry of Health of the USSR No. 755 of 12.08.1977 and No. 48 of 23.01.1985 "On Control over Work Performance with use of Experimental Animals", provisions of the World Medical Association's Declaration of Helsinki adopted in 1964 and amended in 1975, 1983, and 1989) according to the Protocol approved by the Bioethics Committee. All surgical interventions were performed under aseptic conditions and general anesthesia.

An original method for modeling ulcerative lesion of rat colonic wall was developed [7]. The parietal microflora of colon biopsy material was assessed by bacteriological methods including the study of qualitative and quantitative composition of aerobic, facultative anaerobic, anaerobic microflora, fungi of the genus *Candida* within certain time intervals of the experiment using highly selective solid and liquid media, the aerobic and anaerobic cultivation techniques, diagnostic short-term tests and the semi-automatic microbiological analyzer "ATB Expression" (Biomerieux, France) in accordance with the current regulatory documentation (OST 91500.11.0004–2003. Patient Management Protocol. Intestinal Dysbacteriosis. Order of the Ministry of Health of the Russian Federation No. 231 of 09.06.2003). The material was collected according to the methodological guidelines MU 4.2.2039–05 "Technique for Biomaterial Collection and Transportation in Microbiological Laboratories". The samples were delivered to the laboratory within an hour. Upon weight determination (with accuracy to 0.001 g), the biopsy material was thoroughly homogenized in 0.85% sodium chloride solution at the rate of 1:10. Ten-fold dilutions of the homogenate up to 10^{-8}–10^{-11}(weight / volume) were prepared from the produced suspension.

Cultures for an extended set of media for aerobic and facultative anaerobic bacteria (Endo's, Ploskirev's, Levin's media, egg yolk salt agar, meat infusion agar with 5% blood, Bismuth sulphite agar, etc.) were performed from thecorresponding dilutions. For anaerobe recovery, fluid thioglycollate medium, Blaurock medium, Wilson – Blair medium, and anaerobic blood agar were used, to which base 5% laked blood, 10 μg / ml vitamin K and 5 μg / ml hemin were added. For fungus recovery, Sabouraud's medium was used. Anaerobic microflora was cultivated under strict anaerobic conditions using the GEN-box anaer devices (BioMerieux, France) and the gas-generating kit (HiMedia Laboratories, India).

The results were recorded in corresponding time intervals. The result was expressed in CFU / g considering the initial weight of the biopsy material and the homogenate dilution ratio.

The obtained data were presented in the form of median percent with lower and upper the median *Me* with lower and upper quartiles (Q_1–Q_3). The significance of the differences in the obtained data ($p \leq 0.05$) in the compared samples were determined by the Mann – Whitney U test. Statistical processing of the results was performed with the Statistica 10.0 for Windows software package (License No. AXAR402G263414FA-V) [8].

RESULTS AND DISCUSSION

The qualitative and quantitative composition of parietal microflora on the 3rd, 7th, and 10th days of the experiment was assessed using the developed original experimental model of ulcerative lesion of the distal colon segment with wall ischemia and maintained inflammation. Modeling was performed by surgical intervention with parietal recovery, legation and transection of the direct artery branches of the colon with accompanying veins within 3 cm from the urinary bladder base for ischemic damage of the distal segment of the colonic wall. One day after the intervention, the experimental animals received 1% aqueous solution of dextran sulfate sodium orally by free drinking throughout the whole experiment to maintain inflammatory process in the colonic wall.

When assessing quantitative and qualitative composition of the colon parietal microflora of healthy animals, it was determined that 95% of the parietal microbiota was obligate flora represented by *Lactobacillus* spp. (48.7 %), *Bacteroides* ssp. (31.7%), *Peptococcus* spp. (14.6%), *Enterococcus faecium* (2.9%), gram-positive rods (1.2%), *Bifidobacterium* spp. (0.7%), *E. coli* (0.1%), and others (0.1%) (Fig.1, Table 1).

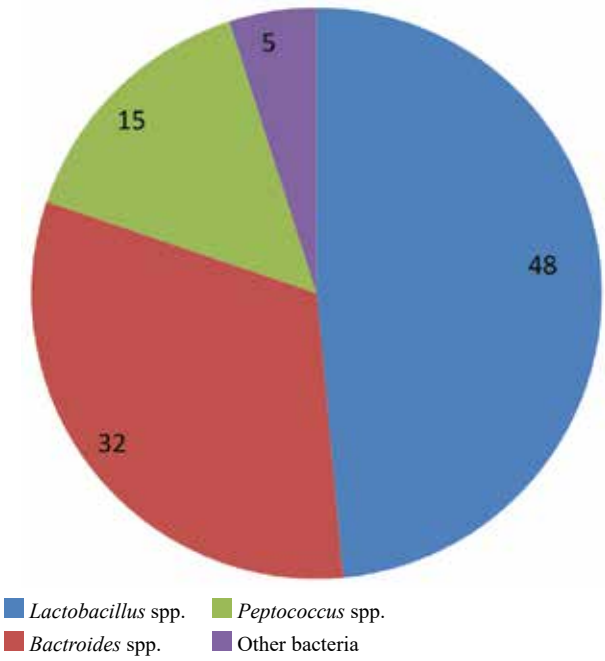

Fig. 1. The percentage of the parietal microflora of the colon in healthy Wistar rats

According to the results of the bacteriological examination, by the 3rd day of the experiment, it was determined that against induced ulcerative colitis, reduction of the total bacterial count was noted by more than 2 times in comparison to healthy animals. In absolute numbers, it was reflected in reduction of the quantity from 8.21×10^9 to 3.92×10^9 due to a significant decrease in the number of *E. coli* (10^6–10^4), *Enterococcus faecalis* (10^7–10^4), gram-positive rods (10^5–10^0), and *Bacteroides* ssp. (10^8–10^5), with an accompanying significant increase in *Bifidobacterium* spp. (10^7–10^8) ($p \leq 0.05$). The comparative analysis of the percentage of parietal microflora in the animals with induced ulcerative colitis on the 3rd day of the experiment showed an increase in *Bifidobacterium* spp. from 0.7% to 38.3 %, *Lactobacillus* spp. from 48.7% to 61.3 %, as well as a reduction of facultative microflora representatives from 51.3 % to 0.4% in comparison with healthy animals.

Table 1

The quantitative and qualitative composition of the colon parietal microflora in the original model of ulcerative colitis (Lg CFU / g), *Me* (Q_1–Q_3)

Parameter		Norm	3rd day	7th day	10th day
		Lg CFU / g			
		Frequency, %			
	1	6.1 (6.0–6.3)	3.7 (3.0–4.3)*	1.7 (0–4.0)*	3.8 (3.6–3.9)*
		100%	100%	50%	100%
	2	3.0 (0–5.0)	0 (0–4.0)	0 (0–0)	0 (0–0)
		66.7%	33.3%		
	3	3.5 (3.5–4.0)	3.0 (0–4.0)	0 (0–0)*	0 (0–0)*
		100%	66.7%		
	4	7.0 (5.0–7.2)	3.75 (3.5–3.9)*	0 (0–0)*	0 (0–0)*
		100%	100%		
	5	1.3 (0–3.0)	1.5 (0–3.6)	0 (0–3.0)	0 (0–0)
		50%	50%	33.3%	
	6	5.0 (5.0–6.3)	0 (0–4.0)*	0 (0–0)*	0 (0–5.0)
		100%	33.3%		33.3%
	7	0 (0–5)	3.0 (0–3.2)	0 (0–0)	0 (0–0)
		33.3%	66.7%		
	8	7.0 (7.0–7.0)	8.0 (8.0–8.0)*	3.0 (0–6.0)*	5.5 (5.0–6.0)*
		100%	100%	50%	100%
	9	9.0 (5.0–9.0)	8.0 (8.0–9.0)	2.5 (0–5.0)*	6.0 (5.0–6.0)
		100%	100%	50%	100%
	10	8.0 (8.0–8.3)	5.0 (5.0–6.0)*	2.5 (0–5.5)*	6.0 (5.0–6.3)*
		100%	100%	50%	100%
	11	0 (0–0)	0 (0–6.0)	0 (0–0)	0 (0–0)
			33.3%		
	12	0 (0–0)	0 (0–0)	0 (0–0)	2.5 (0–5.3)
				16.6%	50%
	13	0 (0–8.3)	0 (0–6.0)	0 (0–0)	6.0 (5.6–6.0)
		33.3%	33.3%		100%

Note: 1 – E. *coli*; 2 – *Proteus mirabilis*; 3 – *Citrobacter freundii*; 4 – *Enterococcus faecalis*; 5 – *Staphylococcus epidermidis*; 6 – *gram-positive rods;* 7 – *Candida* spp.; 8 – *Bifidobacterium*; 9 – *Lactobacillus;* 10 – *Bacteroides* ssp.; 11 – *Actinomyces* spp.; 12 –*Vellonella* spp.; 13 – *Peptococcus* spp*;* * – statistically significant difference by the Mann – Whitney test in comparison with normal values ($p_U \leq 0.05$).

It is known that *Lactobacillus* can produce lactic acid, hydrogen peroxide, lysozyme, and substances with antibiotic activity, such as reuterin, plantaricin, lactocidin, and lactoline. Along with *Bifidobacteria*, *Lactobacilli* interact with enterocytes and stimulate body defense mechanisms, increasing the regeneration rate of mucous membrane, having an effect on synthesis of antibodies to related but pathogenic microorganisms, and activating phagocytosis and synthesis of lysozymes, interferons, and cytokines. *Lactobacilli* produce a numbe*r* of hydrolytic enzymes, particularly, lactase and decomposable lactose, thus preventing the development of lactase deficiency. Moreover, *Lactobacilli* maintain pH of the colon at 5.5–5.6 [9, 10].

When inducing ulcerative colitis during the experiment, in was revealed that on the 3rd day, the lactose negative bacteria appeared accounting for 34.8% from total number of *E. coli*, which is an initial evidence of developing dysbiosis. The significant reduction of the colon bacilli also indicates the developing dysbiosis. It is known that *E. coli* benefits the host organism by synthesizing vitamin K and preventing the development of pathogenic microorganisms in the intestine [11]. A drastic increase of *Bifidobacteria* indicates development of

the compensatory reaction directed to maintain the normal condition of parietal microflora and protective properties of the intestinal wall. *Bifidobacteria* provide physiological defense of the intestinal barrier from the ingress of microbes and toxins into the internal environment and have high antagonist activity in relation to the pathogenic microorganisms and opportunistic pathogens due to generation of organic fatty acids.

In 33% of the examinations, the occurrence of *Actinomyces* was detected. Insertion of *Actinomyces* into the colon mucosa can result in generation of the infectious granuloma reaching surrounding tissues and forming apostasis and fistulae. *Staphylococcus* also contributes to the formation of the suppurative process. An increase in its total concentration of which almost by 3.5 times was noted by the 3rd day of the experiment. *Actinomyces* antigens result in a specific sensibilization and allergic alteration of the body (hypersensibilization of delayed or tuberculin type), as well as antibody formation (complement-fixing, agglutinins, precipitins, etc.) (Fig. 2).

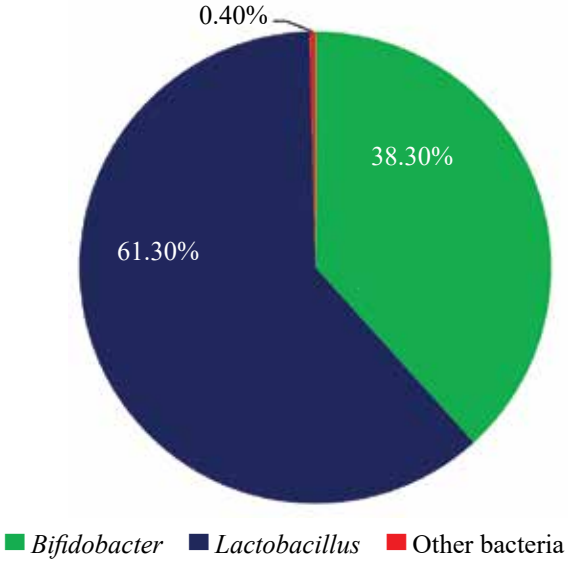

Fig. 2. The percentage of the parietal microflora of the colon in rats under conditions of modeled ulcerative colitis on the 3rd day

By the 7th day of the experiment, against the background of ischemia and reduction of protective properties of the intestinal wall, a significant decrease in the absolute total number of bacteria to 6.25×10^6 was identified in comparison with the indices of healthy animals (Fig. 3). Moreover, a significant difference with the results of the 3rd day of the experiment ($p = 0.025$) was determined. In comparison with indices of microbiota for healthy animals, a significant decrease in the concentrations of *E. coli* (10^6–10^2), *Bifidobacterium* spp. (10^7–10^3), *Lactobacillus* spp. (10^9–10^3), *Bacteroides* spp. (10^8–10^3) ($p \leq 0.05$) was detected along with complete disappearance of some representatives of facultative bacteria, such as P*roteus*, *Citrobacter freundii*, *Enterococcus faecalis*, *Peptococcus,* and gram-positive rods were detected.

During the comparative examination of changes in the percentage composition of parietal microorganisms on the 7th day of the experiment, an increase in the percentage of *Bifidobacterium* spp. by 5.6% (from 38.3 to 43.9%) and reduction of *Lactobacillus* spp. by 31.2% (from 48.7 to 17.5%) were determined. On the 7th day, the percentage of *Bacteroides* spp. increased and was 26.3%. The percentage of *E. coli* also increased: on the 7th day, it was 0.5%. *Staphylococcus epidermidis* accounted for 0.3%. In 33% of examinations, *Vellonellaceae* was detected, the total percentage of which was 11.4% on the 7th day, and during single examinations it was up to 28%.

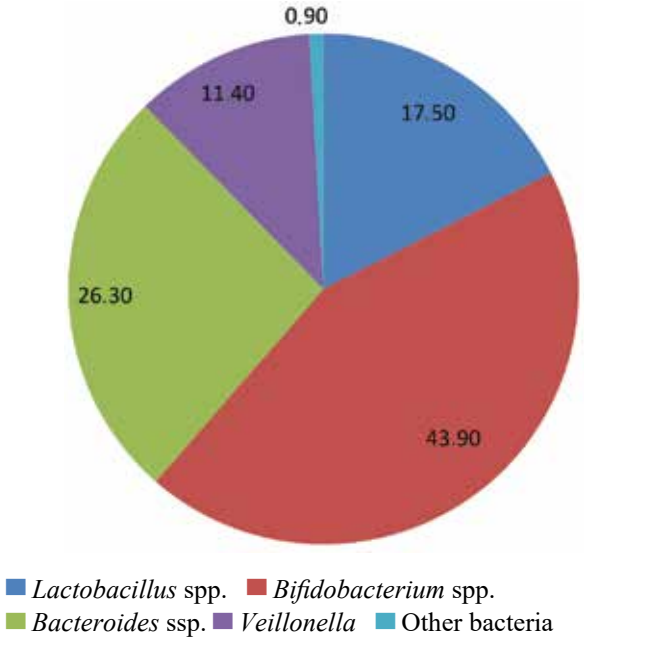

Fig.3. The percentage of the parietal microflora of the colon in rats under conditions of modeled ulcerative colitis on the 7th day

On the 10th day after modeling of ulcerative lesion in the colon wall, an increase in the total concentration of microbes up to 2.39×10^6 in compari-

son with the 7th day of the experiment and reduction of this parameter in comparison with indices of healthy animals were registered. On the 10th day, in comparison with the reference values of healthy animals, there was significant reduction in the concentration of *E. coli* (10^6–10^4), *Bifidobacterium* spp. (10^7–10^6), and *Bacteroides* spp. (10^8–10^6). Additionally, a complete absence of *Proteus mirabilis*, *Citobacter freundii*, *Enterococcus faecalis*, *Staphylococcus,* and gram-positive rod was detected. The prevailing microflora consisted of *Bacteroides* spp. (26.7%) and *Peptococcus* (27.6%), while a smaller part was represented by *Lactobacillus* spp. (17.5%), *Bifidobacterium spp.* (13.8%), and gram-positive rods (4.6%). *Bacteroides* spp. are opportunistic pathogens.

Under conditions of immunodeficiency, which develops when modeling ulcerative colitis using the original method, *Bacteroides* spp. contribute to emergence of purulent inflammation in associations with aerobic bacteria. A wide range of virulence factors, such as capsules, pilli, and outer membrane proteins, contribute to the adhesion processes. Capsular polysaccharide as an aggressive factor protects bacteria from phagocytosis. *Bacteroides* spp. produce a number of enzymes, such as neuraminidase, fibrinolysin, and heparinase, participating in invasion, as well as products of metabolism, such as short-chain fatty acids and biogenic amines, which disrupt the capacity of macrophages and leucocytes. Lipopolysaccharides take part in inactivation of the phagocytosed cells [12]. In 50% of cases, Veillonellaceae bacteria were detected, which account for 9.6% from the total bacterial count. *Veillonellaceae* are among the most common and physiologically significant bacteria of the human colon [13]. In the colon, *Veillonellaceae* are not found very often: in 1 g of feces, the content of this bacterium is in the range of 0–10^8 CFU [14]. Colitis is associated with *Veillonella parvula* (http://www.gastroscan.ru/handbook/118/4113) (Fig.4).

CONSLUSION

Quantitative and qualitative changes in the parietal microflora of the modeled ulcerative lesion of colonic mucosa in male Wistar rats are characterized by the reduction of the bacterial count by the 3rd day, development of dysbiosis due to a decrease in the concentration of *Lactobacillus* and *E. coli*, emergence of fungal microflora, the determination of opportunistic microflora and a compensatory response in the form of a significant increase in *Bifidobacterium*. However, even such a significant increase in the *Bifidobacterium* concentration on the at early stages after the surgery is not sufficient to maintain the normal balance of the intestinal microflora, which is demonstrated by significant reduction of the total parietal concentration of bacteria by the 7th day of the experiment with an increase in the absolute number of opportunistic bacteria.

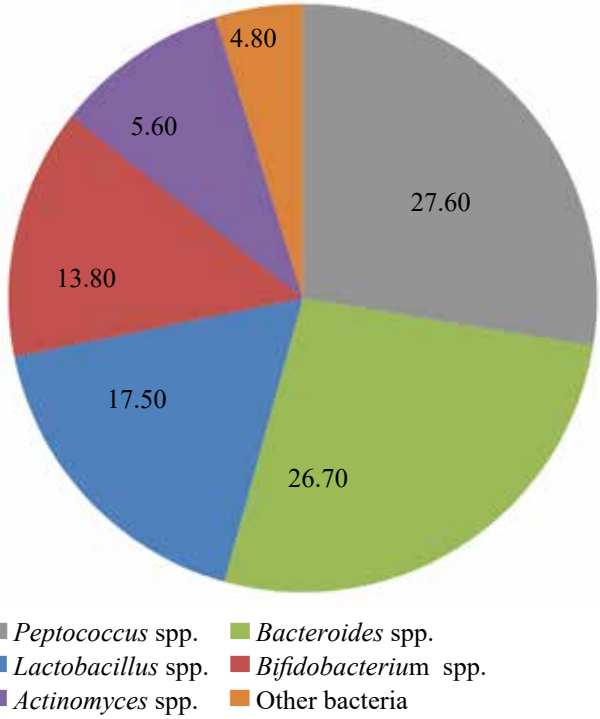

Fig. 4. The percentage of the parietal microflora of the colon in rats under condition of the modeled ulcerative colitis on the 10th day

By the 10th day of the experiment, with a small increase in the total number of parietal bacteria, the predominant microorganisms were *Bacteroides* ssp., the bacteria maintaining the inflammatory process in the colonic mucosa. *Veillonellaceae were* detected in up to 50% of cases, the presence of which is directly associated with the development of inflammation in the colon wall. Further research is obviously required both for understanding complex interaction of macro- and microorganisms and their influence on the development of pathological reactions, and for studying the possibility of correcting these conditions.

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Authors contribution

Kim A.D. – carrying out of experiments, collection of material, drafting of the manuscript. Lepekhova S.A. – conception, formulation of research aims and tasks, critical review, addition of comments and editing of the manuscript. Chashkova E.Yu. – drafting of the manuscript. Koval E.V., Fadeeva T.V., Goldberg O.A. – provision of reagents, materials, animals, and tools for calculation and analysis. Pivovarov Yu.I. – application of statistical, mathematical, computational and other methods for analysis and synthesis of the data.

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Integral assessment of lipoperoxidation processes in women with ovarian hyperandrogenism

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ABSTRACT

Aim. To assess the state of the 'lipid peroxidation – antioxidant defense' system using an integrated indicator (coefficient of oxidative stress) in women with ovarian hyperandrogenism in various periods of reproductive age.

Materials and methods. During an annual preventive medical examination at the Scientific Centre for Family Health and Human Reproduction Problems, 92 women of reproductive age (18–45 years old) were divided into two groups: a group of women with polycystic ovary syndrome (PCOS) ($n = 47$) and a control group of healthy women ($n = 45$). The group of women with PCOS was further divided into subgroups according to age characteristics: the 1st subgroup consisted of women with PCOS of early reproductive age (18–35 years old), and the 2nd group included women with PCOS of late reproductive age (35–45 years old). Practically healthy women of the corresponding ages made up the 3rd and 4th control subgroups. Standard methods were used to study the LPO–antioxidant defense system. The oxidative stress severity was assessed by an integrated indicator: the coefficient of oxidative stress.

Results. An increase in the serum levels of ketodienes and coupled trienes, a decrease in the concentrations of reduced glutathione, α-tocopherol and retinol levels, and an increase in superoxide dismutase (SOD) activity in PCOS women of reproductive age (18–45 years old) were detected, as opposed to the control group. Early reproductive age PCOS women also demonstrated an increase in oxidized glutathione and a decrease in retinol concentrations. In the late reproductive age group of PCOS women, an increased SOD activity was registered. The integrated indicator of oxidative stress in the main group of women with PCOS was 2.5, which shows the enhancement of oxidative processes and imbalance in the LPO–antioxidant defense system. This indicator was the most pronounced (2.8) in women of early reproductive age. In women of late reproductive age, this indicator was equal to 1.9.

Conclusion. The obtained data indicate the development of oxidative stress in women with ovarian hyperandrogenism, which is more pronounced in the group of late reproductive age.

Key words: hyperandrogenism, ovarian dysfunction, polycystic ovary syndrome, reproductive age, pro-oxidants, antioxidants, oxidative stress.

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

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Conformity with the principles of ethics. All patients signed an informed consent to participate in the study. The study was approved by the Committee on Biomedical Ethics at Scientific Centre for Family Health and Human Reproduction Problems (Protocol No. 2.1 of 24.02.2016).

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Интегральная оценка процессов липопероксидации у женщин с овариальной формой гиперандрогении

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РЕЗЮМЕ

Цель. Оценить состояние системы «перекисное окисление липидов – антиоксидантная защита» (ПОЛ–АОЗ) с использованием интегрального показателя (коэффициента окислительного стресса) у женщин с овариальной формой гиперандрогении в различные периоды репродуктивного возраста.

Материалы и методы. В ходе ежегодного профилактического медицинского осмотра 92 женщины репродуктивного возраста (18–45 лет) были разделены на группу женщин с синдромом поликистозных яичников (СПКЯ) (n = 47) и контрольную группу практически здоровых женщин (n = 45). Далее группа СПКЯ была разделена на подгруппы в соответствии с возрастными характеристиками: 1-я подгруппа – женщины с СПКЯ раннего репродуктивного возраста (18–35 лет), 2-я группа – позднего репродуктивного возраста (35–45 лет). Практически здоровые женщины соответствующих возрастов составили 3-ю и 4-ю контрольные подгруппы. В работе использованы общепринятые методы исследования системы ПОЛ–АОЗ. Выраженность окислительного стресса оценивали по интегральному показателю – коэффициенту окислительного стресса.

Результаты. Отмечено повышение в сыворотке крови уровней кетодиенов и сопряженных триенов, снижение восстановленного глутатиона, α-токоферола и ретинола, увеличение активности супероксиддисмутазы у женщин раннего репродуктивного возраста с овариальной формой гиперандрогении по сравнению с группой контроля. Также показано повышение содержания окисленного глутатиона и снижение концентрации ретинола. В группе женщин с гиперандрогенией позднего репродуктивного возраста отмечается увеличение активности супероксиддисмутазы. Интегральный показатель оценки окислительного стресса в общей группе женщин с СПКЯ составил 2,5, что свидетельствует об усилении окислительных процессов, дисбалансе в системе ПОЛ–АОЗ. Наиболее выражен данный показатель у женщин в раннем репродуктивном возрасте (2,8). У женщин в позднем репродуктивном возрасте значение показателя равно 1,9.

Заключение. Полученные данные свидетельствуют о развитии окислительного стресса у женщин с гиперандрогенией овариального генеза.

Ключевые слова: гиперандрогения, дисфункция яичников, синдром поликистозных яичников, репродуктивный возраст, прооксиданты, антиоксиданты, окислительный стресс.

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

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Соответствие принципам этики. Все пациенты подписали информированное согласие на участие в исследовании. Исследование одобрено комитетом по биомедицинской этике при ФГБНУ НЦ ПЗСРЧ (протокол № 2.1 от 24.02.2016).

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INTRODUCTION

Despite being natural for homeostasis, free radical processes sometimes contribute to the development of oxidative stress (OS), which is found in numerous pathological conditions and triggers the aging process. OS is a state when the balance between oxidative and antioxidant systems of cells and tissues has been lost. Enhanced generation of free radicals due to overproduction of reactive oxygen species (ROS) along with weakening of antioxidant defense mechanisms, leads to oxidation of biomolecules with the subsequent loss of their biological functions and / or homeostatic imbalance, potential oxidative damage to cells and tissues, and even their death. Excessive ROS generation can damage such cell components as proteins, lipids, and nucleic acids, which leads to cellular dysfunction [2].

Possible reasons for the decreased antioxidant activity and OS development may include various endocrine disorders (hyperprolactinemia, hyperandrogenism, defective luteal phase etc.). Hyperandrogenism, ovarian dysfunction, which is one of the main manifestations of polycystic ovary syndrome (PCOS), is particularly interesting here [3, 4]. The issue is relevant, as hyperandrogenism is common among women of reproductive age and can be distinguished by high frequency of associated complications and its leading manifestation, which is infertility [5]. Depending on the diagnostic criteria used and the characteristics of the population sample, this complex heterogeneous condition of unknown etiology affects 5–20% of fertile age women [6, 7].

Despite a long history of study, there is still no complete pathogenesis scheme of this disease [8], hence we consider it important to study such pathogenetic factors of many reproductive diseases as an imbalance in the 'lipid peroxidation – antioxidant defense' (LPO–AOD) system. OS development features due to androgen overproduction in ovaries in different periods of reproductive age have not been studied well yet. In our opinion, they need to be investigated thoroughly for further development of antioxidant therapy [9].

According to the existing views, when assessing whether LPO–AOD processes are balanced or not, comparison of individual indicators is not sufficiently informative. In this regard, integrated indicators may be more promising and sensitive. The coefficient of oxidative stress (COS) is often used as an integrated indicator to assess an imbalance between oxidative processes and the antioxidant defense system. This coefficient shows the accumulation of primary, intermediate, and end products of LPO and the activity of various AOD components (enzymes, glutathione, fatsoluble vitamins). The prooxidants / antioxidants ratio can reflect the stage of pathological conditions in the body. Using COS, it is possible to simultaneously assess both LPO and AOD states, as well as to timely assess the degree of imbalance in the LPO–AOD system at any stage of lipoperoxidation [10].

Considering everything mentioned above, the aim of the study was to assess the state of the LPO–AOD system using the coefficient of oxidative stress in women with ovarian hyperandrogenism at different periods of reproductive age.

MATERIALS AND METHODS

We conducted a single-center, cross-sectional, observational study, which included women of reproductive age (18–45 years old), who underwent an annual preventive medical examination (in the period from 2017 to 2019) at the Scientific Centre for Family Health and Human Reproduction Problems. All women were examined during the follicular phase of their menstrual cycle (from day 1 to day 12). The main group comprised 47 women with verified PCOS. PCOS was verified according to ESHRE / ASRM criteria (Rotterdam, 2003) [11]. The control group included 45 gynecologically and somatically healthy women. We further divided the groups into subgroups according to age characteristics: the 1st subgroup consisted of women with PCOS of early reproductive age (18–35 years old), the 2nd group included women with PCOS of late reproductive age (35–45 years old). The 3rd and 4th groups were control subgroups of the corresponding ages.

According to ESHRE / ASRM criteria (Rotterdam criteria), women of reproductive age (18–45 years old) who had two out of three criteria for PCOS (oligo- or anovulation, clinical and/or biochemical hyperandrogenism, polycystic ovarian morphology on the ultrasound) were included in the group with PCOS. Exclusion criteria for the group of women with PCOS were hyperprolactinemia, thyroid disor-

ders, current pregnancy or lactation, removal of the uterus and / or appendages on both sides, endometrial ablation and / or uterine artery embolization, and taking hormonal medications. Inclusion criteria for the control groups were the reproductive age (18–45 years old), regular menstrual periods, and follicular phase of the menstrual cycle. Exclusion criteria for the control groups were current pregnancy or lactation, removal of the uterus and / or appendages on both sides, endometrial ablation and / or uterine artery embolization, taking hormonal medications, and a chronic disease in the medical history.

All women underwent standard clinical and laboratory examinations. Blood was collected in accordance with the standard requirements from the ulnar vein in the morning, on an empty stomach. Plasma and hemolysate of red blood cells were the materials for the study. Standard methods were used to determine the products of LPO and components of AOD: conjugated dienes (CDs), ketodienes and coupled trienes (KD and CT), double bonds (DB), thiobarbituric acid (TBA) reactive substances (TBARS), superoxide dismutase (SOD) activity, reduced (GSH) and oxidized (GSSG) glutathiones and their ratio (GSH/GSSG), α-tocopherol and retinol, total antioxidant activity (TAA).

Measurements were carried out with the help of spectrophotofluorometer BTS-350 (Spain) and Fluorat 02 ABFF-T (Russia). To assess oxidative stress in women with PCOS, the formula for COS calculation was used [12]. To calculate the COS, the following indicators of the LPO–AOD system were used: DB (UL), CDs (umol / l), KD and CT (UL), TBARS (umol / l), SOD (UL), GSH (mmol / l), GSSG (mmol / l), α-tocopherol (umol / l), retinol (umol / l).

$$\mathrm{COS} = \frac{(\mathrm{DB}_i/\mathrm{DB}_n) \cdot (\mathrm{CDs}_i/\mathrm{CDs}_n) \cdot (\mathrm{KD\ and\ CT}_i/\mathrm{KD\ and\ CT}_n) \cdot (\mathrm{TBARS}_i/\mathrm{TBARS}_n)}{(\mathrm{SOD}_i/\mathrm{SOD}_n) \cdot (\mathrm{GSH_i}/\mathrm{GSH_n}) \cdot (\mathrm{A}_i/\mathrm{A}_n) \cdot (\mathrm{E}_i/\mathrm{E}_n)}$$

Where *i* – implies indicators of the examined patient, *n* – indicators of the control group.

The ratio of indicators of the LPO–AOD system of women with PCOS to the average indicators in the control groups was calculated. The value of COS > 1 was considered an increase in the degree of OS, that is, the higher the obtained value of COS, the higher the intensity of lipoperoxidation and the less effective the antioxidant protection.

Statistica 6.1 (Stat-Soft Inc., USA) was used to carry out statistical processing of data. To determine the proximity to the normal law of data distribution, the Shapiro − Wilk test was applied. *Me* (Q_1–Q_3) was used as descriptive statistics for variables with quantitative data. The nonparametric Mann – Whitney test was used in the analysis of intergroup differences for independent samples with non-normal distribution. The differences were considered statistically significant at $p < 0.05$.

RESULTS

The research results showed that ovarian hyperandrogenism is followed by the activation of lipoperoxidation and a decrease in antioxidant protection. A change in the concentration of secondary LPO products (KD and CT), which increased by 43% ($p = 0.0001$) compared to the control value, indicated activation of LPO in the experimental group of women of reproductive age (18–45 years old) with PCOS. During the study of primary (KD) and end (TBARS) products of LPO, no significant differences from the control group were found.

In the same group of women, a decrease in the TAA by 15% ($p = 0.0312$) indicated that the AOD system is under strain. a 9% ($p = 0.0323$) increase in the key SOD anti- oxidant enzyme was registered. At the same time, a 11% ($p = 0.0001$) decrease in GSH and a slight increase in GSSG were detected. This influenced the GSH / GSSG ratio, which was 16% lower in the group of women with PCOS ($p = 0.0065$). However, the content of α-tocopherol and retinol decreased by 12% ($p = 0.0001$) and 22% ($p = 0.0315$), respectively, compared to the control group.

The group of PCOS women of early reproductive age (18–35 years old) did not show statistically significant differences in the content of primary, secondary, and end lipoperoxidation products compared to the control group of the same age. However, the antioxidant defense mechanism changed: a

decrease in the level of TAA by 25% (p = 0.0005) and an increase in the oxidized glutathione concentration by 14% (p = 0.0001) were detected, but the reduced glutathione level remained unchanged. The GSH / GSSG ratio was 13% lower (p = 0.0323) than in the control group. Increased consumption of retinol was observed, which was a consequence of a decrease in its concentration by 26% (p = 0.0012). No significant differences in α-tocopherol levels were found.

In the subgroup of women of late reproductive age (35–45 years old) with hyperandrogenism, only a statistically significant increase in SOD by 9% (p = 0.0004) was found in comparison with the control group of the same reproductive period. When analyzing the peroxidation indicators and the AOD system in the control groups of early and late reproductive age, no statistically significant differences were found. Comparison of early and late reproductive age groups of women with PCOS revealed a statistically significant decrease in the GSSG level by 15% (p = 0.0032) and an increase in the GSH / GSSG ratio by 22% (p = 0.0001) in the late reproductive age subgroup.

Further, to assess the oxidative stress level in women with ovarian hyperandrogenism, the COS formula was used. The obtained COS values of more than 1.0 in the groups of women with PCOS indicated an increase in the OS degree.

The subgroup of women of early reproductive age with PCOS demonstrated more pronounced oxidative stress. COS values confirming dysregulation in the LPO–AOD system in PCOS women are shown in Figure.

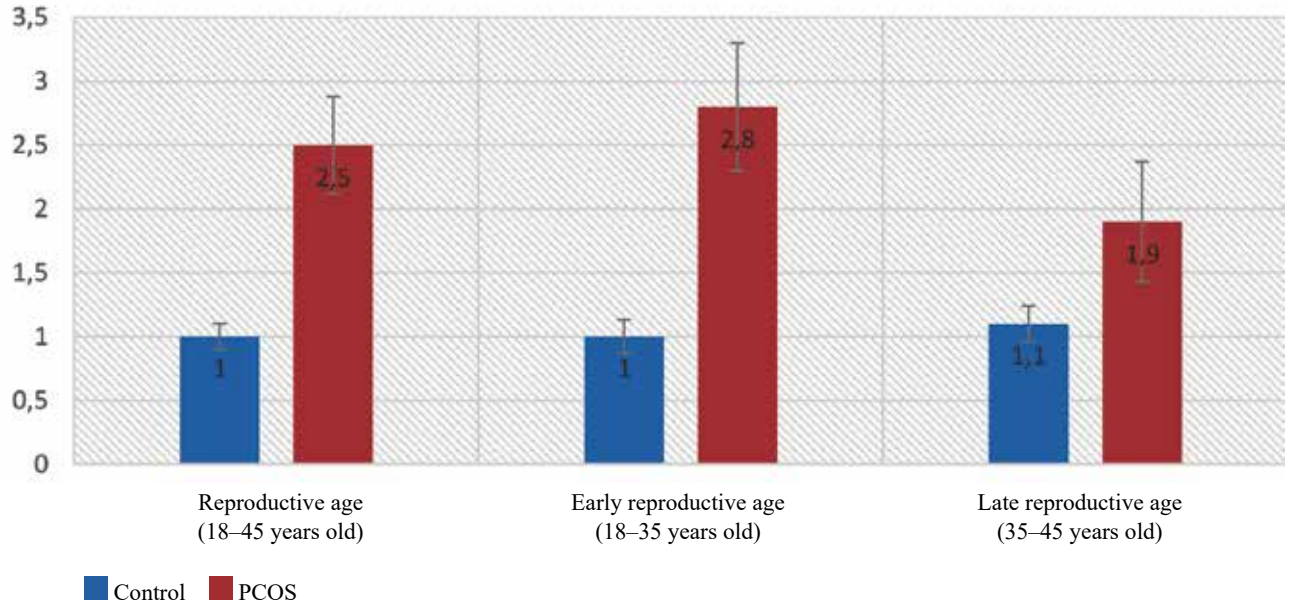

Figure. COS values in women with PCOS and in the control group

DISCUSSION

It is well known that women with ovarian hyperandrogenism have metabolic disorders, and the socalled "mitochondrial" dysfunction, which is closely connected with OS, plays an essential role in their pathogenesis. The pathogenetic role of OS in the development of reproductive disorders and decline of female reproductive function is also well-known [14, 15].

The main group of PCOS women showed activation of lipoperoxidation processes, which is manifested in an increase in the content of secondary LPO products–KD and CT. Increased production of LPO products at intermediate stages of lipid peroxidation, process, in particular, KD and CT, is proved to lead to toxic compound formation and accumulation and provoke extended damage to biopolymers, cytoplasmic membranes, and subcellular structures [16].

Though the most important indicator reflecting increased oxidative degradation of lipids is TBARS products, our study did not show statistically significant differences in this parameter. TBARS are end

products of oxidation indicating a serious damaging effect.

However, according to the existing concepts, free radical oxidation processes do not cause damage if the antioxidant defense mechanism keeps oxidative reactions at the balanced level. The components of the AOD system inactivate reactive oxygen species and inhibit the development free radical chain mechanisms of organic compound oxidation. The balance of the prooxidant and antioxidant components determines the intensity of metabolism and the adaptive potential of the body [17].

The results of the study of the AOD system components in the experimental group of women with PCOS showed increased activity of the main enzyme – superoxide dismutase, as well as a decrease in the blood total antioxidant activity (TAA). The changes in these indicators may reflect tension in the AOD system and a decrease in adaptive and compensatory capabilities in women with ovarian dysfunction [18]. Suppression of LPO processes in the cells at different stages is carried out by both enzymatic and nonenzymatic systems. In PCOS, there is a decrease in the concentration of molecular antioxidants, such as α-tocopherol and retinol. Decreased concentration of α-tocopherol, the most important regulator of oxidative balance, can affect the reproductive function and may contribute to the development of PCOS [9].

Lowering the level of retinol, a component of antioxidant protection, is associated with its increased consumption, since, in addition to fighting free radicals, it also increases the antioxidant effect of α-tocopherol [19]. A simultaneous decrease in the reduced glutathione concentration and a slight increase in the oxidized glutathione level, may be associated with the decreased activity of glutathione reductase and / or increased activity of glutathione peroxidase. Such changes lead to a decline in the total glutathione pool, which indicates a decrease in the contribution of this tripeptide to the anti-radical protection of the body [20, 21].

The study found that in different periods of reproductive age, LPO processes in women with ovarian hyperandrogenism are inactivated by various components of the AOD system. In early reproductive age, the main antioxidant protection is provided by the redox system of glutathione, and in late reproductive age, the processes of lipoperoxidation are mainly regulated by SOD.

The results of our study indicate the development of pronounced OS in women with ovarian hyperandrogenism, as evidenced by COS values. The COS value in the experimental group of women with PCOS equals to 2.5, in women of early reproductive period with PCOS, it is 2.8, and in late reproductive period subgroup it equals to 1.9. This coefficient value indicates a significant imbalance in the LPO–AOD system, highlighting the intensification of LPO processes, especially in early reproductive age women with PCOS.

CONCLUSION

Thus, the presence of hyperandrogenism in women of reproductive age is followed by the activation of adaptive and compensatory mechanisms for preserving the LPO–AOD system homeostasis and preventing LPO end product formation.

In this regard, COS, as an integrated indicator, helps to more objectively describe changes in the LPO–AOD system disorders and is a more sensitive indicator than separate individual components of this system, since it comprises both the products of lipid peroxidation at different stages and the activity of various components of antioxidant protection. COS shows the nature of peroxide processes in peroxide damage in the body and helps to choose rational antioxidant correction strategies. The oxidative stress coefficient can also be used for personalized evaluation of the effectiveness of antioxidant therapy and its correction in different pathological conditions.

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Authors contribution

Kolesnikova L.I., Sholokhov L.F., Grebenkina L. A., Kurashova N.A. – conception and design of the study. Krusko O.V. – implementation of the practical part of the study, analysis and interpretation of data. Belenkaya L.V. – collection of clinical material. Kolesnikov S.I. – critical revision for important intellectual content, final approval of the manuscript for publication.

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Gender differences in self-reported social functioning of patients with chronic coronary artery disease and affective disorders

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ABSTRACT

Aim. To determine gender differences in self-reported social functioning of patients with comorbidity of affective disorders (ADs) and chronic coronary artery disease.

Materials and methods. Self-reported social functioning of 248 cardiac patients (194 men (78.2%) and 54 women (21.8%)) with chronic coronary artery disease (CAD) and ADs was studied using the Social Adaptation Self-evaluation Scale (SASS). The average age of patients with chronic CAD in men was (57.2 ± 6.5) years, and in women (59.3 ± 7.1) years, $p = 0.04$. Qualitative and quantitative indicators were investigated using the Mann – Whitney test, Wilcoxon test, and T-test; $\chi 2$ (Pearson's goodness-of-fit test) was used to estimate the frequencies.

Results. ADs were represented by chronic mood disorders (45%), first-time depressive episodes (DEs) (24%), recurrent DEs (24.5%), as well as bipolar II disorder (BD II) (6.5%). ADs in 42.4% of patients were associated with psychosocial stressors (mainly, loss), $p = 0.02$. Men statistically significantly more often (37.1%, 72/194) than women (16.7%, 9/54) demonstrated limited communication with others as a result of projection mechanisms, a high level of hostility, passive aggressiveness and lack of initiative, typical of patients with ADs, $p = 0.003$.

Conclusion. Social functioning of patients with ADs and chronic coronary artery disease was complicated irrespective of gender. Women were single and bereaved of their children more often than men. Due to the low level of communication outside family and professional setting, most of the patients maintained communication mainly with the family. However, due to ADs, they were not able to feel support from family members and rarely initiated communication with other people (men did it statistically significantly more often than women).

Key words: affective disorders, chronic coronary artery disease, self-reported social functioning, gender differences.

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

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

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Гендерные различия самооценки социального функционирования пациентов с хронической ИБС и аффективными расстройствами

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РЕЗЮМЕ

Цель исследования – определение гендерных различий самооценки социального функционирования пациентов при коморбидности аффективных расстройств (АР) и хронической коронарной болезни.

Материалы и методы. С помощью шкалы самооценки социальной адаптации изучена самооценка социального функционирования 248 больных (мужчин – 194 (78,2%) и женщин – 54 (21,8%)) кардиологического стационара с хронической ишемической болезнью сердца (ИБС) и АР. Средний возраст пациентов кардиологического стационара с хронической ИБС у мужчин составил (57,2 ± 6,5) года, у женщин – (59,3 ± 7,1), $p = 0,04$. Качественные и количественные показатели исследованы с помощью критериев Манна – Уитни, Вилкоксона и Т-критерия, для оценки частот использован $\chi 2$ (критерий согласия Пирсона).

Результаты. АР представлены хроническими расстройствами настроения (45%), впервые возникшими депрессивными эпизодами (ДЭ) – 24%, рекуррентными ДЭ – 24,5%, а также биполярными аффективными расстройствами II типа 6,5%. АР у 42,4% пациентов было связано с психосоциальными стрессорами (преимущественно утратами), $p = 0,02$. Мужчины статистически значимо чаще (37,1%, 72/194), чем женщины (16,7%, 9/54), более ограниченно общались с окружающими в результате механизмов проекции, высокого уровня враждебности, пассивной агрессивности, безынициативности, характерными для больных АР, $p = 0,003$.

Заключение. Социальное функционирование пациентов с АР и хронической ИБС затруднено независимо от пола. Женщины чаще мужчин являются одинокими и пережившими утрату детей. В связи с низким уровнем общения вне семьи и профессиональной деятельности большая часть пациентов поддерживала общение преимущественно в семье. Однако в силу АР, не способны почувствовать поддержку и со стороны членов семьи, они редко инициируют такие пациенты общение с другими людьми (мужчины статистически значимо чаще, чем женщины).

Ключевые слова: аффективные расстройства, хроническая ишемическая болезнь сердца, самооценка социального функционирования, гендерные различия.

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

Источник финансирования. Исследование выполнено в рамках государственного задания (тема «Комплексное исследование клинико-психопатологических закономерностей и патобиологических механизмов формирования и прогредиентности социально значимых психических и поведенческих расстройств с разработкой инновационных методов ранней диагностики, персонализированных стратегий терапии и профилактики» № АААА-А19-119020690013-2).

Соответствие принципам этики. Все пациенты подписали информированное согласие на участие в исследовании. Исследование одобрено локальным этическим комитетом НИИ психического здоровья, Томский НИМЦ (протокол № 6 от 21.06.2017).

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INTRODUCTION

In recent decades, the issue of comorbidity has been actively studied [1–5]. Cardiovascular diseases (CVD) and depression are two common health problems worldwide [1, 3, 6, 7]. Depression is about twice as common in women as in men and has the strongest association with coronary artery disease (CAD) [8]. On average, it is more severe in women than in men and has an earlier age of onset [8, 9].

About half of women under 60 years of age with a history of myocardial infarction (MI) suffer from severe depression [9, 10]. Young women are more likely to die from MI than men. Stress-induced myocardial ischemia is more common in girls than in boys and is capable of leading to chronic dysregulation of neurohormonal systems under stress [10]. This can begin at an early age, creating the basis for an increased cardiovascular risk in women many years before the onset of CAD.

In the third National Health and Nutrition Study (NHANES III), a history of major depression or attempted suicide was associated with an almost 15-fold increase in risk of coronary artery disease in women and a 3.5-fold increase in this risk in men. In a prospective mental health study in women under 40 with depression, the risk of CAD was six times higher than in women of the same age without depression; although depression was not associated with CAD in men or the elderly [11]. Overall, scientific evidence supports a stronger relationship between depression and CAD in younger women.

Depression was also associated with deterioration in quality of life (QoL) in short-term and long-term studies [11]. Less attention was paid to the socio-psychological factors of depression development and the issues of its impact on the functioning of patients. In the study of depressive disorders (DDs), medical aspects were usually studiedstudied, such as diagnosis, clinical presentation, therapy, prevention, and rehabilitation of persons with those disorders [6, 11–15], whereas an integrated approach to the study of depression implied the study of clinical and biological, psychological, and socio-cultural factors for the onset and course of DDs, as well as self-evaluation of the quality of life [16–18]. Surovtseva et al. [18] found that the degree of decrease in self-evaluated QoL and social functioning of patients with ADs was determined by a complex of biological, psychological, and social factors.

There were enough studies on the relationship between depression and QoL deterioration. However, gender differences were studied insufficiently in self-reported social functioning in cases of comorbid depression and chronic coronary disease.

The aim of the study was to determine gender differences in the self-reported social functioning of patients with comorbidity of AD and chronic coronary artery disease.

MATERIALS AND METHODS

In a cardiology hospital, 290 patients with chronic CAD were diagnosed with ADs. To study self-evaluation of social adaptation, the Social Adaptation Self-evaluation Scale (SASS, M. Bosc et al., 1997) was used. Of 290 patients with chronic CAD and ADs, 248 individuals completed the self-evaluation scale of social adaptation, of which 194 (78.2%) people were men and 54 (21.8%) were women. The average age of men was (57.2 ± 6.5) years, and the average age of women was (59.3 ± 7.1) years, $p = 0.04$.

Statistical data processing was performed using the Statistica 8.0 software. Parametric indicators were assessed using the T-test for dependent and independent groups (with normal distribution of features), nonparametric indicators were evaluated according to the Mann – Whitney and Wilcoxon criteria; chi-square (Pearson's goodness-of-fit test) was used for frequency estimates. Data were given as absolute and relative numbers, *n* (%), the median and interquartile range, Me $(Q_1–Q_3)$.

RESULTS

The nosological framework of mood disorders in patients with chronic CAD was characterized by

the prevalence of chronic affective disorders (45%). The first-onset depressive episodes (DEs) accounted for 24% of cases, recurrent DEs – for 24.5%. Bipolar II disorders were registered in 6.5% of cases [7, 13].

When analyzing the contribution of external factors to the development of ADs in patients with chronic CAD, it was found that 42.4% of patients suffered from psychogenic stressors of varying severity (mainly, loss). Thus, among patients with ADs and chronic CAD who had lost a child or were childless, there were 18.5% of women (10 / 54), and only 6.7% of men (13 / 194) (p = 0.02). 35.9% of patients associated the occurrence of mood disorders with health deterioration. In 18.1% of patients, ADs developed autochthonously (Table 1).

Table 1

Association of mood disorders with external psychogenic factors

Parameter	Quantity, *n* (%)
Factors were not specified	9 (3.6)
Mood disorder developed autochthonously	45 (18.1)
Mild and moderate psychogenic factors	68 (27.4)
Severe and critical psychogenic factors	37 (15)
Factors of health deterioration	89 (35.9)
Total	248 (100)

The study of marital status revealed that 17.1% (33 / 194) of men and 53.7% (29 / 54) of women were single (Table 2).

Table 2

Marital status of men and women with ADs and chronic CAD, *n* (%)

Parameter	Men, *n* = 194	Women, *n* = 54
Married	161 (82.9)	25 (46.3)
Single, incl.:	33 (17)	29 (53.7)
– divorced	18 (9.3)	11 (20.4)
– widowed	14 (7.2)	15 (27.8)

Family relations were assessed as “poor” and “satisfactory” by 24.7% of men (48 / 194) and 31.5% of women (17 / 54), without statistically significant differences (p = 0.5).

Almost one-third of men and one-fifth of women had higher education, and about one-third of men and half of women had secondary special and incomplete higher education. About 30% of men and almost 13% of women had secondary education or less (Table 3).

Table 3

Education level of men and women with ADs and chronic CAD, *n* (%)

Education	Men, *n* = 194	Women, *n* = 54
Higher	56 (29)	10 (19)
Secondary special and incomplete higher	67 (35)	27 (50)
Secondary and lower	58 (30)	7 (13)

According to the level of education, men with AD and chronic CAD were distributed approximately equally (by one-third) in the group with higher, secondary special and incomplete higher, and with secondary or lower level education. Among women in this group of patients, persons with secondary special and incomplete higher education prevailed (about half).

56.7% of men (110 / 194) and 66.7% of women (36 / 54) did not work (Table 4).

Table 4

Types of disability in persons with ADs and chronic CAD depending on gender, *n* (%)

Disability	Men, *n* = 110	Women, *n* = 36
Temporary	73 (66.4)	18 (50)
Disability groups 2 and 3	48 (43.6)	3 (8.3)
Retirement age	43 (39.1)	34 (94.4)

Below, the evaluation indicators according to self-report data and the frequency of their presence according to the SASS scale in men and women are presented (Table 5).

The overall mean score on the scale of self-reported social functioning in patients with ADs and chronic CAD corresponded to the level of difficult social adaptation. In men it was 33.9 ± 6.6, and in women it was 34.6 ± 6.5, without the statistical significance of differences (p = 0.5).

2.8% (7/248) of patients with ADs and CAD were socially maladapted, 58.1% (144 / 248) had difficulty in social adaptation, 41.5% (103 / 248) had normal adaptation in society, 0.4% (1 / 248) had excellent adaptation, and 2.8% (7 / 248) were impossible to be evaluated.

In men and women with ADs and chronic CAD, the frequency (p = 0.007) and the severity (p = 0.03) of the conviction that “people from their social environment rarely or never seek communication with them” differed significantly.

Table 5

Differences in self-reported parameters of social functioning between men (n = 194) and women (n = 54) with ADs and chronic CAD

Parameter	Self-evaluation, score, Me (Q_1–Q_3)		p	Symptom presence, n (%)		p
	Men	Women		Men	Women	
Employment	–	–	–	110 (56.2)	36 (66.7)	0.2
Interest in employment	2 (1–2)	2 (1–2)	0.9	79 (40.7)	21 (38.9)	0.8
Satisfaction from employment	2 (1–2)	2 (1–2)	0.5	90 (46.4)	24 (44.4)	0.8
Satisfaction from hobbies	2 (2–2)	2 (1–2)	0.2	47 (24.2)	19 (35.2)	0.1
Evaluation of spending leisure time	1 (1–2)	1 (1–2)	0.8	124 (63.9)	37 (68.5)	0.5
Seeking family communication	2 (2–2)	2 (2–2)	0.3	48 (24.7)	9 (16.7)	0.3
Evaluation of family relationships	2 (2–2)	2 (1–2)	0.9	48 (24.7)	17 (31.5)	0.5
Number of relationships outside the family	2 (1–2)	2 (1–2)	0.7	83 (42.8)	21 (38.9)	0.6
Activity in relationships outside the family	1 (1–1)	1 (1–2)	0.7	153 (78.9)	38 (70.4)	0.2
Evaluation of relationships with other people in general	2 (1–2)	2 (1–2)	0.7	64 (33.0)	17 (31.5)	0.8
Importance of relationships with others	2 (1–2)	2 (2–3)	0.3	53 (27.3)	10 (18.5)	0.3
Frequency of seeking communication with the patient from others	2 (1–2)	2 (2–2)	0.03	72 (37.1)	9 (16.7)	0.007
Observance of public rules by the patient	2 (2–3)	2 (2–3)	0.06	13 (7.70)	1 (1.9)	0.3
Involvement in the life of the society	0 (0–1)	0 (0–1)	0.7	50 (77.3)	41 (75.9)	0.8
Pleasure from search for information	2 (1–2)	2 (1–2)	0.2	73 (37.6)	25 (46.3)	0.25
Interest in information	2 (1–2)	2 (1–3)	0.3	60 (30.0)	20 (37.0)	0.4
Difficulty expressing an opinion	2 (2–2)	2 (2–2)	0.4	16 (8.2)	9 (16.7)	0.1
Feeling rejected	2 (2–3)	2 (2–3)	0.2	8 (4.1)	0 (0)	0.3
Importance of physical attractiveness	2 (1–2)	2 (1–2)	0.3	103 (53.1)	24 (44.4)	0.3
Difficulty dealing with income	2 (2–3)	2 (1–2)	0.2	48 (24.7)	18 (33.3)	0.2
Feeling ability to manage their lives	1 (1–3)	1 (1–3)	0.98	104 (53.6)	29 (53.7)	0.99

There were no other statistically significant differences in the severity and frequency of different levels of social adaptation disturbances which would depend on gender ($p > 0.05$).

DISCUSSION

In patients with CAD, depression was the strongest predictor of QoL [17], especially one-year health-related QoL, even after taking into account functional status and clinical variables [11, 17]. The ratio of women and men in the study group was 1:4, which was associated with the predominance of men among patients with CAD. The average age of men was 2 years less than that of women (57.2 and 59.3 years, respectively), $p = 0.04$.

In patients with chronic CAD in the cardiology hospital, chronic affective disorders were often found (45%). DEs were first-onset (24%) and recurrent (24.5%). BD was found in 6.5% of cases. In 91.7% of cases, the depressive syndrome was polymorphic, more often with anxiety (54.8%) [7, 13].

Women were less likely to have a marriage partner ($p = 0.03$). Men were more likely to remarry than women. A third of women and almost a quarter of men were not satisfied with family relationships ($p = 0.3$). Perhaps, in this regard, the patients did not seek communication and support in the family: 24.7% (48 / 194) among men and 16.7% (9 / 54) among women ($p = 0.3$).

About half of men (43.3%, 84 / 194) and a third of women (33.3%, 18 / 54) worked, including working parttime, unofficially, being retired by age or disability. Of the unemployed individuals, more than 60% of men and 50% of women were recognized as temporarily disabled. More than 40% of men and only 8.3% of women belonged to disability groups 2 and 3, which was probably determined by social support for men, as they did not reach the retirement age. Almost 95% of women and only about 40% of men were retired 27.3% of men (30 / 110) and 5.6% of women (2 / 36) did not work, though being of working age and without medical examination restricting employment, mostly with lower secondary education. Perhaps this was explained by a small number of vacancies with a decrease in tolerance to physical activity and restrictions on the labor market for people of pre-retirement age with a low level of education.

53.1% (103 / 194) of men and 44.4% (24 / 54) of women ($p = 0.3$) evaluated their physical attractiveness as “not very important” or “not at

all important". 8.2% (16 / 194) of men and 16.7% (9/54) of women indicated difficulty in expressing their opinion ($p = 0.1$).

More than half of the patients (78.9% (153 / 194) of men and 70.4% (38 / 54) of women, $p = 0.2$) showed low activity in initiating interactions with other people according to the self-evaluation scale: (1 (1–1) in men and 1 (1–2) in women, $p = 0.7$). Relationships with other people were assessed by them as "of little value" or "of no value" (27.3% of men (53 / 194) and 18.5% of women (10 / 54), $p = 0.3$) Perhaps that was why patients of both sexes maintained relationships with a small number of people 2 (1–2), $p = 0.7$.

On the whole, they negatively assessed relationships with other people (about one-third of patients: 33.0% (64 / 194) of men and 31.5% (17 / 54) of women, $p = 0.8$). Men more often (37.1%, 72 / 194) than women (16.7%, 9 / 54) noted that people around them rarely sought communication with them ($p = 0.003$). This can be explained by mechanisms of projection, high level of hostility, passive aggressiveness, and lack of initiative [18], which are typical for of patients with ADs and lead to objective limitation of communication on the part of people from the environment. Compliance with social rules, good manners, and courtesy was reported as "almost always" by both men and women ($p = 0.06$). At the same time, only 7.7% (13 / 194) of men and 1.9% (1 / 54) of women confessed to violating social rules ($p = 0.3$).

More than three-quarters of patients (77.3% (50 / 194) of men and 75.9% (41 / 54) of women) were not involved in social activities, $p = 0.8$. Social participation was rated as low or absent in men and women (0 (0–1), $p = 0.7$).

They had a reduced ability to organize the environment in accordance with their desires and needs according to the self-evaluation data (1 (1–3), $p = 0.9$). This was typical of more than half of the patients (53.6 (104 / 194) of men and 53.7% (29 / 54) of women, $p = 0.99$).

One-fourth of men (24.7%, 48 / 194) and one-third of women (33.3%, 18 / 54) indicated that they "often" or "always" found it difficult to manage their income, without the statistical significance of differences depending on gender ($p = 0.2$). It was likely connected not only with a low income level, but also with difficulties in decision making and self-doubt.

About one-third of men (30.9%, 60 / 194) and women (37.0%, 20 / 54), $p = 0.4$, denied interest in scientific, technical, and cultural information: according to the self-assessment of the corresponding item in the SASS scale, 2 (1–2) in women and 2 (1–3) in men ($p = 0.3$). Pleasure from searching for information about various things, situations, and people for better understanding was moderate according to the self-evaluation data (2 (1–2)) ($p = 0.2$); 37.6% (73 / 194) of men and 46.3% (25 / 54) of women did not experience pleasure from this activity ($p = 0.25$).

In self-questionnaires, about one-fourth of men (24.2%, 47 / 194) and one-third of women (35.2%, 19 / 54) indicated the absence or reduced pleasure from hobbies ($p = 0.1$).

In general, more than 60% of patients (63.9% (124 / 194) of men and 68.5% (37 / 54) of women) were not satisfied with spending their free time and rated it as "poor" and "satisfactory" (1 (1–2), $p = 0.8$). This could be associated not only with restrictions of activity and exercise tolerance, but also with a decrease in hedonism. Similar results in the group of patients with CAD (taking into account the symptoms of depressive disorder, decreased renal function, and cognitive impairment) were obtained by Dorofeeva et al. [19].

CONCLUSION

About half of the patients in the study group were found to have chronic ADs, and the rest – depressive episodes in the framework of mono- and bipolar ADs. In more than 40% of cases, ADs developed due to exposure to severe or catastrophic psychosocial stressors (loss, including children), which was noted more often in women than in men ($p = 0.02$). More than one-third of patients associated the occurrence of ADs with health deterioration, and one-fifth of the patients had an autochthonous onset of ADs.

Women were less likely to have higher education than men, possibly due to a preference for family values over higher education (during the period of birth and raising children). About half of men and one-third of women worked, including working part-time, unofficially, and being retired by age (95% of women and only about 40% of men) or disability (40% of men and 8% of women). Women were less likely to be married than men ($p = 0.04$). Men were

probably remarried. About one-third of patients of both sexes were not satisfied with family relationships (p = 0.3), one-fourth did not seek communication and support in the family (p = 0.3).

The low significance of relationships with other people was noted by about 30% of men and women, without the statistical significance of the differences. The negative assessment of relationships with others was present in one-third of the patients, irrespective of gender. This probably led to inactivity to initiate interactions with others (over 70% of men and women).

Statistically significant differences (p = 0.003) depending on gender were obtained regarding the conviction that "people around them rarely seek communication with the patients" (37.1% of men and 16.7% of women). This fact could be explained by projection mechanisms, passive aggressiveness (up to 92% of men and women (p = 0.3) reported adherence to social rules), lack of initiative (more than 75% of patients (male and female), p = 0.8, were not involved in social activities). These were behavioral patterns typical of patients with ADs leading to objective limitation of communication on the part of people from the environment.

According to the self-evaluation data, irrespective of gender, the patients had problems not only in sphere of communication, but also in the getting interest and pleasure from searching information (more than one-third of patients, p = 0.25), hobbies (about one-third of patients, p = 0.1), free time (more than 60%, p = 0.5), as well as self-evaluation of their external attractiveness (more than half of the patients, p = 0.3). In patients with ADs and chronic CAD, social adaptation was difficult in almost 60% of cases (irrespective of gender, p = 0.1). Family was almost the only source of support in case of the chronic course of ADs and CAD, which had to be taken into account when planning rehabilitation measures in this group of patients.

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Authors contribution

Lebedeva E.V., Nonka T.G. – carrying out of research, statistical analysis and interpretation of data. Schastnyy E.D., Repin A.N. – critical revision of the manuscript for important intellectual content, final approval of the manuscript for publication.

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Tolerance to colonoscopy preparation with Fortrans and predictors of negative effects

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ABSTRACT

Background. The tolerance of colon cleansing with Fortrans is associated with a number of negative factors. This determines patient compliance and quality of bowel preparation. The relevance of this issue is increasing due to the prevalence of this method in the diagnosis and treatment of colon pathology.

The aim of the study was to investigate tolerance to Fortrans in colon preparation for colonoscopy and factors affecting comfort.

Materials and methods. Before colonoscopy, a questionnaire method was used to study 84 patients who underwent colon preparation with Fortrans. Patient satisfaction with the preparation was evaluated on the visual analogue scale (VAS).

Results. 45 (52.4%) people were satisfied with comfort of the preparation and rated its level as 0–2 points on the VAS. 39 (47.6%) patients were not satisfied with the preparation, a discomfort level of 3–10 points was estimated. Factors affecting patient tolerance of Fortrans administration were determined.

Conclusion. Satisfactory tolerance of the colon preparation with Fortrans was observed in half of the patients, which depended on their psychological state and realized expectations of comfort during the procedure. Predictors of intolerance of colon preparation are side effects of Fortrans, which are largely mitigated by its split intake, as well as incorrigible factors, such as higher education, repeated colonoscopy, and history of constipation.

Key words: bowel preparation, predictors of tolerance, side effects of Fortrans.

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Переносимость подготовки к колоноскопии препаратом «Фортранс» и предикторы, влияющие на ее характер

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РЕЗЮМЕ

Введение. Переносимость очищения толстой кишки фортрансом связана с рядом факторов, которые недостаточно исследованы в рамках рассматриваемой проблемы. Она определяет комплаентность пациента и в итоге качество очистки толстой кишки. Значение переносимости подготовки к колоноскопии возрастает из-за распространенности этого метода в диагностике и лечении патологии толстой кишки.

Цель. Изучить переносимость фортранса при подготовке толстой кишки к колоноскопии и факторы, влияющие на ее характер.

Материалы и методы. На доколоноскопическом этапе методом анкетирования исследованы 84 пациента, прошедшие подготовку толстой кишки фортрансом. По визуально-аналоговой шкале (ВАШ) изучена удовлетворенность пациентами приемом препарата.

Результаты. 45 (52,4%) человек удовлетворены комфортом подготовки, самооценка по шкале ВАШ 0–2 балла, 39 (47,6%) пациентов отметили неудовлетворенность приемом препарата, уровень дискомфорта 3–10 баллов. Выявлены факторы, влияющие на переносимость пациентами приема фортранса.

Заключение. Удовлетворительная переносимость подготовки толстой кишки фортрансом наблюдается у половины пациентов. Это в определенной мере зависит от их психологического состояния и степени исполнения ожиданий комфорта подготовки. Предикторами непереносимости подготовки толстой кишки являются нежелательные проявления фортранса, которые в значительной мере нивелируются раздельным приемом препарата, а также некоррегируемые факторы: высшее образование, повторная колоноскопия, запоры.

Ключевые слова: подготовка кишечника, предикторы переносимости, нежелательные явления при подготовке фортрансом.

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

Источник финансирования. Авторы заявляют об отсутствии финансирования.

Соответствие принципам этики. Все пациенты подписали информированное согласие на участие в исследовании. Исследование одобрено локальным этическим комитетом при Тюменском государственном медицинском университете (протокол № 90 от 17.03.2020).

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INTRODUCTION

Antegrade intestinal lavage is the main preparation method for colon examination. Fortrans, a polyethylene glycol-based medicine, the oldest of this group, remains a common drug for purgation and often serves as a control in various scientific studies which investigate new drugs and ways of preparing the intestines for various diagnostic and therapeutic procedures [1–3].

Unsatisfactory tolerance of colon cleansing is associated with side effects of medications used for this purpose. This is one of the factors influencing preparation tolerance, and it is important, though not the only one. There are other factors, such as

demographic, social, and clinical ones, which are not sufficiently studied within the issue under consideration.

Tolerance of the preparation process determines the patient's compliance and, as a result, the quality of colon cleansing. This is the key to successful colonoscopy [1, 3, 4]. The study of tolerance predictors, their relationship with compliance, and the quality of colon cleansing will help to correct the colonoscopy preparation plan, which will improve the quality of examination and treatment of patients [5, 6]. Studies on patient satisfaction with their colon cleansing medications preparations are warranted and necessary [7]. These studies are carried out on the basis of analyzed patient questionnaires and assessments of the quality of treatment or its stages [8].

The aim of the study was to investigate tolerance of Fortrans in colonoscopy preparation and factors affecting its quality

MATERIALS AND METHODS

A total of 84 patients participated in the study. The average age of the patients was 57.7 ± 11.8 years. There were 39 (46.42%) elderly and old patients. The study included 37 men (44.0%) and 47 women (55.9%). In 53 people, the indication for colonoscopy was a screening study with a positive fecal occult blood test, and they took the drug for the first time. In 31 patients, an operation was planned for endoscopic removal of polyps detected in primary health care facilities, and Fortrans was taken for the second time. Patient preparation was carried out at home. Preparation instructions were given by the endoscopist at the pre-colonoscopy appointment.

Patients were admitted to hospital on the day of the study. Before colonoscopy, all patients were interviewed by the co-author of this study, according to the plan presented in the questionnaire. Patients assessed the degree of discomfort in preparation for the examination on the 10-point visual analogue scale (VAS): 0 – no discomfort and 10 – maximum discomfort. Based on the degree of discomfort, 2 groups of patients were formed, who underwent colon cleansing for colonoscopy satisfactorily or unsatisfactorily, according to their self-assessment.

The study was prospective, non-randomized, and single-center.

RESULTS

Depending on the comfort during preparation, the patients were divided into 2 groups. The 1st group consisted of 45 (52.4%) people, 21 men (46.66%), and 24 women (53.33%), who were satisfied with the comfort during preparation; the VAS scores were 0–2 points. The patients felt no discomfort, or the discom- fort was mild. There were 25 (55.55%) people over 60 years old in the first group.

The second group consisted of 39 (47.6%) patients, 16 men (41.0%) and 23 women (59%), and included 14 (35.9%) patients over 60 years old. They noted dissatisfaction with the preparation, the assessment of discomfort on the visual analogue scale was from 3 to 10 points. No statistically significant differences by age and sex were observed in the studied groups, $p = 0.07$ and 0.6.

The comfort of preparation equal to 0 points was noted by 23 (27.4%) people, 1 point – 15 (17.8%) patients, 2 points – by 7 (8.3%) people. There were 27 (32.1%) patients with the discomfort level of 3–6 points and 12 (14.3%) patients with the discomfort level of 7–10 points. The most common side effects were abdominal syndrome: pain and bloating in 23 (27.4%) people, dyspepsia in 22 (26.2%) patients, including nausea in 18 (21.4%) cases and vomiting in 4 (4.8%) patients. An increase in blood pressure was recorded in 13 (15.5%) patients.

Thus, adverse effects of Fortrans were registered in 58 (69.1%) cases. An increase in the frequency of adverse effects of Fortrans (abdominal pain, dyspeptic syndrome, and increased blood pressure) were statistically significant in patients with an unsatisfactory assessment of tolerance of the medication.

Non-adherence to the instructions for colonoscopy preparation was registered in 28 (33.3%) people (Table 1). In the group with satisfactory tolerance, 12 (26.7%) patients did not follow the preparation methodology, most frequently they noted non-compliance with the diet: 8 (17.8%) people. 16 patients (41.0%) who rated the bowel preparation process as unsatisfactory did not follow the doctor's instructions. The main violations were connected with a decrease in the volume of fluid taken: 11 (28.2%) patients. The differences in non-compliance with the amount of fluid taken were statistically significant ($p = 0.044$) between the groups.

Table 1

Violations of Fortrans intake depending on the preparation tolerance, *n (%)*

Parameter	Number of patients	Preparation tolerance		*p*
		satisfactory, *n* = 45	unsatisfactory, *n* = 39	
Non-compliance with the diet	13 (15.5)	8 (17.8)	5 (12.8)	0.531
Decreased fluid intake	15 (17.8)	4 (8.9)	11 (28.2)	0.044
Total	28 (33.3)	12 (26.7)	16 (41.0)	0.146

The number of violations made it possible to determine the compliance of patients for the entire cohort of the studied groups: 84.5% for diet violation and 82.2% for fluid intake. Among patients with satisfactory tolerance, compliance with treatment in terms of the volume of fluid drunk was 91.1%, in terms of adherence to a diet – 82.2%. In the group with unsatisfactory tolerance, compliance was 71.8% and 87.2%, respectively. The general compliance with treatment was 73.3% in the group with satisfactory preparation tolerance and 59% in the group with unsatisfactory preparation.

The results of assessing satisfaction with colon cleansing in patients with prior experience in preparation and those admitted to the department to remove previously detected polyps were significantly worse than in patients who underwent preparation with Fortrans for the first time, 7 (15.5%) and 38 (84.4%) patients, respectively, $p = 0.001$. Satisfactory assessment of preparation tolerance among patients suffering from constipation was noted by 12 (26.6%) patients and unsatisfactory assessment – by 21 (53.8%) patients, $p = 0.02$. Out of 28 (33.3%) patients with higher and incomplete higher education, 19 (48.7%) people were not satisfied with the preparation, while 9 (20.0%) patients were satisfied, $p = 0.01$.

In the studied cohort, 47 (55.9%) patients had one-phase preparation, and 37 (44.1%) patients had two-phase preparation. The split (two-phase) method was preferred. The type of preparation process depended on the patient's ability to take medication in the morning. Assessment of satisfaction with preparation practically did not differ for both methods of colon cleansing. One-phase preparation was characterized as satisfactory by 53% of patients and unsatisfactory by 46% of patients, and two-phase preparation – by 51% and 48% of patients, respectively (Table 2).

Table 2

Adverse effects of Fortrans in various preparation methods, *n (%)*

Symptom	One-phase preparation, *n* = 47	Two-phase preparation, *n* = 37	*p*
Abdominal syndrome	13 (27.6)	10 (27.0)	0.949
Dyspepsia	26 (33.9)	6 (16.2)	0.001
Nausea	13 (27.6)	5 (13.5)	0.112
Vomiting	3 (6.3)	1 (2.7)	0.432
Increased blood pressure	9 (19.4)	4 (10.8)	0.295
Total	38 (80.8)	20 (54.1)	0.009

Side effects of Fortrans were detected in 38 (80.8%) patients with one-phase colon preparation and in 20 patients with two-phase colon preparation (54.1%), $p = 0.009$. The main adverse effects during one-phase and split preparation were abdominal syndrome, the frequency of which was the same in both groups, 27.6% and 27.0%, respectively, and dyspepsia, whose appearance during split preparation was two times less frequent than in patients with one-phase preparation, 16.2% and 33.9%, respectively, $p = 0.001$ (Table 2).

In one-phase preparation, 6 (12.76%) patients reported violation in adherence to the diet and 13 (27.65%) patients to the fluid intake. In split preparation, the same was noted by 7 (18.9%) and 2 (5.4%), patients respectively.

In one-phase preparation, compliance for adherence to the diet was 87.27%, compliance for the volume of fluid taken was 72.35%. In split preparation, the compliance was 81.9% and 94.6%, respectively. The groups with different preparation methods showed statistically significant differences in adherence to fluid intake, $p = 0.009$.

DISCUSSION

The study is focused on the factors that could affect discomfort of patients when preparing for a colonoscopy with Fortans in various situations. They are closely related to such features as intolerance, dissatisfaction, and tolerance – terms that are close in meaning and are used interchangeably. Only a few studies have examined the topic of tolerance during colonoscopy, at the same time, one of the important criteria for assessing the quality of hospital work is patient satisfaction with the treatment [9]. The prevalence of poor tolerance of preparation, which, due to its discomfort, often exceeds the colonoscopy itself, makes the study relevant [10].

The study analyzing the level of discomfort when taking Fortrans according to the VAS scale showed that 45 (52.4%) patients noted satisfactory tolerance of preparation. Patient satisfaction with Fortrans is reflected in a few publications [11]. The research of S.G. Tereshchenko et al. (2013) studied the level of patient "non-burdensomeness" in preparing for colonoscopy with Fortrans, and the obtained result was similar to our studies (55%) [11].

Thus, only slightly more than half of the patients who underwent Fortrans colonoscopy preparation were satisfied with its quality. The study of Fortrans side effects showed that in the general cohort of patients abdominal pain and bloating were reported by 23 (27.4%) patients. The published studies show significant differences in the incidence of abdominal pain with Fortrans, from no pain to 52% [10, 11]. In the group with satisfactory tolerance, pain syndrome occurred in 6 patients (13.3%), and in the group with unsatisfactory tolerance, it was reported much more often, by 17 (43.6%) patients, $p = 0.002$. Abdominal pain during colon preparation according to V.M. Ussui et al. was a reliable reason for the patient's refusal to undergo a second colonoscopy [9]. Dyspepsia was observed in 22 (26.9%) patients: in 1 (2.2%) patient with satisfactory tolerance of preparation and in 21 (53.8%) people with unsatisfactory tolerance of preparation, $p = 0.001$. The incidence of this syndrome for Fortrans intake also has significant differences: from 12.9% to 96.4% [3, 10].

In our research, increased blood pressure was recorded in 13 (15.5%) patients: 3 (6.6%) patients with satisfactory tolerance and 10 (25.6%) patients with unsatisfactory tolerance, $p = 0.037$. There is a significant relationship between patient dissatisfaction with the preparation and adverse effects of taking the medication. The side effects are the reason for inadequate colon cleansing preparation. Non-adherence to the instructions during colon preparation with Fortrans is not uncommon, which is confirmed by various studies [3, 5, 10, 11].

E.D. Fedorov et al., S.G. Tereshchenko et al. found out that 57.1 and 58% of patients, which is more than half of the studied patients, could not drink the entire intended volume of fluid [3, 11]. Due to the large volumes of fluid taken and the need for long-term adherence to the diet, the drugs of the Macrogol group are characterized by lower compliance of patients with prescriptions.

According to Fedorov E.D. et al. [3] and D.A. Svetyash [40], compliance in taking Fortrans was 82% and 78%, respectively. In our study, compliance in taking Fortrans with satisfactory tolerance was 73% and with unsatisfactory tolerance, it was 59%. There are studies confirming our results: the higher the assessment of satisfaction with the preparation, the greater the adherence to the drug taking, "the patient compliance is influenced by the level of their comfort, confidence and, satisfaction" [9].

Of all the factors that determine preparation tolerance to the chosen medication, the doctor can influence the colon cleansing results only by prescribing a method of its administration: one-phase or two-phase intake of a dose of the drug by the patient. The rest of the factors (demographic, social, clinical) are independent constants determined by patients themselves. Demographic characteristics (age, sex) did not have a significant effect on the preparation tolerance. Of the social factors, only higher education was a statistically significant sign of unsatisfactory tolerance of colon preparation ($p = 0.01$). Adverse effects of Fortrans, constipation and repeated colonoscopy were significant clinical features of dissatisfaction with the preparation for colonoscopy.

While examining tolerance to colonoscopy, S. Hazeldine et al. revealed that patients with repeated colonoscopy also significantly more often noted worse results on procedure tolerance on the VAS [13].

Adverse effects of Fortrans were observed in 38 (80.8%) patients with the one-phase preparation method, and in 20 (54.1%) patients with the split method, $p = 0.009$ (Table 2). Similar results were obtained by other researchers [1]. At the same time, when comparing patient satisfaction in different methods of preparation, the results did not differ. Satisfaction with one-phase preparation was observed in 25 patients (53.1%) and with split preparation in 19 patients (51.3%). Dissatisfaction with the methods was detected in 22 (46.8%) and 18 (48.6%) patients, respectively. There was no difference which would indicate the beneficial impact of any preparation method on comfort of the procedure.

At the same time, the study of the Fortrans adverse effects in groups of patients with different methods of preparation showed their significant differences. In the one-phase method, the side effects were more frequent in comparison with the split method (Table 2). It would be logical to assume that frequent side effects of Fortrans in one-phase preparation method should lead to a decrease in the comfort score in this method of colon cleansing. However, it did not happen, as duration of split prepa- ration, including sleep between phases, was more than 12 hours. There was no quality sleep due to the need to wake up early and take medication for the second time. Then patients had to arrive at the clinic on time and undergo the admission procedure, all this had to be done till 1–3 p.m. (the time of the colonoscopy with the split preparation method).

All nonresident patients risked being late for examination, which did not contribute to their psychological comfort, but, when assessing the tolerance, it was compensated for by less frequent side effects of Fortrans due to a decrease in its intake volume. At the same time, one-phase evening preparation, with its difficulties in taking 4 liters of liquid and high incidence of adverse drug effects, was more convenient for nonresident patients in terms of psychological comfort, which was facilitated by the absence of time pressure, which influenced the overall assessment of the preparation tolerance.

Ultimately, similar assessments of satisfaction with the preparation tolerance were obtained in the groups with one-phase and two-phase preparation for colonoscopy. T. Voiosu et al., using a 10-point visual analogue scale to assess patient satisfaction with the preparation for colon cleansing, also noted that there was no difference in the assessment of comfort depending on the preparation method [12]. L.A. Shafer et al. showed that awakening and taking medication early in the morning in 1/3 of patients preparing for a split colonoscopy caused a negative reaction, which affected the assessment of satisfaction with this preparation method [14].

Patient satisfaction with treatment was defined by M. Tierney et al. as “a multifaceted and individually dependent response with questionable validity” [8]. Analysis of the study results and literature data suggests that the preparation tolerance is not a direct reflection of the degree of adverse drug effects during colon cleansing, but is a multifactorial patient selfassessment of their condition, where the psychological component and its “doubtful validity” are equally important.

The psychological state is formed as a result of patient’s knowledge about the peculiarities of preparation, the need for it, the conditions of being in the clinic, and communication with medical personnel at all stages of preparation and colonoscopy. A lack of assessment of these parameters, understudied comparison of Fortrans tolerance with other medications used for colon cleansing, and single-center nature of the study are the weaknesses of this work.

CONCLUSION

Satisfactory tolerance of colon preparation with Fortrans is observed in half of the patients. It significantly depends on the adverse effects of the drug, which are to a large extent mitigated by its split intake, and a number of unregulated features associated with characteristics of the patients, such as higher education, repeated colonoscopy, and constipation, which must be taken into account when planning colon cleansing.

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Attachment style and accuracy of facial expression recognition in depression

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ABSTRACT

Aim. To investigate links between the attachment style and ability to detect facial emotions using a functional magnetic resonance imaging (fMRI) paradigm in depressed patients.

Materials and methods. Participants diagnosed with mild to moderate depression or dysthymia (19 patients) and healthy volunteers (20 individuals) were to identify one of eight basic emotions on 48 photos by choosing the appropriate answer from two options. Attachment was measured using the Experience in Close Relationships Scale. In addition, depression, alexithymia, and rumination were estimated as other possible correlates.

Results. In the group of patients with depression, anxious attachment score had a negative correlation with the accuracy of angry facial expression detection ($\rho = –0.65$, $p < 0.01$) and a positive correlation with the accuracy of sad facial expression recognition ($\rho = 0.48$, $p < 0.05$). Patients with high total rumination ($\rho = –0.48$, $p < 0.05$) and depressive rumination ($\rho = –0.53$, $p < 0.05$) scores also detected angry facial expression less accurately. None of the mentioned relationships were present in healthy people, however, they demonstrated a correlation of the total number of portraits tagged as “sad” with the brooding rumination score ($\rho = 0\ .53$, $p < 0.05$).

Conclusion. Attachment disruptions in depressed patients may be related to aggravation of the deficit in the ability to detect emotions of others.

Key words: mood disorders, emotions, emotional intelligence, empathy.

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

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Conformity with the principles of ethics. All individuals were notified of the objectives of the study and signed an informed consent to participate in the study. The study was approved by the Ethics Committee at the Research Institute of Molecular Biology and Biophysics, Federal Research Center of Fundamental and Translational Medicine (Protocol No. 1 of 08.06.2016).

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Стиль привязанности и распознавание эмоциональной мимики при депрессии

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РЕЗЮМЕ

Цель. Изучить связи между стилем привязанности и способностью к распознаванию эмоциональной экспрессии в условиях эксперимента с использованием функциональной магнитно-резонансной томографии у пациентов с депрессией.

Материалы и методы. Испытуемые с легким, умеренным депрессивным эпизодом или дистимией (19) и здоровые добровольцы (20) должны были определить одну из восьми базовых эмоций на 48 фотографиях, выбрав из двух вариантов верный. Для оценки стиля привязанности использовался Опросник привязанности к близким людям. Дополнительно оценивался уровень депрессии, алекситимии и руминации как другие возможные корреляты.

Результаты. В группе депрессии выраженность тревожного стиля привязанности обратно коррелировала с точностью определения мимики гнева ($\rho = –0,65$, $p < 0,01$), а положительно – с качеством распознавания печальных лиц ($\rho = 0,48$, $p < 0,05$). Экспрессия гнева также хуже распознавалась пациентами с высоким баллом по шкале руминации в целом ($\rho = –0,48$; $p < 0,05$) и подшкале депрессивной руминации ($\rho = –0,53$; $p < 0,05$). У здоровых людей не выявлены упомянутые связи, однако число портретов, определенных как печальные, было ассоциировано с подшкалой навязчивых мыслей шкалы руминации ($\rho = 0,53$, $p < 0,05$).

Заключение. Нарушения привязанности при депрессии могут быть связаны с усугублением дефицита способности к определению эмоций других людей.

Ключевые слова: аффективные расстройства, эмоции, эмоциональный интеллект, эмпатия.

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

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INTRODUCTION

Emotional intelligence disruptions defined as difficulties in understanding emotions of other people are frequent in depression. Patients with a current depressive episode are worse at deducing emotions from facial expressions than remitted ones, and those with a history of depression perform poorer in this task than people with no lifetime history of depression [1]. This feature is partly related to a dysfunction at the late stage of the perceptual processing leading to a more negative evaluation of the external stimuli [2]. However, a deficit in emotion recognition may be influenced

by other, more specific factors, namely an attachment disruption, which develops and is manifested in the early mother-infant relationships, according to J. Bowlby's original theory [3]. M. Ainsworth et al. defined three main attachment styles, namely secure, anxious, and avoidant; the latter including the dismissive and the fearful subtypes [4].

C. Hazan and P. Shaver [5] hypothesized that the attachment style determines adult behavior in romantic relationships. While people with the secure attachment style expect reliability and safety, those with the anxious style use relationships as a means to cope with a feeling of a threat and are afraid of losing a social bond perceived as defense. The avoidant attachment style is related to suppression of both a feeling of a threat and social needs [6, 7]. People with anxious and avoidant attachment styles, compared to those with the secure style, demonstrate selective attention to social stimuli [6, 7]. Associations between the attachment style and responses to facial expressions in adults are established in a number of studies [1, 6, 8–10]. In depressed patients, the anxious attachment style is frequent [11]. However, both dysfunctional attachment styles are related to a risk of depression [12] and the presence of a comorbid social phobia aggravating the affective symptoms [13].

Multiple studies have focused on neuroimaging correlates of attachment. In such studies, simple tasks aimed at uncovering some features of the attachment style are performed during fMRI scanning [7, 14–16] or EEG recording of event-related potentials [8, 9].

The aim of the study was to explore the associations between the prominence of pathological attachment styles and the accuracy of recognizing basic emotions in healthy participants and in patients with depression using fMRI.

MATERIALS AND METHODS

The study was a part of the project devoted to neuroimaging and psychological markers of depression and evaluation of the effectiveness of its non-pharmacological treatment. The experimental group included individuals aged 18–65 years with the first documented episode of unipolar depression. The participants received no pharmacological treatment and were unwilling to undergo any antidepressant therapy, which seems reasonable, since most patients had mild depression. Exclusion criteria were major psychiatric and neurological disorders as well as MRI contraindications. The experimental group consisted of 21 patients (6 men, 15 women, the average age was 34.3 ± 9.0 years, Beck Depression Inventory (BDI) score was 18.7 ± 10.5) who were diagnosed with a mild depressive episode (F32.0), moderate depressive episode (F32.1), or dysthymia (F34.1) according to ICD-10.

The control group comprised 21 healthy volunteers (6 men, 15 women, the average age was 33.8±8.5 years, BDI score 4.6 ± 4.5). The same set of exclusion criteria was applied, including the absence of a current affective disorder and affective disorders in the medical history. All variables presented as the mean ± standard deviation were normally distributed (Kolmogorov – Smirnov test, $p < 0.4$). The groups had no significant differences in gender ratio and the mean age. No data were obtained for two individuals from the depression group and one healthy volunteer, which resulted in their exclusion from the study.

All the participants signed an informed consent prior to their inclusion in the study. The study was approved by the Ethics Committee at the Research Institute of Molecular Biology and Biophysics, a division of the Federal Research Center of Fundamental and Translational Medicine (Protocol No. 1 of 08.06.2016).

The patients from the experimental group were invited by a licensed private therapist who verified the diagnosis of affective disorder and compliance with other inclusion criteria. The control group was recruited via an advertisement in the social network and by word of mouth from previous participants. Each control matched a patient from the experimental group in gender and age ± 5 years. Those who had given their consent to participate in the study first visited a licensed neurologist to rule out any major neurological disorder and then the International Tomography Center, Siberian Branch of Russian Academy of Sciences, for an imaging session. A reference MRI and a combined EEG-fMRI acquisition were performed with a few emotional paradigms. The behavioral data from one of them are analyzed in the current study. After the scanning session, a brief attention check was done. Namely, the participants looked at a number of photos and indicated those that they had seen during the scan-

ning session. After that, psychological questionnaires were completed. The Experience in Close Relationships and the Ruminative Response Scales were in a paper-and-pencil form, while other questionnaires were filled in via the BOS-Test (Comsib Ltd, Russia) software for psychological assessment. Upon completion of data collection, the participants received a small monetary reward and a CD with the reference structural image.

Psychological variables were assessed using the Experience in Close Relationships Scale [17] (an adaptation of the Experience in Close Relationships Scale [18]), the Ruminative Response Scale [19] (an adaptation of the Ruminative Response Scale [20]), the 26-item Toronto Alexithymia Scale, Beck Depression Inventory [21] (an adaptation of the Beck Depression Inventory [22]), and Zung Self-Rating Depression Scale [23] (in an adaptation [24]). The levels of rumination, alexithymia, and general depression were estimated in order to find out whether results of correlation analysis were related primarily to attachment or to more general variables. Age was also included in the analysis in order to check its possible influence on the main results.

The facial expression recognition paradigm was prepared using the Millisecond Inquisit software. Forty-eight photos of facial expressions (six photos for each basic emotion) from the Face-Place database (M.J. Tarr, Center for the Neural Basis of Cognition and Department of Psychology, Carnegie University, http://www.tarrlab.org/) were presented in a pseudorandom order. There were eight basic emotions, namely anger, confusion, disgust, fear, happiness, sadness, surprise, and a neutral facial expression. Each photo was accompanied by two possible answers, and the participant had to choose the correct one by pressing the corresponding button. The participants saw the photo and the answers on the monitor screen; they were reflected in the head coil mirror of the scanner. We recorded 1) the number of answers in each category, regardless of their correctness and 2) the number of accurate responses in each category which was a sum of correct positive (sensitivity) and correct negative (specificity) answers for all photos which had this answer as an option. Associations between the participants' psychological features and facial expression recognition parameters were established by the Spearman's rank correlation coefficient ρ separately for each group using the IBM SPSS 21.0 software. Due to a small sample size, the statistical power of the analyses in the current study allowed to discover correlations with $\rho = 0.45$ and above.

Thus, the cutoff score for significant correlations is nearly equivalent to an accepted border between weak and moderate correlations ($r = 0.4$) [25]. Since the study is aimed at highlighting prominent links, this obstacle does not seem to be a serious restriction. However, the negative results of the study should be interpreted with caution.

RESULTS

Depressed patients did not differ from healthy volunteers in the general accuracy of facial expression recognition (on average, 73% of correct answers in each group). The correlation analysis demonstrated that depressed participants exhibited a negative correlation between the anxious attachment score and the quantity of correct answers in the "anger" category (see Table 1 and Figure). A positive correlation between the anxiety attachment score and the quantity of correct answers in the "sadness" category was also established in this group.

Table 1

Spearman's rank correlation coefficient of the quantity of correctly recognized portraits for each emotion and attachment scores in depressed and healthy participants

Scale	Facial emotion							
	Anger	Confusion	Disgust	Fear	Happiness	Surprise	Sadness	Neutral
Depressed patients, $n = 19$:								
– anxious;	–0.30	–0.36	–0.14	0.045	–0.22	–0.08	0.19	0.19
– avoidant	–0.65[b]	–0.5	–0.09	0.14	–0.10	0.06	0.48*	–0.11
Healthy volunteers, $n = 20$:								
– anxious;	0.07	0.10	–0.26	–0.17	–0.30	–0.17	0.22	–0.35
– avoidant	0.21	0.14	0.15	0.18	0.14	–0.16	0.14	0.19

Note. * $p < 0.05$; b – $p < 0.05$ with the Bonferroni correction.

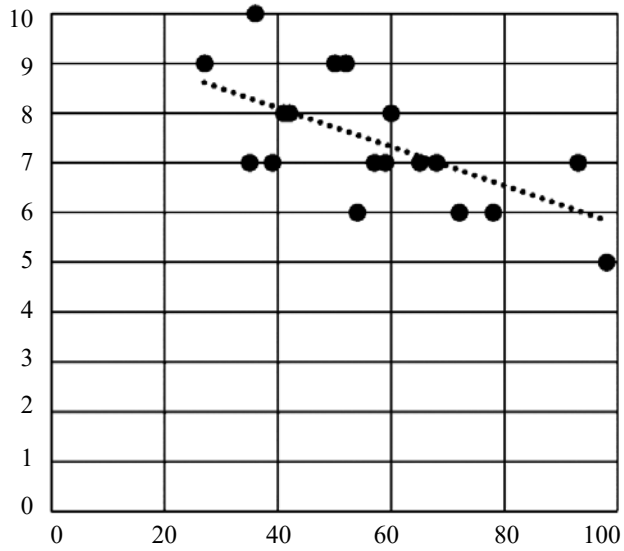

Figure. A scatter plot for anxious attachment score (abscises) and the quantity of correctly recognized anger expressions on photos (ordinates) in the group of depressed patients, ρ = –0.65.

In the depression group, the accuracy of recognizing the angry expression was also associated with general rumination (ρ = –0.48; $p = 0.04$) and depressive rumination (ρ = –0.53; p = 0.02). No such correlations for depression or alexithymia scores were discovered in any group. Correlations between age and facial emotion recognition were observed (Table 2). However, they were unrelated to the results mentioned above. An interesting general trend may be a decline in the facial expression recognition accuracy in healthy volunteers with age.

The correlations listed above did not work for the total number of answers (irrespective of correctness) identifying an emotion on the photo. No such significant correlations were present in the depressed group, while in the healthy group brooding rumination scores had a negative correlation with the total number of answers in the "sadness" category (ρ = 0.53; $p = 0.02$).

Table 2

Spearman's rank correlation coefficient of the quantity of correctly recognized portraits for each emotion and age in depressed and healthy participants

Group	Facial emotion							
	Anger	Confusion	Disgust	Fear	Happiness	Surprise	Sadness	Neutral
Depressed patients, $n = 19$	10	–0.22	0.01	–0.00	0.03	–0.51*	–0.09	–0.03
Healthy volunteers, $n = 20$	0.25	–0.60**	–0.09	–0.39	–0.62[b]	–0.33	–0.39	–0.58**

* $p < 0.05$; ** $p < 0.01$; [b] $p < 0.05$ with the Bonferroni correction.

Lastly, the number of answers in the "happiness" category had a positive correlation with the participants' age in the control group (ρ = 0.61; $p < 0.01$).

DISCUSSION

The most important and the only statistically significant result of the study following multiple comparison corrections is the link between the anxious attachment style and decreased accuracy of angry facial expression recognition established specifically in the depressed group. This association is of special interest because of higher prevalence of the anxious attachment style in depressed patients [11]. An additional correlation analysis demonstrated the absence of a correlation between angry facial expression recognition and age (ρ = 0.1, which would be a negligible association even if it became significant due to a larger sample [25]). The presence of weak yet significant correlations with the rumination scores allows to suppose a role of a more global cognitive style driven mostly by a reaction to social stimuli embodied in the term "attachment".

We found no published results on the relationship between the attachment style and facial expression recognition in depression. In social phobia, which is a frequent condition in depression, individuals with the anxious attachment style show faster reactions to emotional stimuli than to neutral ones [26]. However, this result is unspecific to certain emotions and most likely reflects increased emotional vulnerability, a trait related to anxious attachment [6].

The data previously collected from healthy volunteers of different age differ from ours to a large extent. In an event-related potential study, the differences in the early (50–120 ms) signal components during observation of aggressive and neutral facial expressions were registered only in participants with the avoidant attachment style [8]. In another study, participants with secure and anxious styles reacted more strongly to aggressive body language

than to neutral one [9]. Anxiety which was associated with the anxious attachment style was unrelated to emotion recognition in photos demonstrated subliminally and to corresponding shifts in the brain activity [15].

In a study on priming effects, an avoidant but not anxious attachment style was related to suppression of reactions to angry and sad facial expressions [6]. In adolescents, the secure attachment style was associated with correct facial expression recognition based on both the whole face and eye area alone [27], which implies a deficit in the corresponding ability in abnormal attachment styles, though with no relation to a certain expression kind.

Thus, in healthy volunteers, reactions to angry facial expressions are determined primarily by the prevalence of the avoidant attachment style, while the role of anxious attachment seems to be independent of certain emotions. However, according to our data, in depression, the anxious style interferes with correct recognition of the angry facial expression. It is worth noting that people with anxious attachment use social interactions as a means to fulfil their need for the subjective safety [6, 7], which is why ignoring aggressive facial expressions is considered reasonable, for it reduces social anxiety and may prevent conflicts. In a naturalistic environment, healthy people also interpret angry facial expressions as an aversive stimulus [28]. However, it is possible that only with a combination of depression and anxious attachment the need to avoid aggression becomes strong enough to justify perceptual defense against this category of stimuli.

The core result of the study (a negative correlation of the accuracy of angry facial expression recognition and anxious attachment score specific for the depressed group) should be considered with respect to the limited sample of our study. A bootstrap of the correlation analysis involving 1,000 iterations showed a 95% confidence interval for the correlation coefficient in the group of depressed patients $-0.86 \leq \rho \leq -0.27$. A part of the confidence interval lies in the area of insignificant correlations; however, the entire interval is characterized by negative values. The absolute magnitude of the correlation lies in the range from weak to high / very high [25]. A similar analysis in the group of healthy participants revealed a confidence interval of $-0.27 \leq \rho \leq 0.68$. The fact that the confidence intervals for this correlation do not overlap may be a finding supporting the association specificity.

A positive correlation between the anxious attachment score and the accuracy of sad facial expression recognition also contradicted the data in the group of healthy volunteers. Anxious attachment was associated with a need for close relationships along with a doubt in object accessibility that led to a greater interest in emotional expression recognition [7]. Individuals with the anxious attachment style were less likely to suppress their reactions to sad faces [6]. However, fMRI data suggested that people with this attachment style demonstrated increased cerebral responses to happy and not to sad facial expressions [7]. Responses to sad mimics and, probably, the accuracy of sadness recognition may reflect readiness to interact with a person who is experiencing emotional discomfort and needs support [2].

Thus, the anxious attachment style in depression shapes imperative social needs and requires greater tolerance to a partner's state and readiness to share his or her discomfort. The core characteristics of the sad emotion in depression may also have some influence, making the perception of others' sadness a less negative stimulus. The two core findings of the study discussed above are in full compliance with J. Gray's motivational theory in its current edition (see [29] for theory evolution and its current provisions and [30] for its link with frontal cortical asymmetry and a special role of anger). In the depressed participants, this theory predicts a deficit in the behavioral activation system of the right hemisphere which is responsible for actions aimed at fulfilling person's needs. On the contrary, the fight–flight–freeze system (prevention of external threats) and the behavioral inhibition system of the left hemisphere (behavioral regulation in a motivational conflict) are expected to have increased activity.

Anxious attachment is related to a similar imbalance with a focus on the behavioral inhibition system, and the same is typical of proneness to rumination. Observation of another person's angry facial expression may cause either an angry response or a reaction of fear and anxiety, or both. Thus, any combination of the three motivational systems within a single response is possible, which emphasizes individual differences and creates a favorable environment for studying correlations between the attachment style and the accuracy of facial expression recognition.

Recognition of sadness which is characterized by lesser involvement of the behavioral activation system complies with the aforementioned depression features. However, full compliance of the data with this model would require additional positive correlations of the anxious attachment score with the accuracy of recognizing disgust, fear, confusion, and possibly surprise, and its negative correlations with the correctness of recognizing happiness. The influence of the insufficient sample size in this case is questionable, for the magnitude of ρ in the majority of mentioned correlations is close to zero, while the direction of the relationship in confusion is opposite to an expected one ($\rho = -0.35$). To test a hypothesis that associations of the attachment style and rumination scores with the accuracy of angry and possibly sad facial expression recognition demonstrate an individual case showing the influence of J. Gray's systems on emotional variables, further studies are required with larger samples and inclusion of relevant scales to measure J. Gray's system activity in moderation analysis and in analysis of causal relationships between the variables as regressors / factors.

Our data on the declining ability to detect emotionsof happiness and confusion with age may be of interest for the research on emotional intelligence and empathy in healthy people. It is reflected in a tendency to treat expressions that are hard to recognize as happy ones. It may be explained by a decreased interest in the emotional state of other people (similar to the data on sadness in [2]). Our results are in contrast with the previously published findings [31] stating that recognition of facial expressions of fear and anger worsens with age, while the accuracy of recognizing happiness does not change.

CONCLUSION

The study allowed to obtain novel data on the associations between the attachment style and the accuracy of facial expression recognition by healthy and slightly depressed people. In addition to previously published results on the predominant role of avoidant attachment in the development of atypical reactions to angry facial expressions in healthy participants, a negative correlation between the prominence of anxious attachment and the accuracy of angry facial expression recognition, typical of depression, was identified. The findings point at the need to consider the attachment style as a factor influencing disruptions of emotional intelligence and empathy in depression.

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Authors contribution

Melnikov M.Ye. – conception and design, drafting of the manuscript. Shtark M.B. – conception and design. Bezmaternykh D.D. – development of software for image demonstration and answer collection, collection of corresponding data. Kozlova L.I. – collection of data for psychological testing. Natarova K.A. – selection of patients according to the clinical criteria. All authors participated in discussion on the draft of the manuscript and approved the final version of the manuscript for publication.

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Change in physical performance indicators of the progenies of rats with experimental preeclampsia in early and late pharmacological correction by GABA derivatives

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ABSTRACT

The aim of the study was to assess the changes in physical performance parameters in the progeny of rats with experimental preeclampsia (EP) undergoing early and late pharmacological treatment with gamma-aminobutyric acid (GABA) derivatives.

Materials and methods. The experiments were carried out on the progeny of rats aged 3 (n = 358), 18 (n = 288), and 25 (n = 138) months, born to white outbred female rats with EP modeled by replacing drinking water with 1.8% sodium chloride from the 1st to 21st day of gestation. At the first stage, physical performance of 3- and 18-month-old progeny of female rats with EP after early pharmacological treatment (from hte 40th to hte 70th day of life) with GABA derivatives, such as succicard (22 mg / kg), salifen (7.5 mg / kg), phenibut (25 mg / kg) and a comparator drug, pantogam (50 mg) was studied. At the second stage, succicard (44 mg / kg), salifen (15 mg / kg), phenibut (50 mg / kg) or pantogam (100 mg) had been intragastrically administered in the progeny of rats with EP for 30 days (from the 24th to the 25th month of life). The horizontal rope walking test (HRWT), Rotarod performance test, and forced swim test with weight load (FSTwWL) were used in the study.

Results. The HRWT, Rotarod performance rest, and FSTwWL showed a decrease in muscle strength, coordination and motor activity, and aerobic and anaerobic endurance in rats with EP aged 3, 18, and 25 months as compared to the values in the animals born from intact rats. Succicard, a GABA-derivative, and pantogam, a comparator drug, were effective both in early and late pharmacological interventions, whereas salifen and phenibut were effective only when administered during puberty. As the offspring of EP rats were aging, their muscle strength, coordination, and motor activity were decreasing, while their aerobic and anaerobic endurance was increasing.

Conclusion. Physical performance in the progeny of rats with induced EP aged 3, 18, and 25 months tended to decrease. Pharmacological treatment with GABA derivatives in the adolescent period attenuated EP consequences. When administered during puberty, only succicard and the comparator drug pantogam, had a therapeutic effect. This fact provides evidence that a succicard-based drug can be developed for preventive management of preeclampsia consequences.

Key words: experimental preeclampsia, the progeny of rats, GABA derivatives, physical performance.

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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Показатели физической работоспособности у потомства крыс с экспериментальной преэклампсией при ранней и поздней фармакологической коррекции производными ГАМК

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РЕЗЮМЕ

Цель исследования – оценить изменения показателей физической работоспособности у потомства крыс с экспериментальной преэклампсией (ЭП) при ранней и поздней фармакологической коррекции производными гамма-аминомасляной кислоты (ГАМК).

Материалы и методы. Эксперименты выполнены на потомстве в возрасте 3 мес (n = 358), 18 (n = 288) и 25 мес (n = 138), рожденном белыми беспородными самками крыс с физиологической беременностью и ЭП, моделированной заменой питьевой воды 1,8%-м раствором натрия хлорида с 1-х по 21-е сут гестации. На первом этапе изучали физическую работоспособность 3- и 18-месячного потомства самок крыс с ЭП после ранней фармакологической коррекции (с 40-х по 70-е сут жизни) производными ГАМК сукцикардом (22 мг/кг), салифеном (7,5 мг/кг), фенибутом (25 мг/кг) и препаратом сравнения пантогамом (50 мг). На втором этапе потомству, рожденному крысами с ЭП, в течение 30 сут (с 24-го по 25-й мес жизни) вводили в желудок сукцикард (44 мг/кг), салифен (15 мг/кг), фенибут (50 мг/кг) или пантогам (100 мг). В исследовании использовали тесты «Удержание тела на горизонтальном веревочном канате» (УТнаГВК), «Ротарод» и «Вынужденное плавание с грузом» (ВПсГ).

Результаты. У потомства самок крыс с ЭП в возрасте 3, 18 и 25 мес уменьшились мышечная сила, координационно-двигательная активность и аэробно-анаэробная выносливость в тестах УТнаГВК, «Ротарод» и ВПсГ по сравнению с показателями у животных, рожденных интактными крысами. Производное ГАМК сукцикард и препарат сравнения «Пантогам» были эффективны как при ранней, так и при поздней фармакологической коррекции, салифен и фенибут – только при введении в пубертатном периоде. С возрастом у потомства крыс с ЭП снижались мышечная сила и координационно-двигательная активность, но аэробно-анаэробная выносливость увеличивалась.

Заключение. У потомства крыс, подвергнутых ЭП, в возрасте 3, 18 и 25 мес ухудшалась физическая работоспособность. Фармакологическая коррекция производными ГАМК в адолесцентном периоде ослабляла последствия ЭП. При введении веществ в пубертатном периоде лечебное действие оказывали только сукцикард и препарат сравнения «Пантогам». Это предполагает возможность создания на основе сукцикарда препарата для превентивной коррекции последствий преэклампсии.

Ключевые слова: экспериментальная преэклампсия, потомство крыс, производные ГАМК, физическая работоспособность.

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

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INTRODUCTION

Preeclampsia is a severe pregnancy-related multisystem pathology, which increases the risk of development unfavourable consequences in the offspring both at early stages of life and in the long-term perspective. Impaired formation of the fetoplacental complex and endothelial dysfunction typical of preeclampsia contribute to circulatory deterioration in the "mother-placenta-fetus system", which results in the insufficient nutrient delivery to the fetus and development of chronic hypoxia [1]. At the critical stages of prenatal development, preeclampsia is associated with pathological changes in the embryo's organs and tissues. The progeny born to mothers with this pregnancy complication show physical developmental delays, higher risks of disease development in a long-term perspective and decreased physical performance [2].

To date, no medications with proved efficacy in correcting post-hypoxic disorders occurring at different ontogenesis stages in children delivered by women with preeclampsia, have been developed, nor have strategies of treating the complications of this severe pathology been designed. The search for safe and effective agents to manage the complications of preeclampsia is high on the agenda of pediatric and therapeutic practice.

Earlier studies have demonstrated that the derivatives of gamma-aminobutyric acid (GABA) have an endothelium-, neuro-, and cardioprotective action, demonstrate antihypoxic and antioxidant effects, and enhance the physical work capacity in rats [3–5]. These findings suggest that GABA derivatives can be used to manage the preeclampsia consequences in the offspring.

The aim of the study was to assess the physical performance parameters in the progeny of rats with experimental preeclampsia (EP) undergoing early (from the 40th to the 70th day of life) and late (from the 24th to the 25th month of life) pharmacological treatment with GABA derivatives, such as succicard, salifen, phenibut, and the comparator drug pantogam.

MATERIALS AND METHODS

The experiments were conducted on the offspring of white outbred rats with physiological pregnancy and EP modeled by replacing drinking water with 1.8% sodium chloride from the 1st to 21st days of gestation at the age of 3 *(n* = 358), 18 (*n* = 288), and 25 (*n* = 138) months [3]. The animals were obtained from the Rappolovo Breeding Station (Leningrad Region, Russia). The animals were kept and cared for in the vivarium of Volgograd State Medical University in accordance with the Principles of the Good Laboratory Practice of the National Standard of the Russian Federation GOST P-33044-2014, and the international guidelines of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (The European Convention, 1986). The study also complied with the provisions of the Order of the Ministry of Health of RF No. 199n of 01.04.2016 "On Approving Laboratory Practice Regulations" and the directive 2010/63/EU providing the European Union legislation for the Protection of Animals Used for Scientific Purposes of 22.09.2010. The protocol of the experimental study was approved by Research Ethics Review Board of Volgograd region (Protocol No. 2044-2017 of 25.12.2017).

The animals received GABA derivatives, such as succicard (the composition of 4-phenylpiracetam and succinic acid in the ratio 2:1), phenibut (γ-amino-β-phenylbutyric acid) and salifen (the composition of phenibutr and salicylic acid in the ratio 2:1 ratio). All the substances were synthesized at the Department of Organic Chemistry of Herzen State Pedagogical University. Pantogam served as a comparator drug (hopantenic acid, PIK-PHARMA PRO LTD, Russia; syrup, 100 mg / ml).

The rats' offspring were separated from their mothers on the 39th day after birth. The study involved two stages. At the first stage, the animals were divided into groups: 1, 2 – positive controls – male rats (*n* = 30) and female rats (*n* = 29) delivered by healthy rats and receiving distilled water; 3, 4 – negative controls – male rats (*n* = 30) and female rats (*n* = 30) delivered by the rats with EP and receiving distilled water; 5, 6, 7, 8, 9, 10, 11, 12 – experimental groups – male and female rats (30 animals of each gender) delivered by the rats with EP and receiving the following GABA derivatives: succicard at a dose of 22 mg / kg, phenibut at a dose of 25 mg / kg, salifen at a dose of 7.5 mg / kg or pantogam at a dose of 50 mg / kg. GABA derivatives, pantogam, and distilled water were administered intragastrically once a day the from 40th to

the 70th day of life. The dosage of the agents corresponded to half doses administered in adult rats. These doses of the substances demonstrated the highest pharmacological activity in earlier experiments [3, 4]. Physical performance was explored in rat pups aged 3 months using the horizontal rope walking test (HRWT), when the rats were suspended on a taut horizontal rope grasping it with their forepaws [6], Rotarod performance test [6], and forced swim test with weight load (FSTwWL) [7]. The same tests were employed to study 18-month-old male and female rats from the positive control groups (n = 25 and n = 23), negative control groups (n = 28 and n = 25), and experimental groups 5 (n = 24), 6 (n = 27), 7 (n = 20), 8 (n = 21), 9 (n = 23), 10 (n = 24), 11 (n = 24), and 12 (n = 24).

At the second stage, distilled water was intragastrically administered in male and female rats from the groups of positive (n = 11 and n = 11) and negative control (n = 15 and n = 12). At the same time, males and females in the experimental groups 5 (n = 16) and 6 (n = 9) received succicard at a dose of 44 mg / kg, rats from the experimental groups 7 (n = 11); and 8 (n = 14) received salifen at a dose of 15 mg / kg, animals from the groups 9 (n = 14); and 10 (n = 12) received phenibut at a dose of 50 mg / kg; and groups 11 (n = 7) and 12 (n = 6) received pantogam at a dose of 100 mg. The HRWT, Rotarod performance test, and FSTwWL were used to assess physical performance of the rats at the age of 25 months. The agents were administered at doses which were effective for adult rats [3, 4].

The findings were statistically processed bu STATISTICA v.12.5 software, license number 133-190-095 (StatSoft Inc., USA), using the Mann – Whitney U test, Student's t-test to compare paired samples, Newman – Keuls test, Kruskal – Wallis test with Dunnett's test for multiple comparisons, and Shapiro – Wilk test to assess the samples for normality of distribution. The differences were considered statistically significant at $p < 0.05$. The data are presented in the form $M \pm m$, where M is the mean and m is the error of the mean.

RESULTS

The execution time of HRWT, Rotarod performance test, and FST-wWL in the negative control offspring aged 3, 18, and 25 months was significantly shorter than in the animals delivered by healthy rats. This finding suggests decreased muscle strength, lower balance and motor coordination capacities, and reduced aerobic and anaerobic endurance both at early and late stages of ontogenesis (Table 1, Table 2, Figure).

When executing the HRWT test, 3-month-old offspring receiving GABA derivatives and the comparator drug pantogam, demonstrated a significantly longer time of hanging on a horizontal rope than the rats from the negative control group. At the age of 18 months, the suspension time increased in the male rats receiving succicard and pantogam and female rats receiving salifen and phenibut. At the age of 25 months, the male rats, to whom succicard, phenibut, and pantogam were administered, tended to hang on a horizontal rope longer. The test execution time for the offspring of all groups aged 18 and 25 months was significantly shorter than in the 3-month-old animals. 25-month-old rats showed a shorter suspension time as compared to 18-month-old-rats. This tendency was observed in the male and female rats delivered by rats with EP and females receiving salifen and phenibut (table 1).

Table 1

Dynamics of the muscle strength changes in the offspring of rats with experimental preeclampsia undergoing early and late pharmacological treatment with GABA derivatives ($M \pm m$) during the horizontal rope test

Groups of animals	Gender of rats	Test execution time, s		
		3 months	18 months	25 months
Positive control	Males	50.27 ± 1.58	10.08 ± 0.83 &	7.60 ± 0.91 &
	Females	62.03 ± 1.80	6.83 ± 0.73 &	6.82 ± 0.87 &
Negative control	Males	27.31 ± 1.61 *	6.46 ± 0.64 $&	3.80 ± 0.31 $&>
	Females	37.07 ± 1.99 *	6.33 ± 0.45 &	4.50 ± 0.31 *&<
Progeny of rats with experimental preeclampsia receiving succicard	Males	42.07 ± 1.66 #	8.50 ± 0.69 #&	8.33 ± 0.62 ^&
	Females	57.09 ± 1.77 #	7.19 ± 0.53 &	6.43 ± 0.53 &
Progeny of rats with experimental preeclampsia receiving salifen	Males	41.31 ± 1.35 #	6.25 ± 0.69 &	5.55 ± 0.55 &
	Females	56.37 ± 1.62 #	11.57 ± 0.85 #&	5.71 ± 0.60 &<
Progeny of rats with experimental preeclampsia receiving phenibut	Males	43.55 ± 1.19 #	6.39 ± 0.61 &	6.38 ± 0.43 ^&
	Females	52.13 ± 1.55 #	9.61 ± 0.74 #&	6.00 ± 0.51 &<

Table 1 (continued)

Groups of animals	Gender of rats	Test execution time, s		
		3 months	18 months	25 months
Progeny of rats with experimental preeclampsia receiving pantogam	Males	34.48 ± 1.60 #	8.54 ± 0.68 #&	6.57 ± 0.75 ^&
	Females	51.38 ± 1.49 #	6.96 ± 0.48 @	5.83 ± 0.48 &

Note. The differences are statistically significant ($p < 0.05$) compared with the positive control group: $ – by the Mann – Whitney test, * – by the Student's t-test; compared with the negative control group: ^ – by the Kruskal – Wallis test with the Dunnett's (post hoc) test, # – by the Newman – Keuls test; compared to offspring at the age of 3 months: @ – by the Mann – Whitney test, & – by the Student's t-test; compared with offspring at the age of 18 months: > – by the Mann – Whitney test, < – by the Student's t-test.

The assessment of balance and coordination capacities by the Rotarod performance test demonstrated that 3-month-old male rats receiving salifen and phenibut stayed on the rotating rod significantly longer than the negative control animals. At the age of 18 months, males receiving succicard, salifen, and pantogam and females receiving pantogam managed to stay longer; at the age of 25 months, the length of time the animals stayed on the rod increased in males receiving succicard and pantogam and females receiving succicard.

The test execution time in the offspring aged 18 and 25 months was shorter than in 3-month-old rats (Figure).

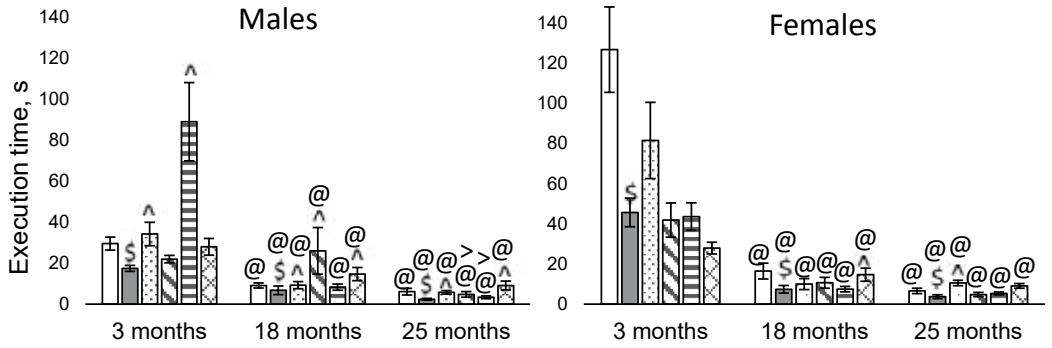

Fig. 1. Dynamics of the coordination and motor activity changes in the offspring of rats with experimental preeclampsia undergoing early and late pharmacological treatment with GABA derivatives ($M \pm m$) during the Rotarod performance test:

The differences are statistically significant ($p < 0.05$) compared with the positive control group: $ – by the Mann – Whitney test, * – by the Student's t-test; compared with the negative control group: ^ – by the Kruskal – Wallis test with the Dunnett's (post hoc) test, # – by the Newman – Keuls test; compared with offspring at the age of 3 months: @ – by the Mann – Whitney test, & – by the Student's t-test; compared with offspring at the age of 18 months: > – by the Mann – Whitney test

When undergoing the FSTwWL test, 3-month-old males rats receiving succicard, salifen, and pantogam, alongside the females receiving succicard, salifen, and phenibut were able to swim much longer compared to the animals of the negative control group. At the age of 18 months, the time of swimming increased in males receiving all the GABA derivatives under study and pantogam, as well as in females receiving salifen; at the age of 25 months, the time of swimming was longer in females receiving succicard and pantogam. In all groups of rats aged 18 and 25 months, the test execution time increased as compared to 3-month-old offspring.

The swimming time of rats aged 25 months showed a statistically significant decrease only in negative control females receiving salifen compared to 18-month-old rats (Table 2).

Table 2

Dynamics of aerobic-anaerobic endurance changes in the offspring of rats with experimental preeclampsia undergoing early and late pharmacological treatment with GABA derivatives ($M \pm m$) during the forced swim test with weight load

Groups of animals	Gender of rats	Test execution time, s		
		3 months	18 months	25 months
Positive control	Males	129.72 ± 2.97	155.88 ± 8.56 &	166.50 ± 7.20 @
	Females	153.38 ± 3.78	189.61 ± 11.48 &	193.56 ± 9.30 &

Table 2 (continued)

Groups of animals	Gender of rats	Test execution time, s		
		3 months	18 months	25 months
Negative control	Males	92.23 ± 2.78 $	125.74 ± 5.02 * &	134.53 ± 4.95 *&
	Females	99.67 ± 2.79 *	148.71 ± 4.17 * &	116.67 ± 4.90 *&<
Progeny of rats with experimental preeclampsia receiving succicard	Males	108.52 ± 3.41 ^	145.71 ± 5.77 # &	154.47 ± 6.40 &
	Females	111.93 ± 2.98 #	166.33 ± 9.48 &	173.00 ± 12.08 #&
Progeny of rats with experimental preeclampsia receiving salifen	Males	127.40 ± 7.78 ^	150.05 ± 8.58 #	147.40 ± 8.83
	Females	124.10 ± 5.26 #	184.38 ± 8.88 #&	148.42 ± 8.28 &<
Progeny of rats with experimental preeclampsia receiving phenibut	Males	99.28 ± 3.26	164.96 ± 6.81 #&	155.50 ± 6.28 &
	Females	121.53 ± 3.23 #	161.74 ± 9.66 @	153.92 ± 9.07 &
Progeny of rats with experimental preeclampsia receiving pantogam	Males	109.84 ± 3.24 ^	156.08 ± 8.30 #&	135.71 ± 7.97 &
	Females	99.38 ± 2.32	169.43 ± 9.52 &	174.20 ± 11.06 #&

Note. All symbols are similar to those in Figure, except: & – the differences are statistically significant ($p < 0.05$) by the Student's t-test compared with offspring at the age of 3 months, < – the differences are statistically significant by the Student's t-test compared with offspring at the age of 18 months.

DISCUSSION

Preeclampsia results in impaired nutrient and oxygen delivery to the developing embryo. Metabolic changes are associated with acidosis and oxidative stress, which contribute to the damage of cell structures and organ and tissue enzymes and may lead to their dysfunction in the postnatal ontogenesis [2]. These factors increase the likelihood of the development of nervous, cardiovascular, respiratory, and other pathologies [8–11] with decreased physical performance at different life stages.

The findings of the conducted experiments have demonstrated that the physical work capacity of the progeny of rats with EP is lower compared to the animals delivered by healthy rats both at early stages (3 months) and later stages (18 and 25 months) of ontogenesis. This was proved by the differences in the execution time of the HRWT, Rotarod performance test, and FSTwWL.

As is known, GABA derivatives have a positive effect on the physical performance parameters and adaptability to physical exertion and increase the physical endurance of rats performing forced dynamic and static activities [5].

In our experiments, early (from the 40th to the 70th day of life) and late (from the 24th to the 25th month of life) pharmacological treatment with succicard, a GABA derivative, resulted in enhanced muscle strength, coordination and motor activity, aerobic and anaerobic endurance as demonstrated by the HRWT, Rotarod performance test, and FSTwWL in 3-, 18- and 25-month-old progeny, respectively, as opposed to the values in the negative control group. Muscle strength and aerobic and anaerobic endurance were significantly higher in 3- and 18-month-old rats which received salifen and phenibut from the 40th to 70th day of life than in the animals delivered by female rats with EP. Late pharmacological treatment with these GABA derivatives did not have a significant effect on the physical performance of rats with EP. The effect of pantogam, the comparator drug, was similar to that of succicard.

Therefore, succicard and the comparator drug pantogam were effective both in early (from the 40th to the 70th day of life) and late (from the 24th to 25th month of life) pharmacological correction, salifen and phenibut were effective only when administered in the puberty period.

The therapeutic effect of GABA derivatives is conditioned by their polytropic pharmacological action. The agents of this group have endothelium-, neuro-, and cardioprotective, as well as antihypoxic and antioxidant effects [3, 4]. Moreover, GABA derivatives have an impact on glucose transport and utilization, increase ATP synthesis in hypoxia, help to overcome energy deficit in cells, and regulate muscle contractions [5, 12]. All these factors contribute to a rise in physical work capacity in the progeny of rats with EP.

In aging rats, there was a decrease in muscle strength, coordination, and motor activity, whereas their aerobic and anaerobic endurance increased. It is likely that the values obtained during the FSTwWL test were influenced by the fact that it was executed at the age of 6 and 12 months, which could be practice for rats [13].

Physical performance was significantly lower in 25- and 18-month-old negative control animals and rats receiving salifen and phenibut. This finding demonstrates the negative effect of EP on muscle

strength, balance and coordination capacities, and aerobic and anaerobic endurance and proves the inefficacy of late (from the 24th to 25th month of life) pharmacological correction of EP consequences using salifen and phenibut.

CONCLUSION

The physical performance of 3-, 18-, and 25-month-old animals delivered by rats with EP is decreased as compared to the progeny of healthy females. GABA derivatives, such as succicard, salifen and phenibut, reduce the negative impact of EP on the offspring undergoing early (from the 40th to the 70th day of life) pharmacological correction. This fact provides evidence that a succicard-based drug can be developed for preventive treatment of preeclampsia consequences in the progeny.

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Authors contribution

Muzyko E.A. – analysis and interpretation of the data, carrying out of the main stages of the experiments, drafting of the manuscript. Perfilova V.N. – analysis and interpretation of the data, critical revision for important intellectual content, final approval of the manuscript for publication. Suvorin K.V. – carrying out of the major stages of the experiments. Tyurenkov I.N. – conception and design of the study, critical revision for important intellectual content, final approval of the manuscript for publication.

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Prevalence of chronic bronchitis against a background of abdominal obesity in young people aged 25–44 in Novosibirsk

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ABSTRACT

Aim. To study the prevalence of chronic bronchitis (CB) against the background of abdominal obesity (AO) in young people aged 25–44 in Novosibirsk.

Materials and methods. A simultaneous population survey of 25–44 year-olds in Novosibirsk was carried out. The study included 906 people (414 men and 492 women). AO was registered with a waist circumference of more than 94 cm in men and more than 80 cm in women. CB was detected according to standardized epidemiological (questionnaire, pulmonary questionnaire) and functional (spirometry) criteria.

Results. The prevalence of AO and CB in the population was 42.9% and 8.7%, respectively; in men – 43.2% and 7.7%; in women – 42.7% and 9.6%. The prevalence of CB against the background of AO in the population was 1.95 times higher ($p = 0.003$) compared with individuals with CB without AO (OR = 2.08 [CI 1.302–3.333]). In women, the prevalence of CB against the background of AO was 2.15 times higher ($p = 0.005$) compared with women with CB without AO (OR = 2.35 [CI 1.267–4.359]). The prevalence of CB in smokers in the population was 1.65 times higher ($p = 0.015$) compared with non-smokers (OR = 1.72 [CI 1.081–2.739]). In male smokers, the prevalence of CB was 2.02 times higher ($p = 0.031$) compared with non-smoking men (OR = 2.15 [CI 1.020–4.514]). The multiple logistic regression analysis showed that in people aged 25–44, the presence of CB is associated with smoking [Exp(B) = 1.966, $p = 0.006$] and the presence of AO [Exp(B) = 2.091, $p = 0.003$].

Conclusion. Significant effects of AO and smoking on the development of CB in the population of 25–44 year-olds on the whole were revealed. At the same time, in men aged 25–44, the relative risk of developing CB is significantly affected by smoking, and in women aged 25–44, by the presence of AO.

Key words: prevalence, abdominal obesity, chronic bronchitis, population aged 25–44, smoking.

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

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Conformity with the principles of ethics. Informed consent was obtained from all participants for examination and processing of personal data. The study was approved by the local Ethics Committee at IIPM – Branch of IC&G SB RAS (Protocol No. 10 of 21.01.2014).

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Распространенность хронического бронхита на фоне абдоминального ожирения у молодых людей 25–44 лет г. Новосибирска

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РЕЗЮМЕ

Цель исследования – изучить распространенность хронического бронхита (ХБ) на фоне абдоминального ожирения (АО) у молодых людей 25–44 лет г. Новосибирска.

Материалы и методы. Проведено одномоментное популяционное обследование населения 25–44 лет г. Новосибирска. В исследование включены 414 мужчин и 492 женщины. АО регистрировали при окружности талии более 94 см у мужчин и более 80 см – у женщин. ХБ регистрировали согласно стандартизованным эпидемиологическим (опросник, пульмонологическая анкета) и функциональным (спирометрия) критериям.

Результаты. Распространенность АО и ХБ в популяции 42,9 и 8,7% соответственно; у мужчин – 43,2 и 7,7%; у женщин – 42,7 и 9,6%. Распространенность ХБ на фоне АО в популяции в 1,95 раза выше (p = 0,003) в сравнении с лицами с ХБ без АО (OR = 2,08 [CI 1,302–3,333]). У женщин распространенность ХБ на фоне АО в 2,15 раза выше (p = 0,005) в сравнении с женщинами с ХБ без АО (OR = 2,35 [CI 1,267–4,359]). Распространенность ХБ у курящих в популяции в 1,65 раза выше (p = 0,015) в сравнении с некурящими лицами (OR = 1,72 [CI 1,081–2,739]). У курящих мужчин распространенность ХБ в 2,02 раза выше (p = 0,031) в сравнении с некурящими мужчинами (OR = 2,15 [CI 1,020–4,514]). Проведенный многофакторный логистический регрессионный анализ показал, что у людей 25–44 лет наличие ХБ ассоциировано с курением [Exp(B) = 1,966; p = 0,006] и наличием АО [Exp(B) = 2,091; p = 0,003].

Заключение. Выявлены значимые влияния АО и курения на развитие ХБ в популяции 25–44 лет в целом. В то же время у мужчин 25–44 лет на относительный риск развития ХБ значимо влияет статус курения, а у женщин 25–44 лет – наличие АО.

Ключевые слова: распространенность, абдоминальное ожирение, хронический бронхит, популяция 25–44 лет, курение.

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

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Соответствие принципам этики. От всех лиц получено информированное согласие на обследование и обработку персональных данных. Исследование одобрено этическим комитетом НИИТПМ – филиал ИЦиГ СО РАН (протокол № 10 от 21.01.2014).

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INTRODUCTION

The prevalence of chronic bronchitis (CB) is high; therefore, the diagnosis and prevention of this pathology are some of the most pressing problems in pulmonology and the internal medicine. According to the official statistics of the Ministry of Health of the Russian Federation, the prevalence of "chronic and unspecified bronchitis and emphysema" is very high and in 2015 amounted to 1.5% in the adult population [1].

In the epidemiological part of the GARD study (Global Alliance Against Chronic Respiratory

Diseases), conducted in Russia, it was shown that coughing for 3 months was observed by 18.9% of respondents, coughing up mucus for at least 3 months was reported by 12.7% of patients, and the clinical picture corresponding to the diagnosis that of CB (the presence of two features at once) was present in 8.6% of participants (95% CI 7.9–9.3). It is important that 22.2% of the survey participants indicated a history of CB diagnosis (95% CI 21.2–23.2), which, according to the authors of the study, significantly exceeds the official statistics [2].

The prevalence of obesity, including abdominal obesity (AO), is also high [3, 4]. According to the ECVD-RF (Epidemiology of Cardiovascular Diseases in the regions of the Russian Federation) epidemiological study, the prevalence of AO in the Russian Federation in people aged 25–64 is 55% (61.8% among women and 44% among men), increasing with age both among men and women ($p < 0.0001$) [5].

In recent years, many studies have been conducted on the effect of AO on the development of cardiovascular and endocrine pathologies. Several studies have also been conducted on the associations between the development of CB and metabolic syndrome (MS). The clinical and functional features of the combined course of CB and AO are described, which, according to the authors, is a predictor of the development of chronic obstructive pulmonary disease (COPD) not only in smokers, but also in people who have never smoked [6]. It has been shown that for people with an increased waist circumference, the risk of developing COPD is 72% higher than for those who have a normal waist circumference [7].

The significant role of smoking in CB development is known and unconditional [8]. On the other hand, chronic inflammation plays an important role in the formation of the whole complex of pathological changes in CB. Abdominal obesity is also a pro-inflammatory condition that increases the risk of chronic diseases [9]. Adipose abdominal tissue produces a large number of cytokines and bioactive mediators, thus causing a proinflammatory state in people suffering from AO, which is also associated with an increased risk of developing bronchopulmonary pathology [10, 11].

Most studies in this area have been conducted with examination of persons older than 45 years. It seems relevant and in high demand to study the associations of abdominal obesity with CB in young people of working and child-bearing age. It is also relevant to obtain new data on the associations of abdominal obesity AO with CB in individuals living in Siberia, i.e. in extreme continental climate, where, according to international epidemiological studies, there is a high prevalence of risk factors for chronic noncommunicable diseases [12]. Therefore, the aim of the present research was to study the prevalence of CB against the background of AO in young people aged 25–44 in Novosibirsk.

MATERIALS AND METHODS

On the basis of IIPM – Branch of IC&G SB RAS, in 2013–2016, a simultaneous population screening was conducted for the population of 25–44 years old in the Oktyabrsky district of Novosibirsk (a typical district of the city) within the framework of the budgetary theme No. 0541-2014-0004 "Monitoring the health status and prevalence of risk factors for therapeutic diseases, their prognosis, and prevention in Siberia". The study was approved by the Ethics Committee of IIPM – Branch of IC&G SB RAS (Protocol No. 10 of 21.01.2014).

To select the sample, the base of the Territorial Fund of Compulsory Health Insurance in the Novosibirsk Region was used. The study included 906 people (average age of 36.5 ± 5.8 years), including 414 men (average age of 36.3 ± 5.8 years) and 492 women (average age of 36.7 ± 5.8 years). There were no age differences between the groups examined. Informed consent was obtained from all persons for examination and processing of personal data.

To identify abdominal obesity (AO), the criteria of the Russian Society of Cardiology (RSC, 2009), waist circumference (OT), were used. A waist circumference of more than 80 cm in women and more than 94 cm in men was considered positive for AO.

To detect respiratory symptoms, the WHO Respiratory Disease Questionnaire and the ECRHS (European Community Respiratory Health Survey) were used. According to the presence of cough in each year for 3 months or more (chronic cough), the respondents were divided into groups: 0 – no, 1 – yes. When studying the anamnesis, the answers to the question "Have you ever been told by a doctor that you have: 1 – chronic bronchitis, 2 – bronchial asthma, 3 – bronchiectasis, 4 – pneumosclerosis, 5 – pulmonary emphysema, 6 – COPD?" were taken into account.

The study of the external respiration function (ERF) by spirometry was carried out according to the recommendations for performing spirometry on the SpiroUSB Micro Medical Limited apparatus. Spirometry results were recorded and processed by the Spida 5 computer diagnostic program. Spirometry findings were interpreted based on the 2017 international recommendations "Spirometry for health care providers. Global Initiative for Chronic Obstructive Lung Disease (GOLD)".

Statistical processing of the results was performed using the SPSS package (v.17.0), including the creation of a database and statistical analysis. The data obtained in tables and text are presented for categorical (nominal) indicators as absolute and relative values – *n* (%). For quantitative variables, a normality test was carried out using the Kolmogorov – Smirnov test, the results are presented as the median and interquartile range – *Me* [25%; 75%], as well as *(M ± SD)*, where *M* is the arithmetic mean value, *SD* is the standard deviation. Feature associations were evaluated using linear regression analysis. The fractional difference in features was calculated using the Pearson's χ^2 test. Differences were considered statistically significant at $p < 0.05$.

RESULTS

In the population, the waist circumference was 85 [75.9; 96] cm. In men it was 1.18 times more ($p = 0.0001$) than in women (92 [84; 100] cm and 78 [71; 87] cm, respectively). The prevalence of AO in the population of 25–44 year-olds was 42.9%; it did not differ between men and women (Table 1).

Table 1

Prevalence of abdominal obesity in the studied population of people aged 25–44, *n* (%)

Index	Abdominal obesity		Total
	Absent	Present	
Men	235(56.8%)	179 (43.2 %)	414 (100%)
Women	282 (57.3%)	210 (42.7%)	492 (100%)
Population	517 (57.1%)	389 (42.9%)	906 (100%)

The obtained result is slightly different from the results of the ECVD-RF study, which describes the average data for the Russian Federation. The prevalence of AO at the age of 25–34 years in men was 23.3%, in women 27.2%. At the age of 35–44 in men, it was 41.4%, in women – 47.4%. The proportion of women with AO prevails [5]. In Novosibirsk, higher prevalence of AO in people aged 25–44 was found (42.9%), while no differences were identified between men and women, which confirms the data on higher prevalence of risk factors for cardiovascular diseases in Siberia [12].

The prevalence of CB in the population aged 25–44 was 8.7%; it did not significantly differ between men and women (Table 2).

Table 2

Prevalence of chronic bronchitis in the studied population of people aged 25–44, *n* (%)

Index	Chronic bronchitis		Total
	Absent	Present	
Men	382 (92.3%)	32 (7.7 %)	414 (100%)
Women	445 (90.4%)	47 (9.6%)	492 (100%)
Population	827 (91.3%)	79 (8.7%)	906 (100%)

The obtained result practically does not differ from the results of the epidemiological part of the GARD study, according to which 8.6% of patients (95% CI 7.9–9.3) aged from 18 to 80 years (more than 7,000 examined residents of the Russian Federation) were diagnosed with CB [2].

To study the associations between AO and CB, the ratio of signs was first analyzed (Table 3).

Table 3

Prevalence of chronic bronchitis depending on the presence of abdominal obesity in the studied population of people aged 25–44

Index	Abdominal obesity		Total
	Absent	Present	
No chronic bronchitis			
Both sexes, *n*, incl.:	485	342	827
– in the subgroup for CB, %	58.6	41.4	100
– in the subgroup for AO, %	93.8	87.9	91.3
– in the population, %	53.5	37.8	91.3
Men, *n*, incl.:	221	161	382
– in the subgroup for CB, %	57.9	42.1	100
– in the subgroup for AO, %	94.0	89.9	92.3
– in the population, %	53.4	38.9	92.3
Women, *n*, incl.:	264	181	445
– in the subgroup for CB, %	59.3	40.7	100
– in the subgroup for AO, %	93.6	86.2	90.4
– in the population, %	53.7	36.7	90.4
Have chronic bronchitis			
Both sexes, *n*, incl.:	32	47	79
– in the subgroup for CB, %	40.5	59.5	100
– in the subgroup for AO, %	6.2	12.1 *	8.7
– in the population, %	3.5	5.2	8.7
Men, *n*, incl.:	14	18	32
– in the subgroup for CB, %	43.8	56.2	100
– in the subgroup for AO, %	6.0	10.1	7.7
– in the population, %	3.4	4.3	7.7

Table 3 (continued)

Index	Abdominal obesity		Total
	Absent	Present	
Women, *n*, incl.:	18	29	47
– in the subgroup for CB, %	38.3	61.7	100
– in the subgroup for AO, %	6.4	13.8 **	9.6
– in the population, %	3.7	5.9	9.6
Entire population			
Number of persons, *n*, incl.:	517	389	906
– in the subgroup for CB, %	57.1	42.9	100
– in the subgroup for AO, %	100	100	100
– in the population, %	57.1	42.9	100

Note. CB – chronic bronchitis, AO – abdominal obesity.
* $p = 0.003$ in comparison with persons with CB without AO by 1.95 times, OR = 2.08 [CI 1.302–3.333]; ** $p = 0.005$ in comparison with women with CB without AO by 2.15 times, OR = 2.35 [CI 1.267–4.359].

It was revealed that the prevalence of CB against the background of AO in the population of people aged 25–44 was 1.95 times higher ($p = 0.003$) compared with individuals with CB without AO (OR = 2.08 [CI 1.302–3.333]). In women, the prevalence of CB against the background of AO was 2.15 times higher ($p = 0.005$) compared with women with CB but without AO (OR = 2.35 [CI 1.267–4.359]). In men, such a fact was not revealed. The data obtained indicate a significant effect of AO in young women on the risk of developing CB.

Since it is known that smoking is one of the significant risk factors for the development of CB, this study also analyzed the ratio of signs to study the association between smoking and CB in the young population (Table 4). The prevalence of CB in smokers in the population of people aged 25–44 was 1.65 times higher ($p = 0.015$) compared with non-smokers with CB (OR = 1.72 [CI 1.081–2.739]). In male smokers, the prevalence of CB was 2.02 times higher ($p = 0.031$) compared with non-smoking men (OR = 2.15 [CI 1.020–4.514]). In women, such a fact was not revealed. The findings indicate a significant effect of smoking in young men on the risk of developing CB.

In order to clarify the obtained results regarding the effect of AO on the development of CB in young people aged 25–44, a multiple logistic regression analysis was carried out, which showed that in people of both sexes, the presence of CB, regardless of other features, including age and sex, is associated with smoking [Exp(B) = 1.966, $p = 0.006$] and the presence of AO [Exp(B) = 2.091, $p = 0.003$].

Table 4

Prevalence of chronic bronchitis depending on smoking status in the studied population of people aged 25–44

Index	Smoking (S)		Total
	No	Yes	
No chronic bronchitis			
Both sexes, *n*, incl.:	547	280	827
– in the subgroup for CB, %	66.1	33.9	100
– in the subgroup for S, %	92.9	88.3	91.3
– in the population, %	60.4	30.9	91.3
Men, *n*, incl.:	215	167	382
– in the subgroup for CB, %	56.3	43.7	100
– in the subgroup for S, %	94.7	89.3	92.3
– in the population, %	51.9	40.4	92.3
Women, *n*, incl.:	332	113	445
– in the subgroup for CB, %	74.6	25.4	100
– in the subgroup for S, %	91.7	86.9	90.4
– in the population, %	67.5	23.0	90.4
Have chronic bronchitis			
Both sexes, *n*, incl.:	42	37	79
– in the subgroup for CB, %	53.2	46.8	100
– in the subgroup for S, %	**7.1**	**11.7** *	8.7
– in the population, %	4.6	4.1	8.7
Men, *n*, incl.:	12	20	32
– in the subgroup for CB, %	37.5	62.5	100
– in the subgroup for S, %	**5.3**	**10.7** **	7.7
– in the population, %	2.9	4.8	7.7
Women, *n*, incl.:	30	17	47
– in the subgroup for CB, %	63.8	36.2	100
– in the subgroup for S, %	8.3	13.1	9.6
– in the population, %	6.1	3.5	9.6
Entire population			
Number of persons, n, incl.:	589	317	906
– in the subgroup for CB, %	65.0	35.0	100
– in the subgroup for AO, %	100	100	100
– in the population, %	65.0	35.0	100

Note. CB – chronic bronchitis, AO – abdominal obesity, S – smoking.
* $p = 0.015$ in comparison with non-smokers with CB by 1.65 times, OR = 1.72 [CI 1.081–2.739]; ** $p = 0.031$ in comparison with non-smoking men with CB by 2.02 times, OR = 2.15 [CI 1.020–4.514]

DISCUSSION

When discussing the results obtained, it is important to note that, on the one hand, in the pathogenesis of chronic bronchitis, along with smoking, genetic factors, deficiency of α1-antitrypsin, surfactant, and transferrin, cold climate, frequent respiratory tract infections, and chronic inflammation play a significant role [8, 13, 14]. On the other hand, with AO, adipose abdominal tissue produces a large amount of cytokines and bioactive mediators, thus causing a proinflammatory condition in people suffering from AO, which may be associated (cause-effect pathogenetic relationship) with an increased risk of developing bronchopulmonary

pathology [10, 11]. In general, predicting the risk of developing chronic bronchitis in young people is an extremely urgent task [2, 15].

The patterns revealed in this study indicate a significant relationship between AO and smoking with the presence of chronic bronchitis in people of the young age group (25–44 years old) in Novosibirsk. At the same time, in men aged 25–44, the relative risk of developing chronic bronchitis is significantly influenced by the smoking status, and in women aged 25–44 – by the presence of abdominal obesity. Therefore, a possible cause-effect pathogenetic relationship between the increased content of inflammatory biomolecules in the body in AO, secreted by visceral adipocytes, and the chronic inflammatory process in the bronchi in CB can only be discussed in relation to women aged 25–44 in Novosibirsk.

CONCLUSION

Thus, taking into account the importance of the problem of abdominal obesity, this study obtained data indicating its significant role in the risk of developing chronic bronchitis in young women aged 25–44 years in Siberia.

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Authors contribution

Ragino Yu.I. – conception and design of the study, analysis and interpretation of the data, drafting and final approval of the manuscript for publication. Kurtukov E.A. – collection and analysis of the literature data. Denisova D.V. – design of the study and organization of data collection. Polonskaya Ya.V. – analysis of the data, drafting of the article. Shcherbakova L.V. – statistical processing of the data.

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Effect of malignant growth and chronic neurogenic pain on neurotrophin levels in rat brain

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Aim. To determine neurotrophin levels in the gray and white matter of the brain in rats with tumor growth associated with chronic neurogenic pain (CNP).

Materials and methods. The study included white outbred male rats (n = 74). In the main group, the CNP model was created (by bilateral sciatic nerve ligation), and after 45 days, M1 sarcoma was transplanted subcutaneously (n = 11) or into the subclavian vein (n = 11). Two comparison groups (n = 13 each) consisted of sham-operated animals with M1 sarcoma transplanted subcutaneously and intravenously, but without CNP. Control groups included animals with CNP and sham-operated animals. Rats were euthanized on the 21st day of carcinogenesis. The enzyme-linked immunosorbent assay (ELISA) was used to determine brain levels of brain-derived neurotrophic factor (BDNF) (R&D System, USA & Canada), nerve growth factor (β-NGF), neurotrophin-3 (NT-3), and neurotrophin-4/5 (NT-4) (RayBiotech, USA).

Results. CNP caused an increase in β-NGF levels in the cortex and white matter and a rise in BDNF levels only in white matter of the rat brain. Chronic pain stimulated M1 sarcoma growth in both subcutaneous and intravenous transplantation. The dynamics of neurotrophin levels in brain structures differed depending on the tumor site.

Conclusion. The results demonstrated that in both normal peripheral tumor growth and in tumor growth against the background of CNP, changes in neurotrophin levels in the brain of experimental animals can reflect the body reaction to chronic pain and stress caused by peripheral tumor growth.

Key words: M1 sarcoma, chronic neurogenic pain, brain, neurotrophins, nerve growth factor, brain-derived neurotrophic factor.

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

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Влияние злокачественного роста и хронической нейрогенной боли на уровень нейротрофинов в мозге крыс

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РЕЗЮМЕ

Цель – изучить содержание нейротрофинов в сером и белом веществе головного мозга крыс при росте опухоли, сопряженном с хронической нейрогенной болью (ХНБ).

Материалы и методы. Работа выполнена на самцах белых беспородных крыс (n = 74). В основной группе животным моделировали состояние ХНБ (путем двусторонней перевязки седалищных нервов) и через 45 сут перевивали саркому М1 подкожно (n = 11) и в подключичную вену (n = 11). Две группы сравнения (в каждой по n = 13) – ложно оперированные животные с перевивкой саркомы М1 подкожно и внутривенно, но без ХНБ. Контрольные группы – животные с ХНБ и ложно оперированные животные. Забой производили на 21-е сут канцерогенеза. Методом иммуноферментного анализа в головном мозге определяли содержание нейротрофического фактора мозга (BDNF) (R&D System, США, Канада), фактора роста нервов (β-NGF), нейротрофина-3, нейротрофина 4/5 (RayBiotech, США).

Результаты. Показано, что ХНБ вызывает повышение уровня β-NGF в коре и белом веществе и BDNF только в белом веществе головного мозга крыс. Обнаружено, что хроническая боль стимулирует рост саркомы М1 в случае подкожной и внутривенной перевивки. При этом динамика уровня нейротрофинов в структурах мозга была различна в зависимости от локализации опухолевого роста.

Заключение. Полученные результаты свидетельствуют о том, что как при обычном росте опухоли на периферии, так и при росте опухоли на фоне состояния ХНБ изменение уровня нейротрофинов в мозге экспериментальных животных может быть отражением реакции организма на хроническую боль и стресс, сопровождающий рост опухоли на периферии.

Ключевые слова: саркома М1, хроническая нейрогенная боль, головной мозг, нейротрофины, фактор роста нервов, нейротрофический фактор мозга.

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

High prevalence and severity of chronic pain syndromes have caused significant intensification of fundamental and clinical research [1]. Nerve damage was discovered to result in complex molecular and biochemical changes in primary afferents, dorsal horn contours (neurons and especially microglia), as well as at higher levels of neuraxis [2]. The participation of neurotrophins in the processes associated with neuronal injury, chronic pain, and allodynia was revealed. The brain-derived neurotrophic factor (BDNF) has neuroprotective and growth-promoting effects on various populations of neurons after injury. However, data on the BDNF effect on pain and allodynia are contradictory [3]. The role of the nerve growth factor (β-NGF) in regulating synthesis of neurotransmitters and neu-

ropeptides of sympathetic and sensory nerve cells [4] and regeneration of primary nociceptive sensory pathways [5] was shown.

Other neurotrophins with β-NGF expression alter the lineages of primary sensory pathways *in vivo* [6]. Neurotrophin (NT-3) is currently being investigated in clinical trials for the treatment of peripheral neuropathies, which are often associated with chronic pain and allodynia [7]. Recently, neurotrophins and their Trk receptors have been found to be highly active in a variety of cancers, including breast, lung, rectal, pancreatic, prostate and liver cancer, myeloma, and lymphoid tumors [8].

In our previous studies, a violation of the mediator status in the mice brain under the influence of chronic neurogenic pain (CNP) and stimulation of malignant growth in the lungs of rats by chronic pain was demonstrated [9, 10]. The aim of this research was to study the content of neurotrophins in the gray and white matter of the brain of rats in a malignant process associated with chronic neurogenic pain.

MATERIALS AND METHODS

The work was carried out on 74 white outbred male rats weighing 180–220 g, which were bred in the vivarium of Rostov Research Institute of Oncology. The animal work was performed in accordance with the rules of the "European Convention for the Protection of Animals Used in Experiments" (Directive 86/609/EEC) and Order of the Ministry of Healthcare of the Russian Federation No. 267 of 19.06.03 "On Approval of Laboratory Practice Rules". The study was approved by the Bioethics Committee of Rostov Research Institute of Oncology.

In the experimental group, the animals were inoculated with a malignant tumor in the presence of CNP. The animals were anesthetized with Xylazine (Xila drug) at a dose of 0.05 ml / kg of body weight, and after 10 minutes – by Zoletil50 at a dose of 10 mg / 100 g of body weight. Then the CNP model was reproduced: the sciatic nerves were ligated on both sides, and the wounds were sutured. 45 days [10] after the CNP reproduction, 11 animals were subcutaneously (s/c) inoculated with M1 sarcoma according to the standard method, 11 animals were injected in the subclavian vein (i/v) with 0.3 ml of the M1sarcoma cell suspension in saline diluted as (1×10^{6}/l).

The comparison groups ($n = 13$ in each case) included sham-operated animals with inoculation of M1 sarcoma in the same area and at the same dose and volume as in the main groups, but without reproducing the CNP model.

The control groups consisted of 13 animals with reproduced CNP and 13 sham-operated animals, which were decapitated at the same time as the rats of the main and the comparison groups (on the 21st day of carcinogenesis).

After decapitation, the brain was quickly removed, the gray and white matter were isolated on ice and used for the preparation of 10% homogenates in 0.1 M potassium phosphate buffer of pH 7.4, containing 0.1% Tween-20 and 1% BSA. The brain neurotrophic factor (BDNF), as well as the nerve growth factor (β-NGF), neurotrophin-3 (NT-3), and neurotrophin-4/5 (NT-4) (RayBiotech, USA) were determined by the ELISA method (R&D System, USA & Canada).

The results were statistically processed using the Statistica 10.0 software. All results were checked for compliance with the law of normal distribution (Shapiro – Wilk test). Data are presented as an arithmetic mean ± standard error of the mean ($M \pm \sigma$). The comparison of quantitative data in independent samples was carried out using the Kruskal – Wallis test; further a posteriori comparisons were performed using the Mann – Whitney test with an adjustment for the significance level.

RESULTS

The average survival of rats with subcutaneous injection of M1sarcoma was 79.2 ± 9.3 days, and with subcutaneous injection of M1 in the presence of CNP – 80 ± 11.8 days. The average tumor volume is shown in Table 1.

Table 1

The effect of chronic neurogenic pain on malignant process reproduction in the subcutaneous fatty tissue of male rats

Parameter	M1 s/c	CNP + M1 s/c
Dead rats from the total number of animals in the group, %	100	100
Tumor foci volume, cm^3, M ± σ	99.6 ± 5.2	145.5 ± 7.1[1]

[1] statistically significant in relation to the tumor volume with subcutaneous inoculation without chronic neurogenic pain ($p < 0.05$).

The average survival after intravenous administration of tumor suspension was 87 days, the maximum one was 128 days. Tumor foci in the lungs

in the presence of CNP appeared in almost all rats; they developed and resulted in the death of the animals (Table 2). In the group of animals with intravenous administration without CNP, one rat died. However, tumor nodes were not found in its lungs; the survival was 36 days longer than the average survival in the main group of animals with CNP.

Table 2

The effect of chronic neurogenic pain on malignant process reproduction in the lungs of male rats

Parameter	M1 i/v	Pain + M1 i/v
Dead rats from the total number of animals in the group, %	17	86
Presence of tumor foci in the lungs, volume, cm^3, $M \pm \sigma$	–	86 55.44 ± 6.2

The presented results unambiguously indicate that CNP not only stimulates malignant tumor growth, but also changes its biological aggressiveness, allowing it to develop in orthotopic inoculation. In this situation, it was interesting to determine the role of neurotrophins in the animals' brain in the manifestation of the altered aggressiveness of the neoplasm.

First of all, the CNP creation in rats was found to result in a change in some neurotrophins in the gray and white matter of animals without tumors (Table 3). Thus, the β-NGF levels increased by 1.5 times and 1.7 times, respectively. The BDNF content increased only in the white matter of the rat brain, by 1.6 times. Statistically significant changes in the content of NT-3 and NT-4 under the influence of CNP were not found.

Next, the content of neurotrophins in the brain of rats with traditional subcutaneous inoculation of M1 sarcoma, in an independent variant and in the presence of CNP was studied. When the tumor volume reached 99.6 ± 5.2 cm^3 (pre-terminal period of life), the level of β-NGF increased by 2.9 times relative to sham operated animals only in the gray matter of the brain, while in the white matter, its level reduced by 1.5 times (Table 3). The levels of BDNF and NT-4 had no statistically significant changes, and NT-3 exceeded the control values by 1.9 and 1.4 times in the gray and white matter of the brain, respectively.

With the growth of M1 sarcoma in the subcutaneous tissue in the presence of CNP, there was an increase in the level of β-NGF in the gray matter of the rat brain by 2.2 times relative to animals without CNP and by 4.1 times relative to rats without a tumor, but with CNP (Table 3). The level of BDNF in the gray matter of these rats was 1.4 times higher than in animals without CNP and 1.7 times higher than in animals with CNP alone.

Table 3

The level of neurotrophins in the brain of rats with different types of M1 sarcoma growth and in the presence of chronic neurogenic pain, $M \pm \sigma$

Parameter	NT-3 (pg/g of tissue)	NT-4 (pg/g of tissue)	BDNF (pg/g of tissue)	β-NGF (pg/g of tissue)
Control rats				
Gray matter	55.0 ± 5.1	11.3 ± 1.9	3,367.6 ± 352.9	362.1 ± 28.4
White matter	58.0 ± 6.3	7.4 ± 0.8	9,170.5 ± 861.7	886.0 ± 74.1
Rats with CNP				
Gray matter	56.1 ± 4.8	15.7±2.6	2,900.7 ± 276.3	553.9 ± 51.8[3]
White matter	67.0 ± 5.3	6.6±0.8	14,393.3 ± 1121.5[3]	1,501.7 ± 126.7[3]
Rats with s/c M1 sarcoma				
Gray matter	104.8 ± 9.2[3]	13.5 ± 1.4	3,511.0 ± 346.9	1,036.2 ± 92.4[3]
White matter	82.2 ± 7.1[3]	6.1 ± 0.7	10,287.7 ± 975.8	588.9 ± 55.3[3]
Rats with s/c M1 sarcoma + CNP				
Gray matter	62.3 ± 5.7[1]	12.7 ± 1.3	5,015.9 ± 423.6[1,2,3]	2,275.8 ± 214.1[1,2,3]
White matter	54.9 ± 4.6[1,2]	6.0 ± 0.7	3,440.6 ± 296.8[1,2,3]	1,452.4 ± 136.5[1,3]
Rats with i/v M1 sarcoma				
Gray matter	81.6 ± 7.9[3]	11.8 ± 1.3	4,534.6 ± 411.8[3]	1,747.3 ± 154.6[3]
White matter	147.7 ± 12.6[3]	6.9 ± 0.8	14,614.7 ± 926.7[3]	1,573.0 ± 168.1[3]
Rats with i/v M1 sarcoma + CNP				
Gray matter	44.4 ± 7.0[1]	12.6 ± 1.3	5,043.7 ± 396.7[2,3]	474.8 ± 49.6[1,3]
White matter	65.9 ± 6.3[1]	8.1 ± 1.0	22,073.7 ± 1654.8[1,2,3]	506.3 ± 45.9[1,2,3]

[1] statistically significant difference from the parameter in the group without CNP; [2] statistically significant difference from the parameter in the group with CNP; [3] statistically significant difference from the parameter in the group of control animals ($p < 0.0056$).

The NT-3 content in this sample was reduced compared to the M1 rats and did not have statistically significant differences from the corresponding control. In the white matter of the rat brain with the growth of M1 sarcoma in the subcutaneous tissue in the presence of CNP, there was an increased level of β-NGF, 2.5 times higher than the corresponding value for animals with the growth of M1 sarcoma in the subcutaneous tissue without CNP, and it did not differ significantly from the CNP values in the control. The BDNF level in the white matter of these rats was almost 3.0 times lower than the value in animals without CNP and 4.2 times lower than the CNP control value. Normalization of NT-3 was identified (Table 3).

In animals in which the tumor process in the lung did not develop after intravenous administration of the tumor suspension, in the gray and white matter of the brain, the β-NGF level increased by 4.8 times and 1.8 times, respectively, the BDNF level – by 1.3 times and 1.6 times, respectively, and the NT3 level – by 1.5 times and 2.5 times, respectively. In gray and white matter of the rats with a developed tumor process in the lung after administration of the tumor suspension in the presence of CNP, only the BDNF level increased by 1.7 times and 1.5 times, respectively, but the β-NGF level in the white matter only decreased by 3 times relative to control rats with CNP (Table 3).

DISCUSSION

This study showed that CNP reproduced by bilateral ligation of sciatic nerves causes an increase in the level of BDNF in the white matter and NGF-β in the cortex and white matter of the rat brain. This is consistent with numerous reference data.

NGF is considered to be a chronic pain mediator [11]. Anti-NGF therapy may be effective in reducing pain in experimental models [12]. BDNF is also involved in the mechanisms of neuropathic and inflammatory pain [13]. Neurotrophins mediate their biological functions through two transmembrane receptors: p75NTR (p75 pan-neurotrophin receptor) and TrkB receptor. Anti-NGF therapy may be useful for treating cancer pain, as it can suppress inflammation and then inhibit nerve sensitization [14]. In pain, BDNF is activated, among others, in the cerebral cortex [13, 15] and the spinal cord [16].

The authors of the present study have not found studies on neurotrophins in the brain of animals with the growth of a malignant tumor on the periphery, as it was shown in this research with the growth of M1 sarcoma in the subcutaneous tissue and the lung.

It was demonstrated that changes in the levels of some neurotrophins in therat brain tissue during subcutaneous growth of M1 were characterized by an increase in the NT-3 level in the cortex and white matter of the brain and a rise in the level of β-NGF in the cortex, while there was a decrease in β-NGF in the white matter. In the meantime, the intravenous administration of the tumor suspension, which did not result in tumor growth in the lung, had similar features in terms of the content of neurotrophins in the brain structures. Thus, an increase in the NT-3 level in the cortex and white matter and a rise in the β-NGF level in the cortex were found. Additionally, an increase in the BDNF content in the cortex and white matter and elevated β-NGF level in the white matter were discovered. It is possible that such changes in the level of neurotrophins in the brain of animals were caused bu the subcutaneous growth of M1 following stress accompanying tumor suspension administration and tumor growth. In case of intravenous administration of M1the changes may be caused not only by a stress response to the administration, but also by effective work of antitumor mechanisms that prevented tumor

BDNF is known to play a critical role in the stress response, as evidenced by its altered expression in the brains of stressed animals [17, 18]. Reports have shown that the functionality of the hypothalamus regions and the prefrontal cortex (PFC) of the brain is required for generation of the response to stress and pain [19]. BDNF is highly expressed in these regions, and its expression changes significantly in response to stress [18]. BDNF and β-NGF play an important role in the survival, differentiation, and plasticity of neurons during development and adulthood. When exposed to stress, they are good candidates for transmitting the influence of stress factors, causing changes in brain functioning [20]. β-NGF is required for the survival, proliferation, and differentiation of neurons in the peripheral and central nervous systems [21].

The comparative analysis of parameters in groups of animals with different variants of tumor growth in the presence of CNP is of great interest. This experiment combines chronic pain, tumor

growth, and stress from tumor suspension administration and further growth of the neoplasm. In case of the traditional subcutaneous growth of M1 sarcoma in the presence of CNP, the change in the NGF level reflected the CNP state, while the change in the BDNF level rather reflected the stress response [22].

Everything is more complicated in case of a tumor in the lung in the presence of CNP. A drastic decrease in the NGF level in the cortex and white matter, as opposed to the group of animals with administered tumor suspension, not accompanied by tumor growth in the lung, rather indicates depletion of this protein in the brain structures. The BDNF content in the gray and white matter indicates a pronounced response to stress, which is confirmed by a change in its expression in the brain of stressed animals [17, 18].

CONCLUSION

Thus, the obtained results indicate that during normal tumor growth both on the periphery and in the presence of CNP, changes in the level of neurotrophins in the brain of experimental animals may reflect the body response to chronic pain and stress accompanying tumor growth on at the periphery.

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Authors contribution

Frantsiyants E.M., Kaplieva I.V. – conception and design of the experiment. Frantsiyants E.M., Kaplieva I.V., Bandovkina V.A. – analysis and interpretation of the obtained results. Bandovkina V.A., Surikova E.I. – drafting and editing of the manuscript, critical revision of the manuscript for important intellectual content. Trepitaki L.K., Neskubina I.V. – carrying out of the experiment. Cheryarina N.D., Surikova E.I. – performance of the enzyme immunoassay. Frantsiyants E.M., Kotieva I.M. – final approval of the manuscript for publication.

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Expression of immunoglobulins in human epithelial tumors and their potential role in carcinogenesis

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ABSTRACT

The traditional view on immunoglobulin (Ig) production only by B-lymphocytes and plasma cells has been revisited. Non-lymphoid tumor cells can also synthesize and secrete Ig with unidentified specificity. Expression of Ig genes was detected in the cells of malignant neoplasms of epithelial origin, such as breast carcinoma, colorectal cancer, prostate cancer, as well as in epithelial tumor cell lines. mRNA of the IgG1 heavy (H) chain constant region, sterile Iɣ-Cɣ transcript, H and light (L) chains of IgG, V(D)J recombination of H and L chain gene segments, as well as RAG1 (recombination-activating gene 1) and RAG2 enzymes, which are required for V(D)J recombination, were found in cancer cell lines and resected carcinoma tissues. IgG produced by cancer cells can be involved in the invasion and metastasis of these cells through interaction with E-cadherin, as well as with the metastasis-associated protein MTA1. Tumor-derived IgG plays an important role in malignant progression via activation of platelets by interacting with their FcγRIIa receptors and inducing the production of low levels of reactive oxygen species. The level of IgG in malignant neoplasms is positively correlated with proliferation markers, stage of progression, and growth and survival of the tumor. These data modernize the current views on the mechanisms of carcinogenesis and create the basis for the search for new diagnostic and prognostic markers in malignant neoplasms, as well as methods of their target therapy. Further in-depth studies of the phenomenon of Ig production by tumor cells will contribute to more effective practical application of the accumulated knowledge in this field.

Key words: immunoglobulin expression, cancer, non-lymphoid cell-derived immunoglobulin, cancer-derived immunoglobulin, carcinogenesis, metastasis.

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Экспрессия иммуноглобулинов в эпителиальных опухолях человека и их потенциальная роль в канцерогенезе

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РЕЗЮМЕ

Традиционное представление о продукции иммуноглобулинов (Ig) только В-лимфоцитами и плазматическими клетками в последнее время подвергается ревизии. Клетки нелимфоидных опухолей также могут синтезировать и секретировать Ig неидентифицированной специфичности. Экспрессия генов Ig выявлена в клетках злокачественных новообразований эпителиального происхождения, таких как карцинома молочной железы, колоректальный рак, рак предстательной железы, а также в эпителиальных опухолевых линиях. В линиях раковых клеток и резецированных тканях карцином были обнаружены мРНК константной области тяжелых (H) цепей IgG1, стерильный транскрипт Iγ-Cγ, H- и легкие (L) цепи IgG, V(D)J-рекомбинация генных сегментов H- и L-цепей, а также ферменты RAG1 (recombination-activating gene 1) и RAG2, необходимые для V(D)J-рекомбинации. Продуцируемый раковыми клетками IgG может быть вовлечен в инвазию и метастазирование этих клеток через взаимодействие с E-кадгерином, а также с белком-1, ассоциированным с метастазированием (MTA1). Опухолевые IgG играют важную роль в злокачественном прогрессировании, активируя тромбоциты путем взаимодействия с их рецепторами FcγRIIa и индуцируя выработку низких уровней активных форм кислорода. Уровень IgG в злокачественных новообразованиях положительно коррелирует с маркерами пролиферации, стадией развития, ростом и выживаемостью опухоли. Эти данные модернизируют представления о механизмах канцерогенеза и создают фундамент для поиска новых критериев диагностики и прогноза течения злокачественных новообразований, а также методов их таргетной терапии. Необходимы дальнейшие углубленные исследования феномена продукции Ig опухолевыми клетками для более эффективного практического использования накопленных в этой области знаний.

Ключевые слова: экспрессия иммуноглобулина, рак, иммуноглобулин нелимфоидного происхождения, иммуноглобулин опухолевого происхождения, канцерогенез, метастазирование.

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INTRODUCTION

Traditionally, only activated B-lymphocytes and plasma cells ale regarded as immunoglobulin (Ig) producers. However, according to some researchers, cells of non-lymphoid origin and non-lymphoid localization can also synthesize and secrete Ig. Expression of Ig genes was detected in malignant cells derived from epithelial tumors, such as breast carcinoma [1], colorectal cancer [2], prostate cancer [3], papillary thyroid cancer [4], lung cancer [5], and in cell lines of epithelial tumors, including cervical (HeLa S3), prostate (PC3), lung (A549), and liver cancer (BCL-7402) [6, 7]. In addition, Ig synthesis and secretion, as well as the expression of their genes, were found in non-malignant cells, including proliferating epitheliocytes [7], neurons [8], and some eye cells [9]. Ig produced and secreted by transformed cells belong to different isotypes (IgG, IgM, IgA and IgE) depending on the tumor type [10]. Ig detected in various human malignancies were found to enhance tumor growth and survival, and the levels of these molecules correlated with the markers of proliferation and the stage of

the neoplastic process [2, 11, 12]. Suppression of Ig production by small interfering RNA (siRNA), which blocks expression of the heavy chain gene of all Ig isotypes, resulted in inhibition of growth and proliferation of various cancer types *in vitro* and *in vivo* [13].

The aim of the study was to review the scientific literature on xpression of Ig by human epithelial tumor cells and potential significance of these molecules in carcinogenesis and metastasis.

MOLECULAR STRUCTURE AND GENETIC BASIS OF IMMUNOGLOBULIN DIVERSITY

Ig produced by classical antibody-producing cells (B-lymphocytes and plasma cells) represent a group of proteins with several structural similarities. The fine structure of Ig molecules was investigated using monospecific monoclonal antibodies produced by hybridomas obtained by fusion of activated B-lymphocytes with plasmacytoma cells. The basic structural unit (monomer) of Ig is composed of two identical light (L) chains with a molecular weight of 22.5 kDa and two identical heavy (H) chains with a molecular weight of 50–75 kDa, which are linked together by non-covalent and disulfide bonds. Both H and L chains contain amino-terminal variable (V) regions involved in antigen recognition and carboxy-terminal constant (C) regions. The C regions of the H chains mediate the effector functions of the antibody, which are not directly associated with antigen recognition. There are two types of L chains differing in the amino acid sequence of the C region: κ and λ. The complete Ig molecule is composed of one or more monomers. In humans, depending on the structural variant of the H chain C region (Cμ, Cδ, Cγ, Cα, and Cε), five classes, or isotypes, of Ig (IgM, IgD, IgG, IgA, and IgE) are distinguished, which differ in molecular weight, charge, amino acid sequence, and carbohydrate content.

The generation of Ig diversity is a result of somatic recombination of gene segments of the L and H chains located on different chromosomes. Each H and L polypeptide chain of Ig is encoded by several genetic elements that are physically separated in germline DNA. However, in B-lymphocytes and antibody-producing cells, these elements join and form a single active gene. The V domain of κ-type L chain is encoded by two different gene segments: Vκ (*Variable*) and Jκ (*Joining*). In germline DNA, these gene segments are far apart, but in the course of lymphocyte differentiation they join. When the combined Vκ and Jκ gene segments join the κ-chain C region gene, which is located relatively close to Jκ, a single active gene is formed. The combinatorial joining of Vκ and Jκ gene segments can provide a large number of L chain variants.

The H chain genes are characterized by more complex organization. Thus, the H chain V domain is formed due to the combinatorial joining of three types of germline gene segments: V_H, D_H (*Diversity*), and J_H. This provides even greater diversity of H chains as compared to L chains [14]. Ig gene rearrangement is mediated by coordinate activation of certain enzymes – V(D)J recombinases. Some of these are only found in developing lymphocytes. The rearrangement process is regulated by the lymphocyte-specific component of V(D)J recombinase that binds and cleaves DNA at specific sites, the so-called recombinant signal sequences. The enzymes required to initiate the DNA cleavage are a complex of two proteins encoded by the *RAG1* (recombination-activating gene 1) and *RAG2* genes. RAG1 is enzymatically active only in combination with RAG2. The level of RAG1 and RAG2 expression is influenced by interleukin-7 (IL-7), a cytokine secreted by stromal cells of the bone marrow [15, 16].

Two additional types of somatic changes in Ig genes contribute to Ig diversity. These are somatic hypermutation in the H and L chain V region genes [17] and changes in the H chain C region genes induced by cytokines during the T-dependent humoral immune response. Class, or isotype, switching from IgM or IgD to IgG, IgE or IgA results from the replacement of Cμ or Cδ exons by Cγ, Cε or Cα exons, respectively, without altering the antigenic specificity [18]. The activation-induced cytidine deaminase (AID) plays a key role in both processes. The exact mechanisms of this enzyme functioning are not fully understood; presumably, it may act as an RNA-editing enzyme [19, 20].

IMMUNOGLOBULIN EXPRESSION IN HUMAN EPITHELIAL TUMORS

The functioning of the humoral arm of the adaptive immune system in patients with various forms of neoplastic diseases, including epithelial tumors, has been the focus of research since the 1960s [21]. Since the 1970s, a number of studies have demon-

strated an increase in the blood serum IgG, IgA, and/or IgM levels in patients with non-hematopoietic neoplasia, including carcinomas of the cervix, breast, oral cavity, larynx, bronchi, kidney, and liver [22–25]. High concentrations of IgA in sa liva have also been detected in patients with laryngeal cancer [26]. For a long time, it was believed that Ig in tumor patients, as in healthy individuals, was produced only by B-lymphocytes and plasma cells. However, at the turn of the XX–XXI centuries, compelling evidence emerged suggesting that transformed epithelial cells may also produce Ig, which goes beyond the classical immunological paradigms.

In 1996, X. Qiu et al., using the immunohistochemistry analysis and Western blotting, first discovered IgG-like molecules in the breast and colon carcinoma cells and showed that these molecules are absent in the non-malignant epithelial cells of these organs [27].

In 1998, Y. Kimoto, using the nested reverse transcription polymerase chain reaction (nested RT-PCR) method, which allows for detection of extremely small amounts of mRNA, detected the transcripts of the H chain C region of IgM, IgD, IgG3, IgG1, IgE, and IgA in human carcinoma cell lines: SW116 (intestinal adenocarcinoma), Hep2 (laryngeal squamous cell carcinoma), MCF-7 and MDA-MB-231 (breast adenocarcinoma), and HC48 (pancreatic adenocarcinoma) [10].

Soon, expression of transcripts of the H chain C region and IgG production in epithelial tumor cells were confirmed by other researchers. Thus, using DNA microarrays, genomic analysis of the gene expression profile in 20 samples of primary human hepatocellular carcinomas revealed the H chain C region genes of IgG3 (*IGHG3*), IgA1 (*IGHA1*), and IgM (*IGHM*) [28].

In 2003, X. Qiu et al. used *in situ* hybridization, immunohistochemical analysis and PCR to demonstrate that human epithelial tumors, including breast, colon, liver, and lung carcinomas at the level of single cells obtained by laser microdissection, as well as cells of stabilized tumor lines produce cytoplasmic and secretory forms of IgG. IgG H chain transcripts and the corresponding protein were detected in the transformed cells. Using FACS analysis and Western blotting, authors detected IgG in long-term cultured human tumor cell lines: such as MCF-7 (breast cancer), HT-29, LOVO (colorectal cancer), BCL-7402 (liver cancer), A549 (lung cancer), CaOV3 (ovarian cancer), and HeLa S3 and HeLa MR (cervical cancer). IgG was also detected in the supernatant of HeLa S3 and HeLa MR cell cultures [11].

M. Li et al. evaluated Ig expression in 7 human epithelial carcinoma cell lines. Using the immunohistochemical staining, Western blotting, and solid phase enzyme immunoassay methods, IgA protein expression was detected in cell extracts and culture supernatants of all tested cell lines [29]. Expression of the IgG1 H chain C region gene (*IGHG1*) and IgG protein as well as RAG1 and RAG2 expression in epithelial tumor cell lines (breast, liver, cervix, prostate, nasopharyngeal, stomach, and colorectal cancer) were studied. The *IGHG1* transcripts and sterile Iγ-Cγ transcripts were detected by nested RT-PCR. The γ-type H chain and κ-type L chain proteins were identified by immunofluorescence and Western blotting. V(D)J recombination of the H and L chain gene segments and RAG1/RAG2 expression were also detected in the above cell lines [6].

In 2006, G. Babbage et al. performed gene analysis of the H chain V-region (V_H) in well-characterized breast cancer cell lines (BT 474, MDA-MB-231, MCF-7, SKBR3.T47D, and ZR-75-1) expressing the epithelial marker EpCAM (epithelial cell adhesion molecule) using nested RT-PCR. V_H gene transcripts were identified in 4 out of 6 cell lines. V_H gene expression was found in approximately 32% of single $EpCAM^+$ cells sorted from 3 tumor lines. In 5 of the 6 identified V_H genes, somatic mutations were detected without intraclonal variation, indicating cessation of mutational activity. V_H genes in the breast cancer cell lines were expressed as either pre- or post-switched transcripts, and in two cell lines, dual (both pre- and post-switched) transcripts were identified: IgG/IgA in SKBR3 and IgM/IgG in ZR75-1. However, at the protein level, the authors were unable to detect extra- and intracellular expression of Ig molecules in 4 selected cell lines using FACS analysis with monoclonal anti-IgG and -IgM antibodies. Analysis of RAG1 and RAG2 expression in each cell line showed the absence of any gene transcripts. When discussing the origin of rearranged V_H genes in tumor cells, the authors did not exclude the acquisition of these

genes as a result of the uptake of B-cell chromatin and its assimilation in the tumor cell genome. [30].

L chains of Ig expressed by cancer cells predominantly belong to the κ-type. Liu H.D. et al. in 2007 determined the expression of κ-chain genes in nasopharyngeal carcinoma cell lines by RT-PCR, Western blotting, and FACS. The expression of κ-chain C region mRNA was detected in abnormal cells of the cervical uterine epithelium in cervicitis and cervical intraepithelial neoplasia, as well as in invasive cervical carcinoma cells, and this expression was higher in dysplasia and carcinoma than in cervicitis [31, 32]. Expression of κ-chains was found in other tumors, such as breast, lung, liver and prostate cancer [6], colorectal carcinoma [33], and gastric cancer [34].

A number of studies were devoted to determining the molecular mechanisms of Ig expression in tumor cells. Both mRNA and proteins of RAG1 and RAG2, which are required for V(D)J recombination, were detected in Ig-positive tumor cell lines, including lung, colorectal, cervical [11], hepatic, prostate, gastric, breast, and nasopharyngeal carcinomas [6]. The expression of AID, which is required for class switching and somatic hypermutation, was detected by nested RT-PCR in 6 breast cancer cell lines [30], as well as in papillary thyroid cancer cells [4]. RAG1 and RAG2 mRNA as well as AID mRNA were detected in lung adenocarcinoma cells but not in cells of adjacent normal tissue or normal lung epithelial cell lines [5].

Importantly, AID transcripts were also detected in mammalian pluripotent tissues, including oocytes and primordial germ cells at a level comparable to AID expression in lymphoid tissues [35]. It was suggested that AID plays a role in epigenetic reprogramming and maintenance of the malignant phenotype. It is also possible that aberrant mutations and genomic instability are associated with high levels of AID expression [36].

B cell generation and Ig production are controlled by regulatory components such as receptor tyrosine kinase Flk2, IL-7 receptor (IL-7R), and transcription factors PU.1 (purine box factor 1), Ikaros, E2A (E box binding protein 2A), EBF (early B cell factor 1), and Pax5 (paired box protein 5) [37–39]. In *E2A*-/- or *EBF*-/- mice, B cell development stopped early in the absence of RAG expression and D_H-J_H rearrangement at the IgH locus. Ectopic expression of E2A and EBF1 together with RAG1 and RAG2 activated D_H-J_H rearrangement in non-lymphoid cells [40]. L. Geng et al., using nested RT-PCR, determined Pax5 expression in the human colon cancer cell line SW480 and EBF expression in several human epithelial neoplasia cell lines, including colon tumors (SW480 and LOVO), cervical cancer (HeLa), breast cancer (Bcap-37), and liver cancer (SMMC-7721) [33].

FUNCTIONAL ROLE OF TUMOR-DERIVED IMMUNOGLOBULINS IN CARCINOGENESIS AND POTENTIAL MECHANISMS OF THEIR ACTION

The functional role of IgG produced by epithelial tumor cells was analyzed in several studies, the results of which suggest that tumor-derived IgG enhances tumor growth and survival. In 2003, X. Qiu et al. showed that blockade of tumor-derived IgG by antisense oligodeoxynucleotides or antibodies to human IgG resulted in activation of programmed cell death and suppression of tumor cell growth *in vitro*. In addition, antibodies to human IgG suppressed the growth of the IgG-producing carcinoma cell line HeLa MR in immunodeficient nude mice [11].

In 2006, Y. Deng et al. determined the Ig expression in HT-29 cells (human colon cancer) and evaluated the effect of Ig on the biological activity of tumor cells. Transcripts of Ig H chain V regions (V_H CDR3) in HT-29 cells were detected by RT-PCR. Transfection of the antisense vector CDR3-pIRES 1 neo into HT-29 cells resulted in a significant decrease in Ig expression as well as in induction of apoptosis and inhibition of cell growth [41]. In human HeLa (cervical cancer) and CNE1 (nasopharyngeal carcinoma) cell lines, the blocking antibodies to α-type H chain (Igα) suppressed growth and reduced cell viability. In addition, Igα blockade led to a decrease in the proportion of HeLa cells that entered the S phase of the cell cycle after pre-synchronization in the G2/M phase [42].

The effects of IgG expression on growth and metastasis were studied in the tissues of squamous cell carcinoma and adenocarcinoma of the lung. The level of IgG expression in 86 lung cancer samples was found to be associated with the clinical stage of the tumor and its metastasis to lymph nodes.

Knockdown of IgG by siRNA resulted in decreased proliferation, migration, and adhesive capacity of cultured tumor cells. The relationship between the expression of IgG genes and the expression of metastasis-associated genes (CD44, E-cadherin, matrix metallopeptidase 9 (MMP9), MMP2, integrin-β1, and metastasis-associated protein MTA1) was evaluated by RT-PCR and Western blotting. Only MTA1 expression was found to be significantly reduced in lung cancer cell lines after inhibition of IgG expression. High MTA1 expression is known to be closely associated with cell invasion of various carcinomas and their metastasis to lymph nodes, as well as with progression of cancer symptoms [43, 44]. Suppression of MTA1 expression by siRNA in lung cancer cells inhibited their ability to migrate and adhere in cell cultures. Apparently, MTA1 is co-expressed with tumor-derived IgG, which may play a key role in the lung cancer metastasis through MTA1 regulation [5].

J. Wang et al. found that suppression of IgG mRNA and protein expression by siRNA in HeLa cervical cancer, Hep2 laryngeal carcinoma, and PC3 prostate cancer cell lines inhibited the growth and proliferative activity of tumor cells. Among 27 detected proteins, which interacted with IgG in HeLa cell culture, the following peptides were found to be closely related to cell growth and oxidative stress: RACK1 (receptor for activated C kinase 1), RAN (Ras-like guanosine triphosphatase), and PRDX1 (peroxiredoxin-1). Negative regulation of tumor-derived IgG was found to reduce the intracellular levels of reactive oxygen species (ROS) and increase to increase the overall cellular antioxidant activity. Several ROS scavengers, including catalase, dimethyl sulfoxide, N-acetylcysteine, and superoxide dismutase, inhibited the growth of IgG-deficient tumor cells through suppression of MARK/ERK (mitogen-activated protein kinase/extracellularly regulated kinase)-mediated signals [45]. Exogenous hydrogen peroxide at a low concentration enhanced the survival of these cells through an increase in the intracellular ROS levels [45, 46].

In 2013, P. Liang et al. showed that blockade of tumor IgG with human IgG antibodies or antisense oligonucleotides enhanced apoptosis and suppressed the growth of T24 and BIU-87 bladder cancer cell lines *in vitro* and the growth of tumor xenografts *in vivo*. In addition, inhibition of IgG expression in T24 cell line increased cell sensitivity to mitomycin C and activated caspase-3. Blockade of IgG expression is thought to induce tumor cell apoptosis through activation of the caspase-dependent pathway [47].

G. Lee et al. assessed the expression and functional role of tumor-derived IgG in OC-3-VGH ovarian cancer cells and in many other cancer cell lines from different human tissue origins by immunohistochemistry and immunofluorescence using RP215 monoclonal antibodies. RP215 specifically recognize tumor-associated CA215 antigen, which is expressed in secretory and membrane-bound forms in most cancer cells and consists mainly of tumor-derived IgG H chains. A unique glycosylated H chain epitope of tumor-derived IgG, which reacts with RP215, was characterized. CA215 was shown to be homologous to the H chain of human B cell-derived IgG1 with the molecular mass of 50–70 kDa with the exception of the high content of serine and threonine residues in the V region. A significant correlation of high tumor-derived IgG expression with low level of differentiation and late stage of cancer was revealed using RP215 antibodies [48, 49].

The same research group showed the expression of other proteins of the Ig superfamily in various cancer cell lines. Using MALDI-ToF MS (Matrix-Assisted Lazer Desorption / Ionization Time-of-Flight Mass Spectrometry) analysis, molecular homology of CA215 was revealed not only to H-chains of tumor IgG, but also to T-cell receptors (TCR) and Ig-like adhesion molecules. Using RT-PCR and cDNA sequencing, significant expression of the TCR-α and TCR-β genes, as well as the adhesion molecules CD47, CD54, CD58, and CD147, was found in the vast majority of cancer cell lines tested. In contrast, TCR co-receptors and co-stimulators, such as CD3, CD4, and CD8, were rarely expressed, which indicates the non-functional nature of TCR in tumor cells. These data were supported by the results of immunohistochemical staining and Western blotting of cancer cell lines, as well as cancer tissue samples. It was hypothesized that the expression of Ig superfamily proteins may be related to immune protection and proliferation of cancer cells during carcinogenesis and cancer progression [50].

Immunohistochemical analysis of tumor tissue samples taken from 100 patients with colorectal cancer made it possible to establish that tumor-de-

rived IgG, detected using RP215 antibodies, were expressed in cancer nests of tumor tissues, but not in stromal cells of colorectal tissues. It was found that high level of expression of tumor-derived IgG was a prognostically unfavorable factor. Expression of these IgG was also detected in 3 out of 5 colorectal cancer cell lines tested. Knockdown of tumor-derived IgG in SW480 cells suppressed their proliferation *in vitro*. Blockade of the tumor-derived IgG expression in SW480 cells prior to their subcutaneous injection into nude mice inhibited the growth of the implanted tumor *in vivo*. Direct interaction of IgG of tumor origin with E-cadherin and β-catenin was found. In normal cells, these adhesion molecules form a single complex located in the adherens junctions on the cell membrane. As a result of the knockdown of tumor-derived IgG, the expression of E-cadherin at the adherens junctions increased, while the expression of c-Myc oncogene decreased. The authors speculated that tumor-derived IgG might cause dissociation of E-cadherin from the complex with β-catenin and activate β-catenin / c-Myc-mediated signaling by increasing nuclear translocation of β-catenin, thereby promoting invasion and metastasis [51].

In 2015, Q. Liao et al., using RP215 antibodies, showed that cancer cells with high level of IgG expression were characterized by increased ability to migrate, invade, and metastasize *in vitro* and *in vivo [52].* IgG knockdown in cancer cell lines and in breast cancer resulted in significant inhibition of tumor cell proliferation, migration, and invasion, as well as in induction and enhancement of tumor cell apoptosis [52, 53].

Recently, it has been found that IgG of tumor origin activated platelets by direct interaction with their FcγRIIa receptors [54], which was reflected by increased secretion of dense granules by platelets [55]. It is known that platelets can regulate tumor growth, angiogenesis, and metastasis [56–58], which is associated with the functions of surface receptors and secreted products, such as thromboxane, platelet growth factor [59], and vascular endothelial growth factor [60]. Tumor-related thrombotic complications are one of the leading causes of death in cancer patients. Patients with thrombosis are more likely to have distant metastases, and their one-year survival rate is lower than that of patients without thrombotic complications [61–63].

CONCLUSION

This review presents in chronological order the results of key studies on Ig expression by epithelial tumor cells and the significance of these molecules in carcinogenesis and metastasis. Since the late 1990s, there has been rapid progress in this scientific field from random ("paradoxical") findings of extra-lymphoid Ig production to detailed immunological, genetic, and clinical characteristics of this scientific phenomenon. This information, together with the data on Ig production by normal proliferating epithelial cells, central neurons, and eye cells, substantially modernizes the traditional concepts, according to which V(D)J recombination and Ig production are characteristic only of B-lymphocytes and plasma cells, which requires revision of the classical immunological paradigms in the field of humoral immune response.

The expression of Ig, mainly IgG, of unidentified specificity in epithelial tumors and cancer cell lines of different organ origin is worth noting. Tumor-derived IgG has been shown to be structurally and functionally distinct from the antigen-specific Ig (antibodies) produced by B cells and to be involved in cancer cell growth and survival. Using different methodological approaches, independent research groups consistently excluded factors that could influence the final study results in order to confirm the tumor origin of Ig. Today, there is no doubt about the universal (or near-universal) ability of epithelial tumor cells to produce Ig.

The search for new molecular markers of tumorigenesis is an important and promising direction in immunodiagnostics and immunotherapy of malignant neoplasms [64]. The expression of Ig, mainly IgG, by cancer cells closely correlates with the clinical stage, the degree of pathological changes in tumor, and metastasis to lymph nodes. A positive correlation between IgG expression and clinical parameters of the tumor process suggests the possibility of determining the expression of tumor-derived IgG in oncological practice for diagnostic and prognostic purposes.

The molecular mechanisms and the biological and clinical significance of Ig production by non-lymphoid cells, especially cancer cells, require further in-depth investigation. It remains to be established whether Ig of non-lymphoid origin have

the same functions as Ig produced by B cells or plasma cells. The similarities and / or differences in the mechanisms of Ig production by lymphoid and cancer cells should be elucidated. The question remains whether Ig expression in tumor cells is the cause or the result of cell transformation. It is useful to clarify whether tumor-derived Ig can be used as a therapeutic target in neoplastic diseases. It is likely that the answers to these questions could form the basis for the development of methods for the selective blockade of IgG produced by cancer cells in the therapy of malignant neoplasms.

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Advanced heart failure

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ABSTRACT

The authors of the article have analyzed the problem of advanced heart failure (AHF). Despite significant and, without exaggeration, revolutionary achievements in clinical pharmacology, cardiac surgery, and implantation arrhythmology, the number of patients with chronic heart failure (CHF) in many countries is not decreasing, and in some states, for example, in Russia, it is increasing. At the same time, unfortunately, immediate and long-term results of the so-called optimal therapy of CHF are often disappointing for both the patient and the doctor. In 2007, experts from The Heart Failure Association of the European Society of Cardiology proposed the term advanced heart failure (AHF) to refer to CHF in which optimal drug therapy, as well as cardiac resynchronization therapy, are not effective, which causes repeated hospitalizations and justifies the need for advanced treatment methods such as heart transplantation and mechanical circulatory support, and/or transition to palliative care. The opinions of experts from the established cardiological communities in the Old and New Worlds on the definition, diagnostic criteria, and treatment of AHF have been changing over time. Unfortunately, this evolution has not yet arrived at a consensus. The lecture consistently addresses the issues of terminology, diagnosis, prognostic stratification, and routing of patients with AHF, as well as short- and long-term strategies for treating these patients.

Key words: advanced heart failure, definition, indicators, prognostic stratification, clinical markers, biomarkers, imaging, exercise test, co-morbidity, management strategies, mechanical circulatory support, heart transplantation

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Прогрессирующая (advanced) сердечная недостаточность

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РЕЗЮМЕ

Приводится анализ проблемы прогрессирующей сердечной недостаточности (ПСН). Несмотря на существенные, не будет преувеличением сказать – революционные, достижения клинической фармакологии, кардиохирургии и имплантационной аритмологии, число пациентов с хронической сердечной недостаточностью (ХСН) во многих странах не сокращается, а в некоторых, например в России, увеличивается. При этом, к сожалению, нередко непосредственные и отдаленные результаты так называемой оптимальной терапии ХСН вызывают разочарование как у пациента, так и у врача. В 2007 г. эксперты Ассоциации сердечной недостаточности Европейского общества кардиологов предложили термин ПСН для обозначения ХСН, при которой оптимальная медикаментозная терапия, а также сердечная ресинхронизирующая терапия не являются эффективными. Это является причиной повторных госпитализаций и обосновывает необходимость применения таких передовых методов лечения, как трансплантация сердца и механическая поддержка кровообращения, и (или) перехода к паллиативной помощи. Согласованные позиции экспертов авторитетных кардиологических сообществ в Старом и Новом Свете, касающиеся определения, критериев диагностики и лечения ПСН, менялись со временем, но, к сожалению, их эволюция до сих пор не завершилась полным консенсусом. В лекции последовательно рассматриваются вопросы терминологии, диагностики, прогностической стратификации и маршрутизации пациентов с ПСН, а также краткосрочной и долгосрочной стратегии лечения этих больных.

Ключевые слова: прогрессирующая сердечная недостаточность, определение, индикаторы, прогностическая стратификация, клинические маркеры, биомаркеры, визуализация, тест с физической нагрузкой, сопутствующие заболевания, стратегии ведения, механическая поддержка кровообращения, трансплантация сердца.

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

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INTRODUCTION

Chronic heart failure (CHF) is a notorious medical and social problem that belongs to the priorities of national health systems in almost all developed and developing countries [1, 2]. This is explained by the fact that despite the significant and even revolutionary achievements in clinical pharmacology, cardiac surgery, and implantation arrhythmology, the number of patients (especially those with heart failure with preserved left ventricular (LV) ejection fraction (EF)) with this disabling, costly, and often deadly condition does not decrease in many countries. In some countries, for example in Russia, it increases [3–6]. At the same time, immediate and long-term results of the so-called optimal therapy of heart failure are, unfortunately, often disappointing for both the patient and the doctor [7, 8].

The aim of this lecture was to consider contemporary views on the problem of advanced heart failure (AHF), the prevalence of which in the population of patients with CHF ranges from 1 to 10% [9, 10].

TERMINOLOGY

In 2007, experts from the Heart Failure Association (HFA) of the European Society of Cardiology (ESC) proposed the term AHF to refer to heart failure, in which optimal drug therapy, including diuretics, renin-angiotensin-aldosterone system (RAAS) inhibitors, beta-blockers (unless these drugs are not contraindicated and are well tolerated), and cardiac resynchronization therapy (if there are appropriate indications) are not effective. It is considered not effective if the following persist: objective signs of severe cardiac dysfunction, such as severe systolic and/or diastolic LV dysfunction, high ventricular filling pressure and increased natriuretic peptide blood levels, which are associated with heart failure corresponding to III-IV functional class (FC) according to NYHA (New York Heart Association), dyspnoea and/or fatigue at rest or with minimal exertion, as well as episodes of fluid retention and/or peripheral hypoperfusion at rest.

All of the above is the reason for repeated hospitalizations (≥ 1 case over the past six months) and justifies the need for using such advanced treatment methods as heart transplantation and mechanical circulatory support, and/or the transition to palliative care [11]. The studied cases included not yet fatal patients with refractory heart failure requiring consideration of the use of a circulatory assist device and/or heart transplantation and patients with end-stage heart failure in which irreversible changes in the end-organs cause contraindications to surgery and palliative care is the only possible option (for example, inotropic drug infusions, ultrafiltration or peritoneal dialysis and end-of-life care) were in question.

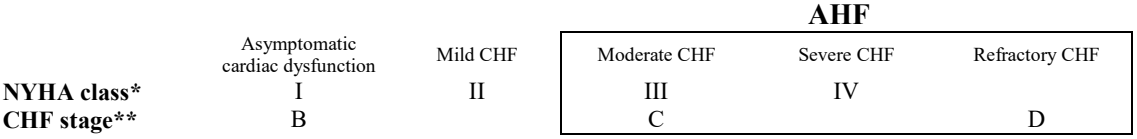

Fig. 1. Position of advanced heart failure (AHF) in chronic heart failure (CHF) classifications [11]: * Classification of the New York Heart Association; ** Classification by the American Heart Association and the American College of Cardiology

Well-known difficulties of precise CHF functional class (FC) diagnosis, associated with the subjectivity of a patient and a doctor in determining what limitation to physical activity is slight or significant and what exertion is habitual for the patient, were described many times in the «Bulletin of Siberian Medicine» among other sources [12–14]. This subjectivity naturally leads to low reproducibility of the results of assessing CHF FC in the same patient by different doctors. CHF affects mainly elderly people. Taking into account polymorbidity, the informativity of the exercise test performed to objectify FC (a 6-minute walk test, for instance) is often unacceptably low in these patients, since the distance traveled by the patient can be affected not only by myocardial, but also by coronary or respiratory failure and other factors [14].

At the same time, some doctors and researchers give a rather subjective interpretation of the NYHA classification. They identify intermediate FC values (for example, III-IV) and attempt to introduce additional gradations into the classification. One of these

is IIIb class which is not categorically identified as "advanced". This class is vaguely characterized as a more serious disturbance of the functional status than in FC III, on the one hand, but not as severe as in CHF corresponding to FC IV, on the other hand [14, 15]. Medicine does not belong to exact sciences, but such an argument cannot be understood, as if you are being convinced that more than two results are possible after flipping a coin – not only "heads" or "tails", but also the coin's hanging in the air.

As for repeated hospitalizations, this is a controversial criterion, since some patients with AHF may often seek unplanned medical care and receive it in an outpatient setting (for example, in the United States in the emergency department), and some patients are hospitalized for reasons not directly related to CHF (for example, exacerbation of the underlying disease or comorbid pathology, or disturbances of heart rhythm and conduction). Most often, unplanned hospitalizations in these patients are caused by acute heart failure (including so-called acute decompensated heart failure) [8] and circumstances related to refractory CHF [11].

It is important not to allow for confusion of concepts. AHF is a form of CHF, which even in rapidly developing decompensation is fundamentally different from acute heart failure [1]. D.V. Preobrazhenskiy et al. [12] rightly consider the concepts of "heart failure" and "chronic heart failure" as synonyms, since speaking of acute heart failure, it is customary to indicate its specific form, such as pulmonary edema, cardiogenic shock, or acute pulmonary heart (it does not matter whether this preceded CHF or not). Nevertheless, in the special medical literature, there is also an opposite point of view, according to which acute heart failure includes episodes of acute cardiac decompensation in patients with CHF in the absence of the clinical presentation of pulmonary edema and cardiogenic shock. We quote what we cannot understand: "Acute decompensated heart failure (first-time, decompensation of CHF) – poorly expressed symptoms of acute heart failure that do not meet the criteria for cardiogenic shock, pulmonary edema ..." [16]. Apparently, all clinicians have encountered primary and secondary refractory disease in patients with CHF (it is important to recall pseudo-refractoriness that can be associated with the patient's non-compliance) with the treatment. There are no generally accepted criteria for verifying this condition (similar to those for resistant hypertension [17]). This is probably why repeated attempts to introduce the refractory phase (stage) of CHF in its classification are met with reasonable resistance from the leading cardiologists of Russia. Yu. N. Belenkov at the "Classification of chronic heart failure" round table at the annual (14.12.2001) conference of the Society of Specialists in Heart Failure, objected to such a pro- posal as follows: "You cannot introduce a refractory phase. Refractory to what? We do not make a classification for ourselves, but a classification for everyone. Your refractory patient and my refractory patient are different patients because we have plasmapheresis, ultrafiltration, and artificial LV" [18].

Finally, the final (terminal) stage of CHF should be distinguished from AHF. According to ESC experts [11], the main difference between them is the presence of a certain degree of reversibility in the severity of CHF manifestations when applying cutting-edge treatment methods. The ambiguous phrase "a certain degree of reversibility" dictates the need to search for informative discriminant signs. Doctors in the absence of the latter should not rush to pronounce a sentence on a patient with CHF.

Such indistinct criteria were the subject of deserved criticism and the reason for revising the definition of AHF for it to take into account the evaluation of the effectiveness of new classes of drugs (such as sinus node If-channel inhibitors and angiotensin receptor and neprilysin inhibitors), characteristics of comorbid pathology, the condition of the end-organs, and other variables neglected by the ESC experts in 2007. The opinions of experts of reputed cardiological communities in the Old and New Worlds regarding the definition and criteria for diagnosis and treatment of AHF have changed over time, but, unfortunately, their evolution has not yet ended in full consensus, and none of the proposed interpretations are indisputable [9, 19]. In this regard, it is worth quoting the titles of the works of well-known cardiologists, who vividly designated the problem: "Advanced heart failure and end-stage heart failure: does a difference exist?" [20], "A changing trend toward destination therapy: are we treating the same patients different-

ly?" [21], "Rise of the machines? Left ventricular assist devices for treatment of severe heart failure" [22], "Mechanical ventricular assistance as destination therapy for end-stage heart failure: has it become a first-line therapy?" [23].

DIAGNOSTIC CRITERIA

Obviously, in order to speak about AHF with confidence, it is necessary to justify the presence of heart failure itself in the patient first. The principles of CHF diagnosis are well developed and described in numerous recommendations [3, 5, 24]. Modern criteria for the diagnosis of AHF, as a rule, include signs first formulated in 1998 by K.F. Adams Jr. and F. Zannad [25]: LV EF value determined at rest is less than 30%, CHF corresponds to FC III-IV, or maximum oxygen consumption is less than 14 ml / kg / min. However, even among patients hospitalized with acute heart failure, at least half have normal LV FV values. The absence of LV global contractile dysfunction should not contradict the diagnostic conclusion about AHF in the presence of other symptoms and signs of this condition [9].

Detailed criteria for the diagnosis of AHF, formulated in current memorandums of the HFA ESC [9], the American Heart Association (AHA) and the American College of Cardiology (ACC) [26], as well as the Heart Failure Society of America (HFSA) [27], are presented in Table 1.

After reviewing the criteria presented in Table 1, many clinicians are likely to have questions. The greatest list of questions, perhaps, is caused by AHF criteria presented in AHA/ACC recommendation, since they do not specify whether all criteria are obligatory for verification of AHF, they contain inaccurate wording (e.g., "frequent", "usually") and do not include any characteristics of a state of cardiac contractile and lusitropic functions. It should be noted that the North American experts focused strictly on CHF and discussed AHF briefly in the context of CHF [26]. However, in the absence of information about the presence and severity of global (segmental) systolic and diastolic ventricular dysfunction, as well as their remodeling, detection of CHF is not always infallible, and the diagnosis itself is not irreproachable [14, 28–32].

In this regard, the recommendations of the HFA ESC 2018 look more reasonable [9]. They em- phasize a thorny path at differential diagnosis, since the symptoms and signs indicated in paragraphs 1 and 4 (Table 1) can be the result of not only cardiac dysfunction, but other conditions (for example, severe lung disease, non-cardiac cirrhosis of the liver, or, most often, renal failure of mixed etiology). However, these patients have a low quality of life and a bad prognosis and require the same attention as someone in whom heart failure is the only existing illness.

Table 1

Most common criteria for diagnosing AHF [9, 26, 27]

HFA ESC, 2018	AHA / ACC, 2013	HFSA, 2015
All the following criteria must be present despite optimal guideline-directed treatment: 1. Severe and persistent symptoms of heart failure [NYHA class III (advanced) or IV]. 2. Severe cardiac dysfunction defined by reduced LVEF ≤30%, isolated RV failure (e.g. ARVC) or non-operable severe valve abnormalities or congenital abnormalities or persistently high (or increasing) BNP or NT-proBNP values and data on severe diastolic dysfunction or LV structural abnormalities according to the ESC definition of HFpEF and HFmrEF. 3. Episodes of pulmonary or systemic congestion requiring high-dose intravenous diuretics (or diuretic combinations) or episodes of low output requiring inotropes or vasoactive drugs or malignant arrhythmias causing >1 unplanned visits or hospitalizations over the last 12 months.	1. Repeated (≥2) hospitalizations or ER visits for HF in the past year 2. Progressive deterioration in renal function (e.g. rise in BUN and creatinine) 3. Weight loss without other cause (e.g. cardiac cachexia) 4. Intolerance to ACE inhibitors due to hypotension and/or worsening renal function 5. Intolerance to beta-blockers due to worsening HF or hypotension 6. Frequent systolic blood pressure <90 mmHg 7. Persistent dyspnoea with dressing or bathing requiring rest 8. Inability to walk 1 block on the level ground due to dyspnoea or fatigue	The presence of progressive and/or persistent severe signs and symptoms of HF despite optimized medical, surgical, and device therapy. It is generally accompanied by frequent hospitalizations, severely limited exertional tolerance, and poor quality of life and is associated with high morbidity and mortality. Importantly, the progressive decline should be primarily driven by the HF syndrome. Indicators of advanced HF in the setting of optimal medical and electrical therapies that should trigger consideration of referral for evaluation of advanced therapies include: • Need for intravenous inotropic therapy for symptomatic relief or for maintaining end-organ function • Peak VO_2 <14 mL/kg/min or <50% from the predicted value • 6MWT distance <300m • ≥2 HF hospitalizations in the last 12 months • >2 unscheduled visits (e.g. ER or clinic) in the last 12 months

Table 1 (continued)

HFA ESC, 2018	AHA / ACC, 2013	HFSA, 2015
4. Severe impairment of exercise capacity with inability to exercise or low 6MWTD (<300 m) or pVO2 (<12–14 mL/kg/min), estimated to be of cardiac origin. In addition to the criteria mentioned above, extra-cardiac organ dysfunction due to heart failure (e.g. cardiac cachexia, liver, or kidney dysfunction) or type 2 pulmonary hypertension may be present, but are not required.	9. Recent need to escalate diuretics to maintain volume status, often reaching daily furosemide equivalent dose >160 mg/day and/or use of supplemental metolazone therapy 10. Progressive decline in serum sodium, usually to <133 mEq/L 11. Frequent ICD shocks	• Worsening right ventricular HF and secondary pulmonary hypertension • Diuretic refractoriness associated with worsening renal function • Circulatory–renal limitation to RAAS inhibition or beta-blocker therapy • Symptoms of progressive/persistent CHF (NYHA functional class III-IV) • Increased 1-year mortality (e.g. 20–25%) predicted by HF survival models (e.g. SHFS, HFSS, etc.) • Progressive renal or hepatic end-organ dysfunction • Persistent hyponatraemia (serum sodium <134 mEq/L) • Recurrent refractory ventricular tachyarrhythmias; frequent ICD shocks • Cardiac cachexia • Inability to perform ADL

Note. HFA – Heart Failure Association; ESC – European Society of Cardiology; AHA / ACC – American Heart Association /American College of Cardiology; HFSA – Heart Failure Society of America; 6MWT - 6-minute walk test; ACE – angiotensin-converting enzyme; ADL – activities of daily living; BNP – B-type natriuretic peptide; BUN – blood urea nitrogen; CRT – cardiac resynchronization therapy; ER– emergency room; HF – heart failure; HFSS – Heart Failure Survival Score; ICD – implantable cardioverter-defibrillator; LV – left ventricular; LVEF – left ventricular ejection fraction; NT-proBNP – N-terminal pro-B-type natriuretic peptide; HfmrEF – heart failure with mid-range ejection fraction; HfpEF – heart failure with preserved ejection fraction; ARVC – arrhythmogenic right ventricular cardiomyopathy; NYHA – New York Heart Association; PCWP – pulmonary capillary wedge pressure; RAAS – renin-angiotensin-aldosterone system; RAP – right atrial pressure; SHFS – Seattle Heart Failure Score; pVO2 – peak exercise oxygen consumption.

PROGNOSTIC STRATIFICATION

Accurate and timely prognosis is important for any disease, but in such a severe pathology as AHF, the results of predictive stratification are of particular importance. In order to justify referring the patient with chronic heart failure in a specialized center (e.g., heart failure clinic), it is enough to detect pronounced decompensation. However, to select patients requiring the use of advanced treatment methods, such as heart transplantation and artificial left ventricle, and to determine the optimal time for this therapy, it is necessary at first to have a substantiated assumption on unacceptably high risks of death in the absence of aid from an organ transplant surgeon or specialists in mechanical circulatory support [9].

A fairly extensive list of indicators of adverse prognosis in AHF is presented in Table 2 [9]. It is logically grouped by ESC experts according to the method of obtaining information (markers obtained in a clinical study and by performing simple instrumental tests; biomarkers evaluated in the laboratory; results of heart and vascular imaging; and exercise testing) and the presence of comorbid pathology.

As you can see, the prognosis in AHF patients is associated with a large number of factors with an obvious linear relationship (from weak to almost functional) between many of them. For example, the quality of life in a patient with CHF is related to gender, parameters of LV remodeling (geometric and electrophysiological) and its functional state, the level of asthenia and the severity of depression, as well as the presence of metabolic syndrome (obesity and indicators reflecting the severity of systemic inflammation and oxidative stress), iron deficiency, and coronary insufficiency [33–39]. Depression of heart rate variability in these patients is directly related to age, heart remodeling, the degree of systolic and diastolic LV dysfunction, the presence of diabetes mellitus and diabetic autonomic neuropathy, as well as the clinical severity of heart failure, and, conversely, the effectiveness of CHF treatment [40–43]. Hyperuricemia can be one of the manifestations of metabolic syndrome or chronic kidney disease [44–46]. The number of cardiac and extracardiac factors affecting the level of the so-called cardiac biomarkers is generally difficult to calculate [47–53].

Due to the multicollinearity between risk factors, the partial prognostic value of each of them is difficult to determine and, therefore, it is not easy to distinguish significant independent predictors from

randomly detected and not carrying any additional information ones. The way out of the problematic situation in clinical practice can be the use of multi-factor models of prognosis, which, for obvious reasons, have their advantages over monofactor models [9, 54, 55].

Table 2

Clinical markers and parameters obtained by instrumental research

General clinical		
Age Male sex ↑ QRS duration Longer HF duration Higher NYHA class Lower and labile SBP and lower DBP and MAP Lower pulse pressure	↑ HR in sinus rhythm but not in atrial fibrillation Reduced HR variability Recent /recurrent HF hospitalizations Changed haemodynamic profiles* Cardiomegaly S3 Poor quality of life	Reduced peripheral muscle strength Rales Edema JVD Hepatomegaly Ascites
Biomarkerss		
Copeptin Low sodium Cardiomyocyte injury - Troponin Cardiomyocyte stress - Higher BNP and/or NT-proBNP - Increased NT-proBNP over time - ANP - MR-proANP Inflammation - CRP	- ESR Oxidative stress and fibrosis - ST2 - Galectin-3 - GDF-15 - MR-proADM - Lower LDL - Uric acid - Low T3 - Albuminuria	
Imaging		
Echocardiography - Lower LVEF - Large areas of hypo/akinesis - LV dilatation - Diastolic dysfunction - Mitral regurgitation - Aortic stenosis - LV hypertrophy - LV mass	- Low LV RS at rest - No LV GLS increase in the dobutamine stress test - Pulmonary congestion by lung ultrasound - Inflammation and fibrosis on CMR - Poor viability of the myocardium on stress echocardiography and CMRR - Reduced miBG uptake	
Cardiopulmonary exercise test		
tests pVO2 Short distance in the 6-min walk test V_E/V_{CO2} slope		
Co-morbidity		
Cardiovascular - Ischemic heart disease/prior myocardial infarction - Prior transient ischemic attack/stroke - Peripheral arterial disease - Atrial fibrillation - Ventricular arrhythmia, sudden cardiac death, ICD shocks Non-cardiovascular - Chronic kidney disease - Diabetes - Chronic obstructive pulmonary disease - Sleep apnoea and Cheyne–Stokes breathing	- Smoking - Anemia - Higher red cell distribution width - Higher white blood cell count - Iron deficiency - Liver dysfunction and low albumin - Depression - Senile asthenia - Cachexia - Cognitive dysfunction - Diuretic resistance	

Note. ANP − atrial natriuretic peptide; BNP − B-type natriuretic peptide; CMR − cardiovascular magnetic resonance; CRP − C-reactive protein; DBP − diastolic blood pressure; ESR − erythrocyte sedimentation rate; GDF-15 − growth differentiation factor 15; HF − heart failure; HR − heart rate; ICD − implantable cardioverter-defibrillator; JVD − jugular venous distention; LDL − low-density lipoprotein; LV − left ventricular; LVEF − left ventricular ejection fraction; miBG − metaiodobenzylguanidine; MAP – mean arterial pressure; MR-proADM − mid-regional pro-adrenomedullin; MR-proANP − mid-regional pro-atrial natriuretic peptide; NT-proBNP − N-terminal pro-B-type natriuretic peptide; NYHA − New York Heart Association; pVO2 − peak exercise oxygen consumption; SBP − systolic blood;pressure; VE/VCO2 − minute ventilation–carbon dioxide production relationship; S3 – gallop rhythm.

* is detected according to the presence/absence of congestion (or hypoperfusion) signs.

Out of more than 100 multi-factor predictive models proposed for patients with CHF, the most well-validated are SHFM (Seattle Heart Failure Model), HFSS (Heart Failure Survival Score), MECKI (Metabolic Exercise test data combined with Cardiac and Kidney Indexes), INTERMACS (Interagency Registry for Mechanically Assisted Circulatory Support), MAGGIC (Meta-Analysis Global Group in Chronic Heart Failure), BIOSTAT-CHF (A Systems Biology Study to Tailored Treatment in Chronic Heart Failure), BCN Bio-HF (Barcelona Bio-Heart Failure), and UCLA score (University of California, Los Angeles) [9]. An unacceptably low survival rate during the year in most cases is indicated by an assessment of the prognosis at a level not exceeding 80% (for example, on the SHFM or MAGGIC model) [9].

ESC experts emphasize the prognostic value of the treatment-related factor [9]. Non-compliance by a doctor or patient with the provisions of modern recommendations for treatment of CHF (particularly refusal to use a β-blocker) is associated with a worsening prognosis. However, it should be understood that the term AHF is applicable only to describe the phenotype of treated CHF. If the patient for one reason or another did not receive optimal therapy, then regardless of the clinical severity of decompensation, it is too early to talk about this form of heart failure.

PATIENT ROUTING

All patients with CHF should undergo regular checkups for timely detection of progression of symptoms and signs. The described mnemonic "I need help" is useful for identifying patients with AHF who need a timely referral to a specialized center which uses advanced methods of heart failure treatment that are not available in an ordinary clinic. It stands for the following: I (**I**notropes), N (**N**YHA class), E (**E**nd-organ dysfunction), E (**E**jection fraction), D (**D**efibrillator shocks), H (**H**ospitalizations), E (**E**dema/escalating diuretics), L (**L**ow blood pressure), P (**P**rognostic medication) [56].

Unfortunately, patients with AHF are often referred to advanced heart failure centers too late. The routing scheme developed within the framework of the active screening concept, the goal of which is timely referral of these patients to the appropriate specialized medical centers, is presented in general form in Figure 2 [9]. The concept of active screening justifies referral (if there are appropriate indications) to a local heart failure clinic of patients with CHF symptoms corresponding to NYHA FC II.

When planning the timing of specialized medical care for patients with AHF, you can use the classification of heart failure severity phenotypes provided in the INTERMACS registry (Interagency Registry for Mechanically Assisted Circulatory Support) [9, 57] (Table 3).

In general, only the 2nd, 3rd, and 4th phenotypes are unconditionally related to AHF, since the 1st type corresponds to acute heart failure and the 5th–7th types – to non-severe CHF.

SHORT-TERM TREATMENT STRATEGY

Since a reasonable conclusion about AHF leaves little hope for the success of pharmacotherapy aimed at hemodynamic, neurohumoral, volumetric, myocardial, and immune unloading of the heart, according to the figurative expression of V. Pernias et al. [58], the solution to the problem lies outside the heart (heart transplantation or implantation of a circulatory assist device). However, in a situation where the patient's clinical condition is rapidly deteriorating or there is a reason for assuming reversible dysfunction of end-organs, active pharmacotherapy or temporary mechanical circulatory support may be required. Such a short-term strategy can be lifesaving for a patient who is on the waiting list for a transplant or implantation of a circulatory assist device [9].

The use of non-glycosidic inotropes and vasoconstrictors should be limited to cases of acute decompensated heart failure, low cardiac output syndrome, and cardiogenic shock [9, 59–62]. Allowed medicines include vasoconstrictors (for example, norepinephrine and vasopressin), inotropes with vasoconstrictor properties (for example, dopamine and adrenaline), as well as inodilators, among which, according to some experts, the most promising is levosimendan (its use is permissible in the absence of a pronounced decrease in systolic blood pressure – >85 mmHg) [9, 60].

The cornerstone of congestion correction in these patients is loop diuretics. For the purpose of the so-called decongestion (not to be confused with

anticongestant therapy in the otorhinolaryngologic practice) in case of refractory edema syndrome, including the one associated with the "braking phenomenon", they should be used (after or against the background of systemic arterial hypotension correction) intravenously at a high dose, in combination with one another, with neurohumoral modulators, and drugs improving renal filtration (for example, aminophylline or a "renal" dose of dopamine) and increasing oncotic blood pressure (albumin, blood plasma) [5, 9, 63, 64].

In patients with normal or elevated systemic blood pressure, a combination of diuretics with vasodilators may be effective, the most promising of which are serelaxin (a recombinant analog of human relaxin-2), low doses of nesiritide (a recombinant form of human brain natriuretic peptide), and the vasopressin antagonist tolvaptan (especially in case of hyponatremia of dilution) [63, 65, 66]. Finally, the use of drugs from the group of the sodium-dependent glucose co-transporter type 2 inhibitors (such as dapagliflozin or empagliflozin) in combination therapy can help to shift the process from the "dead point" and achieve the coveted euvolemia in refractory edema syndrome [63, 67, 68].

If other methods of dehydration are ineffective, it is possible to use extracorporeal ultrafiltration (gentle regimes using a minimum volume of extracorporeal blood and an ultrafiltration rate of no more than 250 ml / hour are preferable) and peritoneal dialysis [5, 9, 69, 70].

Temporary (usually from several days to several weeks) mechanical circulatory support (mono- and biventricular) can be indicated in the development of cardiogenic shock, as well as in a situation where it is necessary to gain time deciding on a heart transplant or implantation of an artificial ventricle (ventricles). For this, percutaneous transluminal methods are currently available, including intra-aortic balloon counterpulsation and Impella® ventricular support systems, as well as paracorporal devices (for example, the so-called tandem heart), including combining mechanical circulatory support with blood oxygenation (venoarterial extracorporeal membrane oxygenation) [9, 71–76].

The following terms are used to describe various technologies for temporary mechanical circulatory support in discrete clinical situations [24]:

1. "Bridge to decision" / "Bridge to bridge" – is used in patients with cardiogenic shock existing until haemodynamics and end-organ perfusion are stabilized to exclude contraindications for long-term mechanical circulatory support (for example, brain damage after resuscitation) and consider additional therapeutic options, including long-term ventricular assist device therapy or heart transplantation.

2. "Bridge to candidacy" – the use of temporary mechanical circulatory support can lead to improved function of the end-organs and give the right to heart transplantation to those patients for whom it was previously contraindicated;

3. "Bridge to transplantation" – mono- and biventricular mechanical circulatory support to keep alive patients at high risk of death waiting for a heart transplant (application may take several months or even years since only 10% of these patients will receive a donor heart within a year);

4. "Bridge to recovery" – mechanical circulatory support to keep patients alive until their cardiac function is restored to a level sufficient to remove the circulatory assist device (usually we are talking about a partially reversible cause of CHF, such as acute myocarditis or peripartum cardiomyopathy).

LONG-TERM TREATMENT STRATEGY

Conventional surgical treatment is aimed at correcting etiological factors, as well as the leading mechanisms underlying CHF. We are talking, for example, about revascularization of ischemic but viable myocardium in patients with LV EF < 35%, prosthetics of the aortic valve in severe symptomatic aortic valve stenosis with an average pressure gradient > 40 mm Hg. or in severe aortic regurgitation in all patients with symptoms and asymptomatic patients with LV EF ≤ 50%, as about surgery to correct mitral regurgitation (endovascular placement of the mitral valve clip theoretically looks more justified in a situation of high perioperative risk), including secondary (due to LV dilation) severe mitral insufficiency (especially in patients with LV EF < 30%), which cannot be corrected with the help of pharmacotherapy and electrophysiological methods of treatment [9].

Long-term mechanical circulatory support in the framework of "definitive treatment" technology can be considered as an alternative to heart transplantation in patients with end-stage CHF, in which

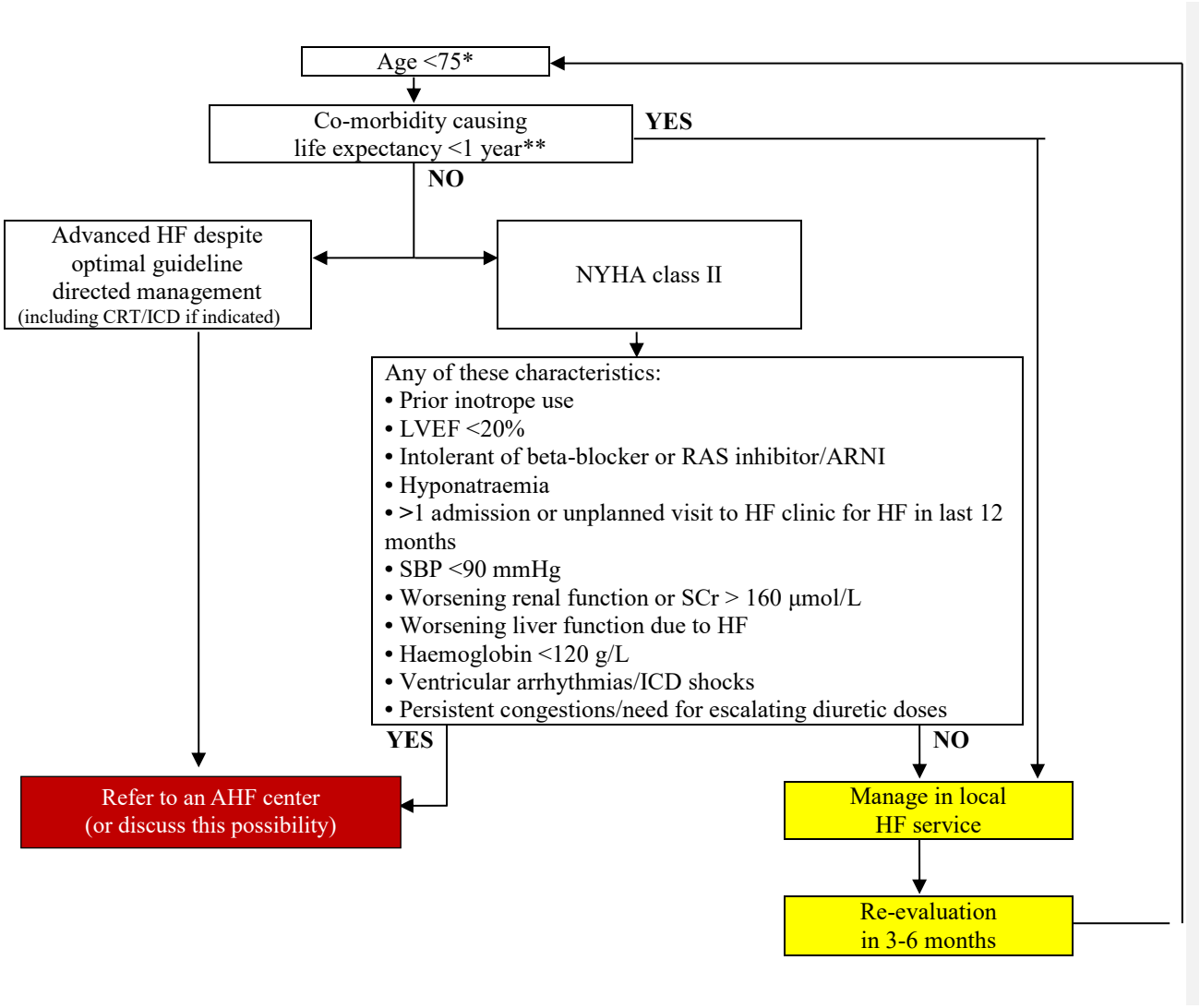

Fig. 2. Triage of patients with advanced heart failure (HF) and appropriate timing of referral [9]: ARNI − angiotensin receptor–neprilysin inhibitor; COPD − chronic obstructive pulmonary disease; CRT − cardiac resynchronization therapy; ICD − implantable cardioverter-defibrillator; LVEF − left ventricular ejection fraction; NYHA − New York Heart Association; RAS – renin-angiotensin system; SBP− systolic blood pressure; SCr − serum creatinine.

* >75 years with good functional status apart from HF (mono-organ disease); ** e.g. incurable cancer, dementia, severe COPD

for objective or subjective reasons, transplantation is not feasible (see below). Naturally, such devices must be implanted and administered only in centers with professionally educated doctors to reduce the risk of complications (secondary infection, pump thrombosis, bleeding, thromboembolism, and dysfunction of the device itself), which, despite continuous improvement in technology, remains a serious problem [9, 24, 77].

The results (patient survival and quality of life) of implantation of circulatory assist devices largely depend on the correct selection of patients for this intervention and the type of device chosen. High survival rates are usually observed among people under 70 years of age, without diabetes, renal failure or cardiogenic shock. In carefully selected groups of patients, implantation of second- or third-generation continuous axial or centrifugal flow devices (that are more effective than devices that use pulsatile pumps) generally provides better results than optimal drug therapy in patients dependent on inotropes.

Table 3

Phenotypes in patients with heart failure [9, 24, 57]

Phenotype	Time frame for intervention
INTERMACS 1: Critical cardiogenic shock Patients with life-threatening hypotension despite rapidly escalating inotropic support, critical organ hypoperfusion, often confirmed by worsening acidosis and/or lactate levels. «Crash *and burn*»	Definitive intervention needed within hours: ECLS, ECMO, percutaneous support devices
INTERMACS 2: Progressive decline Patients with a declining function despite intravenous inotropic support, (may be manifested through worsening renal function, nutritional depletion, and inability to restore volume balance). Also describes declining status in patients unable to tolerate inotropic therapy. «*Sliding on inotropes*»	Definitive intervention needed within few days: ECLS, ECMO, LVAD
INTERMACS 3: Stable but inotrope-dependent Patients with stable blood pressure, organ function, nutrition, and symptoms with continuous intravenous inotropic support (or a temporary circulatory support device or both), but demonstrating repeated failure to wean from support due to recurrent symptomatic hypotension or renal dysfunction. «*Dependent stability*»	Definitive intervention elective over a period of weeks to few months: LVAD
INTERMACS 4: Resting symptoms Patients can be stabilized close to normal volume status but experience daily symptoms of congestion at rest or during ADL. Doses of diuretics generally fluctuate at very high levels. More intensive management and surveillance strategies should be considered, which may in some cases reveal poor compliance that would compromise outcomes with any therapy. Some patients may shuttle between 4 and 5. «*Frequent flyer*»	Definitive intervention elective over a period of weeks to few months: LVAD those in
INTERMACS 5: Exertion intolerant Patients feel comfortable at rest and with ADL, but are unable to engage in any other activity, living predominantly within the house. Patients are comfortable at rest without congestive symptoms, but may have underlying refractory elevated volume status, often with renal dysfunction. If underlying nutritional status and organ function are marginal, patients may be more at risk than INTERMACS 4 and require definitive intervention. «*Housebound*»	Variable urgency, depends upon maintenance of nutrition, organ function, and activity: LVAD
INTERMACS 6: Exertion limited Patients without evidence of fluid overload feel comfortable at rest, with ADL and minor activities outside the home but fatigue after the first few minutes of any meaningful activity. Attribution to cardiac limitation requires careful measurement of peak oxygen consumption, in some cases with haemodynamic monitoring to confirm the severity of cardiac impairment. «*Walking wounded*»	Variable, depends upon maintenance of nutrition, organ function, and activity level: LVAD / Discuss LVAD as option
INTERMACS 7: Advanced NYHA class III A placeholder for more precise specification in future, this level includes patients who are without current or recent episodes of unstable fluid balance, living comfortably with meaningful activity limited to mild physical exertion. «*Placeholder*»	Transplantation or circulatory support may not currently be indicated

Note. ADL – activities of daily living; ECMO – extracorporeal membrane oxygenation; NYHA – New YorkHeart Association; ECLS – extracorporeal life support; ECMO – extracorporeal membrane oxygenation; INTERMACS – Interagency Registry for Mechanically Assisted Circulatory Reviews and lectures

The survival rate after this operation is comparable to the early survival rate after heart transplantation (2-year survival at the level of 76–85%) [9, 24, 77, 78].

The main pros and cons of long-term mechanical circulatory support, formulated by EACTS (European Association for Cardio-Thoracic Surgery) experts [79], are presented in Table 4.

Severe right ventricular dysfunction (for example, in the presence of significant tricuspid regurgitation) is usually considered as one of the main contraindications to implantation of a LV assist device, but it is not an obstacle to heart transplantation [24]. If it is predicted that severe right ventricular dysfunction will be potentially reversible, then temporary (from days to several weeks) extracorporeal devices for mechanical support of the right ventricle can be used in addition to an implanted LV assist device [24]. In patients with irreversible right ventricular dysfunction secondary to left ventricular heart failure, the use of long-term biventricular mechanical circulatory support (using two implantable / extracorporeal pumps or a so-called total artificial heart) should be considered [79–81].

Table 4

Recommendations for evaluation and selection of patients for long-term mechanical circulatory support* [79]
LT-MCS implantation should be considered in patients with the following (Class of recommendation IIa, level of evidence - B): • New York Heart Association functional class IIIB–IV and • Ejection fraction <25% and At least one of the following criteria: – INTERMACS 2–4** – Inotrope dependence – Progressive end-organ dysfunction – pVO_2 <12 ml/kg/min – Temporary MCS dependence
LT-MCS implantation may be considered in patients with (Class of recommendation IIb, level of evidence - B): • New York Heart Association functional class IIIB–IV and • Ejection fraction <25% and a need: – To reverse elevated pulmonary vascular resistance or potentially reversible renal failure in potential heart transplant candidates – To allow time for transplant contraindications to be reversed, such as recent cancer, obesity, and recovering drug and alcohol dependence in potential heart transplant candidates
Patient characteristics associated with a high risk of poor outcome after implantation of a left ventricular assist device (Class of recommendation IIa-III, level of evidence – B-C): • LT-MCS in patients with advanced age, after careful evaluation of comorbidities and senile asthenia, should be considered. • LT-MCS in patients with peripheral vascular disease, depending on its severity, may be considered. • LT-MCS in patients with active systemic bacterial/fungal infection is not recommended. • In patients with well-controlled HIV, hepatitis B or hepatitis C, LT-MCS should be considered. • In patients with diabetes with poor glycaemic control or end-organ complications, LT-MCS may still be considered. • LT-MCS may be considered in patients with chronic dialysis. • LT-MCS implantation in patients with haemostatic deficiencies and coagulopathies may be considered. • LT-MCS implantation in patients with untreated aortic regurgitation or mechanical aortic valve is not recommended. • LT-MCS in patients with untreated severe mitral stenosis is not recommended. • LT-MCS implantation in patients with irreversible liver dysfunction, as diagnosed by liver enzyme laboratory tests and the Model of End-stage Liver Disease score, is generally not recommended. • In patients with poor neurological and cognitive function, LT-MCS implantation is not recommended. • Frail patients and patients with limited mobility may be considered for LT-MCS implantation after careful evaluation. • LT-MCS in patients who are living alone or who are suffering from depression should, after careful evaluation, be considered. • LT-MCS implantation in patients who suffer from dementia is not recommended. • LT-MCS implantation in patients with active substance abuse, not willing to cease the abuse, is not recommended. • LT-MCS implantation in patients with malignancies may be considered, if expected survival is >1 year.

Note. LT-MCS – long-term mechanical circulatory support; INTERMACS – Interagency Registry for Mechanically Assisted Circulatory Support; pVO_2 – peak exercise oxygen consumption; HIV – human immunodeficiency virus.

* It is recommended that reversible causes of heart failure are ruled out; ** Patients with the INTERMACS 3 phenotype will benefit the most [9].

Despite the lack of well-organized controlled studies, the cardiological community is dominated by the view that heart transplantation at the final stage of CHF improves survival (1-year survival rate of about 90% and a median survival rate of 12.2 years), physical performance, and the quality of life to a much greater extent compared to conventional treatment, provided that the appropriate selection criteria are carefully met (the gold standard of treatment for refractory CHF) [9, 82].

Indications for heart transplantation largely coincide with those for long-term mechanical circulatory support, and the list of contraindications is longer and includes additional points, such as high pulmonary vascular resistance or transpulmonary pressure gradient and recently treated cancer [9] (Table 5).

Table 5

Indications and contraindications to heart transplantation [9]

Patients to consider	1. End-stage HF with severe symptoms, a poor prognosis, and no remaining alternative treatment options 2. Motivated, well-informed, and emotionally stable 3. Capable of complying with the intensive treatment required postoperatively
Contraindications	1. Active infection 2. Severe peripheral arterial or cerebrovascular disease 3. Pharmacologic irreversible pulmonary hypertension (LVAD should be considered with a subsequent re-evaluation to establish candidacy) 4. Cancer (a collaboration with oncology specialists should occur to stratify each patient as to their risk of tumour recurrence) 5. Irreversible renal dysfunction (e.g. creatinine clearance <30 mL/min) 6. Systemic disease with multiorgan involvement 7. Another serious comorbidity with a poor prognosis 8. Pre-transplant BMI >35 kg/m^2 (weight loss is recommended to achieve a BMI <35 kg/m^2) 9. Current alcohol or drug abuse 10. Any patient for whom social supports are deemed insufficient to achieve compliant care in the outpatient setting

Note. LVAD − left ventricular assist device; BMI − body mass index; HF − heart failure.

If we recall shortage of donor hearts, the problem of graft rejection, and lack of effective treatment for coronary artery disease of the transplanted heart, it becomes clear why the number of patients receiving permanent mechanical circulatory support is constantly increasing (for example, in Germany alone in 2016, 1,000 implantations of LV mechanical support devices were performed). At the same time, the number of heart transplants in the world has braked at the level of 1994 (about 5,000 per year) [5, 9, 83].

CONCLUSION

AHF is a form of CHF, progression of which has reached a stage where traditional evidence-based therapy becomes ineffective. Patients at this stage persist with symptoms and signs of severe heart failure, often accompanied by episodes of acute decompensation that are associated with an adverse prognosis. It is important to raise awareness of this form of heart failure, since its prevalence in the multilion population of patients with CHF can reach 10%, and it requires timely application of advanced treatment methods, such as heart trans- plantation and mechanical circulatory support and/or transition to palliative care.

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Biochemical, molecular genetic and clinical aspects of COVID-2019

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ABSTRACT

The 2020 coronavirus infection pandemic has potentiated a large number of studies in the world on the etiopathogenesis and clinical and morphological manifestations of COVID-2019 infection. This review presents biochemical, molecular genetic, and clinical aspects of COVID-2019.

Key words: COVID-19, coronavirus, cytokines, polymorphism.

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Биохимические, молекулярно-генетические и клинические аспекты COVID-2019

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РЕЗЮМЕ

Пандемия коронавирусной инфекции в 2020 г. потенцировала проведение большого числа исследований в мире в области этиопатогенеза и клинико-морфологических проявлений COVID-2019. Представлены биохимические, молекулярно-генетические и клинические аспекты COVID-2019.

Ключевые слова: COVID-19, коронавирус, цитокины, полиморфизм.

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INTRODUCTION

The causative agent of the infection, a new coronavirus SARS-CoV-2, which was previously undetected, was identified by Chinese researchers on January 7, 2020 [1]. On February 11, 2020, the new coronavirus infection was named COVID-2019 (Corona VIrus Disease 2019), and the virus causing it was renamed to SARS-CoV-2 (Severe acute respiratory syndrome coronavirus 2) [2]. The coronavirus pandemic in 2020 has potentiated a large number of studies in the world on the etiopathogenesis and clinical and morphological manifestations of COVID-2019 infection.

CHARACTERISTICS AND PATHOGENESIS OF COVID-2019

The first stage of the virus life cycle is receptor adsorption of the viral particle on the surface of the target cell as a result of specific binding of the first subunit of the S1 spike protein to the cell receptor. For SARS-CoV-2, this is the angiotensin-converting enzyme 2 (ACE2) [3–10]. The mechanism of virus-ACE2 binding depends on the cellular serine protease TMPRSS2 [9, 10].

The *ACE* gene is characterized by genetic deletion/insertion (D/I) polymorphism in intron 16, which is associated with changes in circulating and tissue ACE protein concentrations. The D allele is associated with reduced ACE2 expression. Although ACE2 and ACE have only 42% amino acid identity, they both act as carboxypeptidases, cleaving the amino acids from the carboxyl end of the peptides [11]. The D/I polymorphism has significant geographical differences [12]. J.R. Delanghe et al. compared the frequency of the D allele of the *ACE1* gene obtained in 25 different European countries with the prevalence and mortality of COVID-2019. The prevalence and mortality from COVID-2019 infection are inversely correlated with the frequency of the D allele [13].

After receptor binding to the surface of the target cell, the subsequent stages begin. The receptor-mediated endocytosis ends with the penetration of a viral nucleocapsid into the cytoplasm of the host cell, where virion RNA acts as an mRNA for the synthesis of two extended polyproteins pp1a and pp1ab with a length of about 2,000 and 7,000 amino acid residues, respectively. The pp1ab polyprotein includes pp1a and is formed as a result of the ribosome ignoring the stop signal in 20–30% of cases due to a hairpin that displaces the reading frame. Polyproteins ppla and pplab do not exist in the cell as single molecules and are cotranslationally cut by proteases into 16 non-structural (i.e., not part of the virion) proteins that regulate further replication and, in particular, transform the folds of the endoplasmic reticulum into a kind of "factory" for the later stages of virus replication.

One of the most important non-structural proteins is RNA-dependent RNA-polymerase (RdRp), which synthesizes a complementary virion strand of negative-sense RNA, which, in turn, acts as a matrix for synthesis of genomic RNAs that will enter daughter virions. In addition, RdRp synthesizes a series of subgenomic negative-sense RNAs (sgRNAs) on a genomic RNA matrix with a chain break and its transfer to the 3'-end of the matrix, as a result of which all sgRNAs in this series have the same 5' and 3' flanks and central parts of varying degrees of nesting in each other. After that, sgRNAs are used as a matrix for synthesis of positive-sense subgenomic matrix RNAs, from which structural proteins are read. Assembly of daughter virions occurs in the endoplasmic reticulum, and they then leave the host cell by exocytosis [14–17].

Previously, several proteins that can interact with the SARS coronavirus nucleocapsid protein

(SARS-CoV) had been identified. Glycoprotein α-2-Heremans-Schmid (AHSG), required for macrophage deactivation by endogenous cations, was associated with inflammatory process regulation. Rs2248690 variant of the *AHSG* gene (AOR, 1.63; 95% CI, 1.30–2.04) was associated with susceptibility to atypical pneumonia. Rs2248690 affects the transcriptional activity of the *AHSG* gene promoter and, thus, regulates the level of AHSG in the blood. The AA rs2268690 genotype, which leads to a higher concentration of AHSG protein in the blood, is protective for the development of SARS [18].

The site of entry of the pathogen SARS-CoV-2 is the epithelium of the upper respiratory tract and epithelial cells of the stomach and intestines. The initial stage of infection is the penetration of SARS-CoV-2 into target cells that have ACE2 receptors, which are represented on the cells of the respiratory tract, kidneys, esophagus, bladder, ileum, heart, and central nervous system. However, the main and quickly achievable target is the alveolar cells of type II (AT2) in the lungs, which determines the development of pneumonia. The role of CD147 in SARS-CoV-2 cell invasion was also discussed [19].

The pathogenesis of coronavirus infection begins with colonization and destruction of upper respiratory tract epithelial cells by coronavirus. With insufficient immunity, the process passes to the alveoli and is accompanied by the destruction of the surfactant, excessive exudation, and a drastic decrease in gas exchange. In patients who had the disease, type-specific immunity develops and the affected areas of the alveolar walls are replaced with connective tissue [20].

CLINICAL FEATURES OF COVID-2019

Clinical variants and manifestations of COVID-2019 are acute respiratory viral infection (only the upper respiratory tract lesion); pneumonia without respiratory failure; pneumonia with acute respiratory failure; acute respiratory distress syndrome; sepsis and septic (infectious-toxic) shock [19]. At the same time, more than 30% of patients develop hypoxemia (SpO2 is less than 88%) [19]. COVID-2019 can have various manifestations, ranging from no symptoms or mild illness to severe pneumonia [21].

Clinical symptoms of COVID-2019 are fever (in 87.9% of those seeking medical help), usually low-grade temperature (up to 37.5 °C in 56.2%); respiratory symptoms, such as cough (67.7%); in severe cases, shortness of breath (18.6%) and symptoms of intoxication: fatigue and weakness (38.1%), headache (13.6%), dyspepsia (5%) and diarrhea (3.7%). The most frequent manifestations of severe cases are pneumonia (76%) and hypoxia (38%) [22].

Clinical forms of COVID-2019 are the following: asymptomatic (1–3% of cases); mild (with only upper respiratory tract damage); moderate (pneumonia without respiratory failure); severe (pneumonia with respiratory failure, respiratory rate (RR) ≥ 30 per minute, saturation ≤ 93%, PaO_2 / FiO_2 oxygenation index < 300, or the appearance of infiltrates in the lungs in the form ground-glass opacity, occupying more than 50% of the lungs within 24–48 hours); and very severe (critical) form (pneumonia, sepsis, septic shock, multiple organ failure).

Approximately 10–15% of mild and moderate cases (81–82% of all infected patients) develop into severe ones [23]. About 15–20% of severe cases become very severe. A group of high-risk mortality from COVID-2019 should include elderly patients with concomitant diseases, especially with cardiovascular system damage [24]. In patients over 60 years of age, more severe clinical manifestations, greater severity, and a longer course of the disease were detected compared to patients under 60 years of age [21].

The morphological substrate of COVID-2019 infection is diffuse alveolar damage. The virus causes increased permeability of cell membranes and increased transport of albumin-rich fluid to interstitial lung tissue and alveolar lumen; thus, interstitial and alveolar edema develops. In this case, the surfactant is destroyed, which leads to the collapse of the alveoli. Acute respiratory distress syndrome (ARDS) develops following drastic gas exchange disturbances [25–27]. ARDS is very often confirmed in fatal cases of human SARS-CoV-2 infection [28].

Stages of ARDS development are the following. The exudative (acute) stage is manifested through alveolar type I cell damage, increased alveolar-capillary membrane permeability, interstitial and alveolar edema, and filling of the alveoli with leucocytes, red blood cells, and products of damaged cells (alveolar flooding, impaired function and production of endogenous surfactant). The second stage proliferative (subacute) with alveolar type II cell dam-

age, fibroblast migration in the alveolar exudates, proliferation of alveolar type II cells, and reduction of lung edema. The third stage is a fibroproliferative (chronic) phase with oblit- eration of the alveoli and advanced fibrosis of the pulmonary parenchyma.

BIOCHEMICAL AND MOLECULAR GENETIC ASPECTS OF COVID-2019

Genetic susceptibility and inflammatory cytokines have been shown to be associated with ARDS. More than 40 genes, especially *ACE2*, *IL-10*, *TNF*, and *VEGF*, are closely associated with ARDS development [29]. Elevated blood levels of interleukin-6 (IL-6) and interleukin-8 (IL-8) were shown to be associated with an adverse ARDS outcome [30].

It was demonstrated that rapid virus replication and cell damage caused by the virus down-regulation of ACE2 and antibody buildup are responsible for aggressive uncontrolled lung inflammation caused by SARS-CoV-2 [31]. The beginning of rapid replication of the virus can lead to the mass death of epithelial and endothelial cells, causing excessive production of proinflammatory cytokines and chemokines. Research data show that SARS-CoV-2 infection increases markers of inflammation, such as CRP, IL-6, IFNγ, and TNFα [32, 33, 34], which is assumed to be facilitated by a sustained inflammatory response and cytokine storm [35, 36].

It was found that patients infected with COVID-2019 have high blood levels of IL1-β, IFNγ, IP10, and MCP-1, which probably results in activated responses of type 1 helper cells (Th1). In addition, patients requiring hospitalization have higher concentrations of GCSF, IP10, MCP-1, MIP-1a, and TNFα than patients who do not require hospitalization, suggesting that the cytokine storm is associated with the severity of the disease. COVID-2019 infection also initiates increased secretion of type 2 helper (Th2) cytokines (for example, IL-10), which suppress inflammation [33]. To characterize the effect of coronavirus on cytokine and chemokine production in the acute phase of the disease, the blood levels of cytokines and chemokines in patients who were confirmed to have COVID-2019 were analyzed. These data were compared in patients in the intensive care unit (ICU) and patients with a milder form of the disease (comparison group) [33]. Blood cytokines and chemokines (IL1-β, IL1-RA, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8 (CXCL8), IL-9, IL-10, IL-12 p70, IL-13, IL-15, IL-17A, eotaxin (CCL11), FGF2, GCSF(CSF3), GMCSF (CSF2), IFNγ, IP10 (CXCL10), MCP-1 (CCL2), MIP-1α (CCL3), MIP-1β (CCl4), DGFB, RANTES (CCL5), TNFα, and VEGFA) were measured using the 27-PLEX panel for human cytokine analysis of the Bio-PLEX200 system (Bio-Rad, Hercules CA, USA). Blood samples were also taken from 4 healthy people as a control for cross-comparison. It was shown that the initial concentrations of IL-1-β, IL-1RA, IL-7, IL-8, IL-9, IL-10, FGF, GCSF, GMCSF, IFNγ, IP10, MCP-1, MIP-1α, MIP-1β, PDGF, TNFα and VEGF were increased both in ICU patients and in the comparison group, as opposed to the controls. Blood levels of IL-5, IL-12, p70, IL-15, eotaxin, and RANTES (CCL5) were the same in healthy individuals and in patients infected with COVID-2019. Further comparison between ICU patients and comparison group showed that blood concentrations of IL-2, IL-7, IL-10, GCSF, IP10, MCP-1, MIP-1a and TNFα were higher in ICU patients than in the comparison group [33]. J. Lei et al. showed that patients infected with COVID-2019 had increased neutrophils, proinflammatory cytokines, C-reactive protein, D-dimer,and ESR in the blood [37].

Although the mechanism of heart damage is not fully described, there is evidence documenting the effect of COVID-2019 on the cardiovascular system. Thus, a study by C. Chen et al. confirmed that among a group of patients (n = 120) with COVID-2019, there was an increase in troponin (n = 12, 10%) and NT-ProBNP (n = 33, 27.5%), which indicated myocardial damage [38]. Two studies of patients in critical condition demonstrated that 23% (n = 12) of people developed myocardial damage [39], and 33% (n = 7) of patients developed cardiomyopathy [40]. In a study of 138 patients with COVID-2019, 10 patients (7.2%) were diagnosed with acute myocardial damage based on an increase in highly sensitive cardiac troponin I (hs-cTnI), and 8 of them were admitted to the ICU [41]. In another retrospective study, cardiac troponin I (cTnI) was significantly elevated in 33.3% of severe and 100.0% of critical patients [42]. C. Huang et al. and co-authors also identified an increase in the number of COVID-2019 patients with acute myocardial damage and critical conditions leading to ICU ad-

mission [33]. It was also shown that the mortality rate from COVID-2019 pneumonia was significantly higher among patients with cardiovascular or cerebrovascular diseases, which contributed to high mortality in these patients [43].

An increase in the biomarkers of myocardial necrosis in patients with COVID-2019 can provide predictive information for assessing the progression of the disease and the development of adverse events. Since some of the biomarkers are not specific to the myocardium, an increase in these indicators in the blood during the development of adverse events in COVID-2019 patients may reflect damage not only to the myocardium but also to other vital organs or tissues. Thus, in a study of 99 patients with COVID-2019, 75 patients had an increase in the lactate dehydrogenase level, and 13 patients had an increase in creatine kinase [44]. Elevated levels of lactate dehydrogenase were also shown in other studies of patients with laboratory-confirmed COVID-2019 [22, 32]. Renal dysfunction in patients with COVID-2019 was expressed by increased levels of urea and creatinine [41, 45], which was associated with direct exposure to the virus and hypoxia.

Increasing evidence suggest that abnormal biochemical processes in the liver are closely related to the severity of COVID-2019. A study of a cohort of 1,099 patients with COVID-2019 showed that 39.4% of individuals had AST > 40 U / l and 28.1% of individuals had ALT > 40 U / l, and most of the patients had a severe course of the disease [22]. In another multicenter retrospective study involving 32 patients, the average levels of ALT, AST, and bilirubin in the blood of severe and critical patients were significantly higher than in the controls [46]. ALT and AST levels in the blood of patients in severe and critical cases were significantly higher than in mild and moderate cases in other studies [33, 41, 47–49]. According to some data [45, 49], about a quarter of the deceased patients had elevated levels of procalcitonin. It was shown that an increased procalcitonin level (more than 0.5 μg / l) was associated with a fatal outcome (93% probability). Other authors demonstrated that the procalcitonin level was usually within normal values upon admission to the hospital but might increase in patients admitted to the ICU [33, 41, 44].

A retrospective analysis of 99 patients with COVID-2019, held in Wuhan Jinyintan hospital, showed that 36% of patients had increased D-dimer, 16% – a decrease in activated partial thromboplastin time (APTT), 6% – in increase in APTT, 30% – shortened prothrombin time (PT) and 30% – an increase in PT [44]. A retrospective analysis of routine parameters of the coagulation system in 183 patients with COVID-2019 showed that fibrin degradation products (FDP) and D-dimer in the blood of non-survivors were significantly higher than in survivors, whereas PT and APTT were significantly prolonged [50]. A retrospective analysis of 138 patients with COVID-2019 also confirmed that blood levels of D-dimer increased after admission to the hospital [41].

Previous studies demonstrated that elevated D-dimer is an independent risk factor for acute respiratory distress syndrome and mortality in patients with COVID-2019 [48]. Analysis of literature data shows that patients with a severe course of the disease than in patients with mild COVID-2019 [32, 45, 48, 49]. In a series of cases from China, an increase in the concentration of D-dimer in the blood during hospitalization (> 1 μg / ml) was associated with the risk of in-hospital mortality, which was 18 times higher than among patients with normal D-dimer concentrations [49].

It is reported that COVID-2019 should be considered as a disease that leads to increased thrombosis, and it is even proposed to rename COVID-2019 to MicroCLOTS (microvascular COVID-2019 lung vessel obstructive thromboinflammatory syndrome). The authors suggest that in predisposed people, viral alveolar damage is followed by an inflammatory reaction and microvascular pulmonary thrombosis. This progressive endothelial thrombo-inflammatory syndrome can also affect the microvascular bed of the brain and other vital organs, leading to multiple organ failure and death [51].

It was found that severe coronavirus disease can be complicated by coagulopathy, namely disseminated intravascular coagulation, which is quite prothrombotic in nature with a high risk of venous thromboembolism. The incidence of venous thromboembolism among patients with COVID-2019 in the ICU is high. D-dimer level can help in early recognition of these high-risk patients, as well as in prediction of the outcome. Preliminary data show

that in patients with severe COVID-2019, anticoagulant therapy appears to be associated with lower mortality in a subpopulation that meets the criteria for sepsis-induced coagulopathy, or with a markedly elevated D-dimer. Recent recommendations suggest that all hospitalized COVID-2019 patients should receive thromboprophylaxis or full anticoagulant therapy if such indications are present [52]. It is also advisable to use thromboprophylaxis in patients with confirmed coagulation system activation (increased concentration of D-dimer in the blood) upon admission [53].

A number of studies demonstrated a possible link between human leukocyte antigen (HLA) polymorphism and SARS-CoV susceptibility. HLA-B*4601, HLA-B*0703, HLA-DRB4*01010101, HLA-DR B1*1202, and HLA-Cw*0801 are associated with a genetic predisposition to SARS-CoV infection. A possible protective effect was observed for several other HLA alleles, including HLA-Cw*1502 and HLA-DRB1*0301. Genetic variation of HLA A, B, and C, may affect the sensitivity and severity of SARS-CoV-2. It was found that HLA-B*4601 has the smallest number of predicted peptides binding SARS-CoV-2, it is likely that individuals with this allele may be particularly vulnerable to COVID-2019 [54]. HLA-B*1503 demonstrated the greatest ability to represent highly conserved SARS-CoV-2 peptides that are common to human coronaviruses, suggesting that it may provide cross-protective T-cell-based immunity.

Individual genetic variations can help explain different immune responses to the virus in a population. Determining the type of HLA with simultaneous testing for COVID-2019 can improve assessment of the severity of the disease in patients. After developing a vaccine against the SARS-CoV-2 virus that causes COVID-2019, people with high-risk HLA types may have priority for vaccination [55]. In addition to HLA polymorphism, SARS-CoV and MERS-CoV infection susceptibility is also correlated with mannose-binding lectin (MBL) gene polymorphism associated with antigen presentation [56].

A group of researchers from the University of New York identified three signs indicating likely (70–80%) development of acute respiratory distress syndrome and pneumonia: the presence of myalgia, increased hemoglobin levels, and a slight increase in alanine aminotransferase [57].

Researchers at Wuhan University claim that the role of red blood cells in the pathophysiology of COVID-2019 is underestimated. The coefficient of variation in the width of the distribution of red blood cells (RDW) is a predictor of the severity of the condition [58]. According to the new data [59], the SARS-CoV-2 virus is able to secrete non-structural proteins ORF1ab, ORF10, and ORF3a, which easily penetrate the cell membrane of the red blood cell and displace the bivalent iron atom from the porphyrin core of the beta chain of the hemoglobin molecule. A single iron atom is able to transport 4 molecules of oxygen. Thus, destruction of hemoglobin inside the red blood cell takes place The released iron ion contributes to further oxidation of organic molecules. Microhemolysis and hemolytic anemia occur. The authors attribute the occurrence of respiratory failure primarily to the resulting hemoglobin deficiency and oxidative damage initiated by iron ions and hemolysis. In addition to these three non-structural proteins that displace iron from the porphyrin core, the surface glycoprotein of the virus and the protein ORF8 can bind to the heme, which further strengthens the hemolytic potential of the virus. Excess porphyrins in red blood cells can accelerate cell lysis and development of hemolytic anemia [60]. It is hypothesized that critical COVID-2019 patients may experience a form of acquired acute porphyria [61]. Iron settles in the lung tissues, catalyzing oxidative processes and fibrosis [61, 62].

In a meta-analysis of 9 publications containing data from 1,779 COVID-2019 patients, mild thrombocytopenia (140×10^9/l, an average decrease by 31×10^9/l) was observed in patients with a more severe course and was associated with a risk of mortality and severe complications with a five-fold relative risk (OR 5.1) [63]. In the deceased patients, there was an even more pronounced decrease in the number of platelets (123×10^9 /l, decrease by 48×10^9/l).

Z. Varga et al. presented data indicating that SARS-CoV-2 infection contributes to the induction of endotheliitis in several organs as a direct consequence of the virus (noted in the presence of viral bodies) and an inflammatory response of the host. In addition, induction of apoptosis and pyroptosis may play an important role in endothelial cell damage in patients with COVID-2019 [64].

Due to the dominance in the pathogenesis of acute distress syndrome and pneumonia caused by COVID-2019, oxygen delivery disorders associated with intra-erythrocyte and microcirculatory disorders, intravascular coagulation, erythrocyte hemolysis, microthrombosis in lung vessels and intaralveolar fibrin formation, endotheliitis, the clinical and laboratory presentation fits into the framework of chronic hemolytic microthrombovasculitis and secondary chronic disseminated intravascular coagulation (DIC) syndrome.

Rs12252-C/C single-nucleotide polymorphism in the *IFITM3* gene (interferon-induced transmembrane protein 3) is a risk factor for severe influenza and was also detected in a patient with COVID-2019. Interferon-induced transmembrane protein-3 of the rs12252-C genetic variant is associated with disease severity in COVID-2019. Homozygotes for the rs12252 allele in the *IFITM3* gene are associated with a more severe course of the disease depending on age. This confirms the role of *IFITM3* in the pathogenesis of the disease and the possibility of early targeted intervention in high-risk individuals [65]. Susceptibility to the development of pulmonary fibrosis after COVID-2019 may also have a genetic component [66–68].

Molecular genetic studies of patients who have undergone COVID-2019 of varying severity can reveal host body features that explain why some patients carry the disease asymptomatically or in a mild form, while other patients are in a critical condition [69]. The causative agent of the COVID-2019 pandemic outbreak, SARS-CoV-2, is a member of the Coronaviridae family of shell viruses with a single-stranded (ss) RNA genome [70]. SsRNA viruses are recognized by the host's immune system, its first line of defense, through innate pattern recognition (PRR) receptors, such as Toll-like receptor 7 (TLR7), which is a primary sensor for extracellular or endosomal structures of virus-derived nucleic acids.

Gender differences in TLR7 responses were reported for people: female individuals are able to better tolerate hepatitis C, which is also typical of COVID-2019 [71–73]. When binding viral nucleic acid motifs, TLR7 induces expression of type I IFNs (IFN-α and IFN-β) and expression of the recently described type III IFNS family (IFN-λ1–4). Type III IFN is activated during viral infection of the lung and liver epithelium [74]. It was shown that common genetic variations of the germ line at the locus of the type III *IFN* gene determine the host's ability to cope with infection caused by the hepatitis C virus (HCV), a type of ssRNA virus that is tropic to liver epithelial cells. It is assumed that one of the possible variants is a dinucleotide polymorphism in the *IFML4* gene (rs368234815/rs11322783 [TT/GG]), which determines the host's ability to encode the functional protein IFN-λ4 [75].

The knockout variant TT of the *IFNL4* gene is favorable for destroying the virus and resolving the infection, presumably by deactivating the control mechanism of IFNα-desensitizing action that antagonizes the effectiveness of IFNα [76]. In the world, the frequency of favorable knockout variant TT is 0.841, 0.689, and 0.293 among Asian, European, and African populations, respectively. People of different populations may differ in their susceptibility to other RNAviruses, not just to hepatitis C. The study of genetic predictors of disease severity will reveal new targets for intervention against SARS-CoV-2 and COVID-19 infection.

Several international online platforms have been created to concentrate the genetic data of patients obtained by different groups of researchers (https://covid19-hpc-consortium.org; https://www.covid19hg.org/; https://bigd.big.ac.cn/). Platforms involve the exchange of data for their comprehensive analysis.

CONCLUSION

Despite the fact that the lung is the main organ damaged by the virus, COVID-2019 is currently considered a systemic disease affecting a wide range of other vital organs, such as the heart, liver, and kidneys [46, 77, 78]. However, it remains largely unclear whether organ and tissue damage in patients with COVID-2019 is a direct or indirect consequence of viral infection. The clinical features and prognosis of the disease differ in patients of different ages, which can help clinicians worldwide establish risk stratification for all patients with COVID-2019.

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Authors contribution

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The role of neurotrophic growth factors in the pathophysiology of bronchial asthma associated with obesity

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ABSTRACT

Bronchial asthma (BA) and obesity are common diseases with a tendency to a steady and progressive increase in the number of patients. A combination of these diseases is one of the major problems of modern medicine, requiring close attention due to a decrease in the quality of life, poor control over the course of the primary disease, and an increase in the frequency and duration of hospitalization. The association between asthma and obesity is obvious. However, detailed mechanisms underlying it require further investigation. In the last decade, in the formation of the phenotype of BA combined with obesity, much attention has been paid not only to the immune, but also to the neurogenic mechanisms of inflammatory response. It is known that the functioning of all parts of the nervous system can be controlled by neurotrophic growth factors due to their ability to influence many signaling mechanisms. Currently, there is evidence that neurotrophic factors are involved in the pathogenesis of bronchopulmonary and metabolic diseases. The review is devoted to detailed investigation of the mechanisms of neurogenic inflammation in obesity and asthma with participation of neurotrophic factors that may play a significant role in the formation of the obese–asthma phenotype. The study of new mechanisms involved in the pathogenesis of asthma and obesity will make it possible to find common therapeutic targets for this asthma phenotype.

Key words: obesity, bronchial asthma, neurotrophic factors.

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Роль нейротрофических факторов роста в патофизиологии бронхиальной астмы, сочетанной с ожирением

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РЕЗЮМЕ

Бронхиальная астма (БА) и ожирение являются широко распространенными заболеваниями с тенденцией к неуклонно прогрессирующему увеличению числа больных. Сочетанное течение данных заболеваний представляет собой одну из серьезных проблем современности, требующих пристального внимания в связи со снижением качества жизни, ухудшением контроля течения основного заболевания, повышением частоты и длительности госпитализации. Если взаимосвязь между БА и ожирением очевидна, то детализация данных механизмов находится в стадии изучения. В последнее десятилетие в формировании фенотипа БА, сочетанной с ожирением, пристальное внимание уделяется не только иммунным, но и нейрогенным механизмам воспалительной реакции. Известно, что контроль над функционированием всех отделов нервной системы способны осуществлять нейротрофические факторы роста благодаря их способности влиять на множество механизмов сигнализации. В настоящее время имеются данные об участии нейротрофических факторов в патогенезе бронхолегочных и метаболических заболеваний. Обзор посвящен детализации механизма нейрогенного воспаления при ожирении и БА с участием нейротрофических факторов, которые могут играть существенную роль в формировании фенотипа БА, сочетанной с ожирением. Изучение новых механизмов, вовлеченных в патогенез БА и ожирения, позволит найти общие терапевтические мишени для данного фенотипа БА.

Ключевые слова: ожирение, бронхиальная астма, нейротрофические факторы.

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ASTHMA AND OBESITY

Asthma is a widespread disease with a tendency to a steady increase in the number of patients, which is a serious medical and social problem. Globally, about 300 million people suffer from asthma, which accounts for 4–8% of the world population [1]. The number of asthma patients in the world is expected to increase to 400 million within the next five years. High prevalence of this pathology is associated with a decrease in the quality of life of patients, difficulties in controlling the symptoms of the disease, and high treatment costs [2]. According to the International Primary Respiratory Group (IpCRg), one of the reasons for the lack of asthma control is the presence of concomitant pathology, in particular obesity [3].

According to M. Ng et al., more than 600 million adults are obese (BMI ≥ 30 kg / m^2) and about 2.1 billion are overweight (BMI ≥ 25 kg / m^2) [4]. It is predicted that obesity will be diagnosed in 18% of working age population by 2025 [5]. The growing prevalence of obesity and overweightness [6] is observed in both developed and developing countries [7], which harms the global economy. The prevalence of adult obesity has increased from 15.1% in 1980 to 20.7% in 2015; the prevalence of childhood obesity has increased from 4.1% to 4.9% over the same period. In 2015, 417,115 deaths and 14,448,548 disability-adjusted life years (DALYs), years of life changed or lost due to disability, associated with obesity were registered, which accounts for about 10% of the total number of deaths and 6.3% of DALYs among people of all age groups [8]. Despite the progress in studying the etiopathogenesis of obesity and development of new approaches to its treatment, this pathology remains one of the most serious modern problems that resulted in the World Health Organization (WHO) statement on the need to stop the pandemic by 2025 [9].

Overweightness and obesity occur twice more frequently in asthma patients than in the general population [10]. Asthma associated with obesity is one of the urgent medical and social problems requiring particular attention because of a decrease in the quality of life of patients, poor control over the course of the primary disease, and an increase in the frequency and duration of hospitalization [11]. A close relationship between obesity and asthma allows to consider their combination not only as a

comorbid condition but also as an independent phenotype of the disease [12]. This relationship is obvious; however, detailed mechanisms underlying it are still being studied [13]. Several concepts have been proposed to explain the existing relationship [14]. The leading ones are immunological and hormonal concepts revealing the role of systemic inflammation in obesity in the pathogenesis of asthma [15, 16]. At the same time, there has been a growing interest in the role of the nervous system in the pathophysiology of these diseases and its contribution to development of the obese-asthma phenotype.

In the vast majority of cases, asthma is an allergic disease and the immune system plays an important role in its development. A relevant and promising direction of modern research is the study of the neurogenic component of the inflammatory response in this pathology and the role of the nervous system in the development of allergic reactions in asthma [17, 18]. Bidirectional communication between neurons and immune cells was detected. An imbalance in the immune-neuronal communication results in the initiation of neurogenic inflammation. The immune system activates sensory neurons, thereby mediating bronchial hyperresponsiveness, while the interaction between neurons and immune cells results in the development of Th2-mediated immune response [19, 20].

There is increasing evidence that the association between obesity and neurological disorders affects both the peripheral (PNS) and central (CNS) nervous systems [21]. One of the new research directions is focused on studying the neural regulatory mechanism of metabolism in the white adipose tissue [22, 23]. Adipose tissue hormones are responsible for energy homeostasis in the body; therefore, excessive fat accumulation leads to impairment of metabolic processes in various organs and tissues [15, 24]. Obesity is associated with the development of non-insulin-dependent (type 2) diabetes [25], metabolic syndrome [26], neuropathy [27], and several other diseases. Using a mouse model of diabetic neuropathy (leptin-deficient BTBR ob/ob mouse) [21], the role of dyslipidemia accompanying obesity in the development of nervous system dysfunction was demonstrated [28]. Metabolic inflammation in CNS was observed in high-fat diet-induced obesity models [29]. The increased expression of microglia and astrocyte markers in the brain was demonstrated [30]. The same diet-initiated inflammation in the hypothalamus during the first day had persisted for a long period [31]. Currently, metabolic inflammation is being studied in close connection with neurological disorders in obesity [32–34].

Following the presented data, it is clear that the study of new mechanisms in the pathophysiology of asthma and obesity will make it possible to find promising therapeutic targets for treatment of asthma associated with obesity. Neurotrophic factors (NTFs) control the functioning of all parts of the nervous system due to their ability to modulate many signaling mechanisms [35]. Currently, there is evidence that neurotrophic factors are involved in the pathogenesis of neurodegenerative, skin, cardiovascular, psychiatric, bronchopulmonary, and metabolic diseases. Detection of neurotrophins and their receptors in the lungs attracted great attention of researchers to the study of their role in asthma pathophysiology [36, 37]. The involvement of NTF-signaling in the innervation of airways, epithelium, and smooth muscles as well as its presence in immune cells was established. A large body of data emphasizes the key role of sensory neurons in NTF-mediated bronchial hyperresponsiveness. At the same time, studies on the role of neurotrophic factors in the pathophysiology of obesity are few and focused mainly on their role in maintaining energy balance [38].

The review is devoted to detailed investigation of the mechanism of neurogenic inflammation in obesity and asthma with the participation of neurotrophic factors that can play a significant role in the development of asthma associated with obesity.

NEUROTROPHIC GROWTH FACTORS

Neurotrophic factors are a large group of polypeptide compounds. These factors play an important role in the development and functioning of the central and peripheral nervous systems, as well as the immune system. NTFs are involved in regulation of cell growth and differentiation by activating mitogen-activated protein kinases (MAP kinases).

Neurotrophic factors include several families and biomolecules with common properties. The main classification system is based on amino acid sequence homology of neurotrophic factors and in-

cludes four families (neurotrophins, the CNTF (ciliary neurotrophic factor) family, the MANF (mesencephalic astrocyte-derived neurotrophic factor) family, and the GDNF (glial cell line-derived neurotrophic factor) family). However, there are differences in the existing classification among some authors, which makes it possible to differentiate a larger number of families and biomolecules that also belong to neurotrophic factors [39].

Neurotrophins are the neurotrophic growth factor family that include neurotrophin-3 (NT3), neurotrophin-4/5 (NT4/5), brain-derived neurotrophic factor (BDNF), and nerve growth factor (NGF). The family of ciliary neurotrophic factors, or neurokines (neuropoietic cytokines), includes CNTF, leukemia inhibitory factor (LIF), interleukin-6 (IL-6), cardiotrophin-1, cardiotrophin-2, prolactin, growth hormone, leptin, interferons-α/β/γ, and oncostatin M. The MANF family includes MANF (arginine-rich, mutated in early-stage tumors (ARMET)) and cerebral dopamine neurotrophic factor (CDNF).

Another family of neurotrophic growth factors is called glial cell line-derived neurotrophic factors (GDNF-family ligands) and includes GDNF, neurturin (NRTN), artemin (ARTN), and persephin (PSPN). There is a family of neurotrophic factors that consists of epidermal growth factor (EGF), neuregulin, and transforming growth factors alpha and beta (TGFα and TGFβ). The ephrin family contains several members (ephrin A1, A2, A3, A4, A5, B1, B2, and B3).

In addition, some biomolecules were also identified as neurotrophic factors, such as insulin-like growth factor-I/2 (IGF1/2), vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), interleukin-1, -2, -3, -5, -8 (IL-1, -2, -3, -5, -8) and a number of others.

This review summarizes the available literature data on neurotrophins, the most studied family of neurotrophic growth factors, and describes their role in the pathophysiology of asthma and obesity. Special attention is paid to insulin-like neurotrophic factors, the role of which in asthma and obesity is being actively studied today in connection with the emergence of new data on the mechanisms of their action. Based on analysis of the available literature data, it was shown that further study of neurotrophins and insulin-like neurotrophic growth factors is promising as a therapeutic target in asthma associated with obesity.

THE FAMILY OF NEUROTROPHINS AND THEIR ROLE IN THE PATHOPHYSIOLOGY OF ASTHMA ASSOCIATED WITH OBESITY

Neurotrophins (NTFs) are ligands for high-affinity protein tyrosine kinase receptors (TrkA, TrkB, TrkC) that interact with the low-affinity non-tyrosine kinase receptor p75NTR. The p75NTR receptor belongs to TNF receptors. The interaction of NGF with this receptor induces apoptosis at certain stages of cell development. Other functions of NTFs are realized through protein tyrosine kinase receptors. The Trk receptors impact on survival, differentiation, and functional properties of neurons. In particular, the TrkB receptor initiates phosphorylation processes, which leads to an increase in synaptic plasticity. Each of the receptors interacts with a specific site of the neurotrophin molecule triggering a respective signaling cascade [40].

Neurotrophins are produced by smooth muscle and neuroendocrine cells of the lungs. Therefore, NTFs can be involved in the asthma pathogenesis both through interaction with Trk receptors and through an alternative pathway modulating allergic inflammation and airway dysfunction by influencing airway innervation.

In addition to the known effect of neurotrophins on the nervous system, a wide range of regulatory effects of these growth factors on the immune system are described in the literature [35]. Immune cells synthesize NTFs that can bind to receptors expressed by the same cells, influencing the activity of immune system cells through autocrine and / or paracrine interactions [39]. Several types of immune cells (including dendritic cells, mast cells, eosinophils, macrophages, and T and B lymphocytes) are the main source of NTFs in the development of inflammation, in particular, allergic inflammation in asthma.

It is known that $CD4^+$ and $CD8^+$ T lymphocytes express NGF, BDNF, and their receptors. NTFs and their receptors are also found on T helper 1 and T helper 2 cells. It was shown that B lymphocytes synthesize NGF and NT3. Macrophages were reported to have the ability to produce NGF, BDNF, and NT4. It was found that monocytes are able to express protein tyrosine kinase receptors (TrkA), and

polymorphonuclear eosinophils in the bone marrow produce TrkB and TrkC receptors. Eosinophils can not only express neurotrophin receptors but also can store these mediators. The exposure to the allergen from bronchoalveolar lavage fluid (BALF) was accompanied by an increase in NTF receptor expression on eosinophils. In addition, members of the NTF family have the ability to increase the viability of endobronchial eosinophils [40]. Therefore, NTFs not only play an important role in the functioning of the central and peripheral nervous systems, but also have an immunoregulatory effect in allergic diseases, in particular, asthma.

Brain-derived neurotrophic factors

BDNF is produced by epithelial cells, smooth muscle cells, sensory neurons, and some immune cells, such as T cells, macrophages, and mast cells [36, 40]. BDNF stimulates growth of neurons, axons, and dendrites and affects cell apoptosis. The action of this factor is mediated by the ERK signaling pathway (mitogen-activated protein kinase (MAPK) signaling pathway), which is named after ERK (extracellular signal-regulated kinase), the central MAP-kinase, and PI3K/AKT/mTOR signaling pathway, the components of which are enzymes phosphoinositide 3-kinase (PI3K), AKT, and mTOR kinases, that are responsible for proliferation of smooth muscle cells [41]. It was shown that BDNF interacts with TrkB and p75NTR receptors. TrkB expression was found on $CD45^+$ lymphocytes, mast cells, alveolar type 2 cells, and eosinophils [42].

Trigger factors of bronchopulmonary diseases increase BDNF expression by smooth muscle cells [43]. The expression of this factor was found in the airway epithelium [42]. The elevated expression of BDNF is observed in asthma [44], which suggests its involvement in the processes of bronchial remodeling and hyperresponsiveness. It was established that patients with severe asthma have higher levels of mature BDNF isoforms [45]. V. Aravamudan et al. found that type 2 cytokines can regulate the BDNF level in asthma [40]. The relevance of further study of BDNF effects in the airways is undoubted.

The neurotrophic activity of BDNF and its role in inflammation, metabolism, and pathogenesis of cardiometabolic diseases are summarized by the term "triact" that explains the interactions between the brain, the immune system, and the adipose tissue [46]. There is evidence that the level of this factor is closely associated with increased body weight, obesity, type 2 diabetes, and development of metabolic syndrome [47]. Under experimental conditions, it was demonstrated that the elevation of BDNF level improves metabolic regulation by influencing insulin sensitivity in hepatocytes and the function of pancreatic beta cells [48]. It was found that decreased activity of BDNF and its TrkB receptor in the hypothalamus leads to a significant increase in body weight in rodents [49]. However, some results indicate a complex and contradictory relationship between obesity and BDNF levels in children [50]. According to L. Sandrini et al., obesity is not associated with lower circulating BDNF levels [51]. It should be noted that the mechanisms of the development of neurological disorders in obesity are not completely understood. New strategies targeted at BDNF are being developed for treatment of obesity, diabetes, and neurological disorders [52]. It was shown that therapeutic interventions aimed at increasing BDNF expression can have a beneficial effect on the metabolic function, thereby improving neurocognitive parameters in patients with obesity or type 2 diabetes [53]. Obviously, BDNF may be a promising therapeutic target in asthma associated with obesity.

Neurotrophin-3 and neurotrophin-4

Recently, much attention has been paid to the role of NT3 and NT4 in the pathophysiology of bronchopulmonary diseases. Mast cells and eosinophils synthesize NT3. Alveolar macrophages constitutively express NT3 and produce BDNF and NGF in response to allergic stimuli, while interstitial macrophages constitutively express only BDNF. The NT4 factor is involved in the innervation of the lungs [42]. The TrkB receptor has the highest affinity for NT3 and NT4. The TrkC receptor is only activated by NT3. Both NT3 and BDNF, acting through TrkB and TrkC, are able to induce nitric oxide production, thereby facilitating bronchodilation. Patients with asthma exhibit an increased NT3 level in BALF [42, 54]. In a rodent model of allergic asthma, it was demonstrated that the use of NT3 results in switching from noncholinergic innervation to cholinergic one [55]. At the same time, the role of NT3 and NT4 in obesity as well as their contribution to the pathophysiological mechanism

of development of asthma associated with obesity has not been studied.

Nerve growth factors

NGF is one of the most studied members of the protein family of neurotrophic factors. The TrkA receptor has the highest affinity for NGF. The presence of p75NTR is necessary to increase the affinity of TrkA for neural growth factor. This factor is synthesized by astrocytes and has a neurotrophic effect. In addition to the ability of NGF to stimulate the survival of neurons and their repair after damage described by R. Levi-Montalcini [56], it is involved in the control of the main cellular processes, such as oxidative stress, apoptosis, and neurogenesis. Data are indicating that NGF regulates the survival and activity of immune cells, fibroblasts, cardiomyocytes, epithelial cells, mast cells, and adipose tissue cells [57], which makes the study of its role in the pathophysiology of asthma and obesity relevant.

The results of experimental studies carried out on rodent models indicate the relationship between the enhanced NGF level and the development of allergic inflammation. For example, exposure to tobacco smoke was accompanied by an increase in NGF expression [58]. Asthma patients are characterized by an elevated NGF level [59] that correlates with the level of eosinophils, the main effector cells in this pathology [60]. There is evidence of a simultaneous increase in the number of mast cells and NGF levels in chronic inflammation [61]. NGF is produced by both $CD4^+$ and $CD8^+$ T lymphocytes. Under experimental conditions, the role of NGF in modulating the balance of Th1 and Th2 responses of T cells in asthma was shown [62]. Enhanced NGF secretion by Th2 cells was identified, which may be directly related to allergic asthma. In addition, NGF is involved in airway remodeling in asthma [59]. Genomic studies revealed the relationship between NGF, rs6330, and TrkA rs6334 in asthma patients [54].

It should be noted that the role of NGF in the pathophysiology of chronic inflammation in asthma remains to be investigated. The modulation of NGF level affecting the activity of immune cells will make it possible to correct intersystem relationships in asthma. It is one of the topical research fields focused on the search for a new strategy for treating this pathology.

Recent study results have shown that the levels of two neurotrophic factors, NGF and BDNF, are altered in cardiometabolic diseases (atherosclerosis, obesity, type 2 diabetes, metabolic syndrome). These observations have underlain the hypothesis of metabotropic deficiency of NGF/BDNF that plays an important role in the pathogenesis of cardiometabolic diseases [38, 46]. The concept of NGF metabotrophicity is based on the fact that two neurotrophic factors, NGF and BDNF, can act as metabotrophins due to their involvement in maintaining cardiometabolic homeostasis [38].

The data presented above confirm that further studies on the role of these factors in the development of asthma associated with obesity are required.

THE ROLE OF INSULIN-LIKE NEUROTROPHIC FACTORS IN THE PATHOPHYSIOLOGY OF ASTHMA ASSOCIATED WITH OBESITY

The system of insulin-like neurotrophic factors includes ligands (IGF-1 and IGF-2), high-affinity proteins (IGFBP 1–6) that bind them, and receptors. This hormonal network is involved in the processes of cell proliferation, differentiation, and apoptosis.

IGF-1 synthesized by hepatocytes is the most important mediator of the biological action of growth hormone. IGF-1 is one of the proinflammatory mediators involved in the pathogenesis of various diseases, in particular metabolic disorders, inflammatory bronchopulmonary pathology, and lung cancer [63, 64]. The expression of IGF-1 signaling components was observed in the cells of the respiratory tract, smooth muscles, lung parenchyma, and in alveolar macrophages. H. Lee et al. demonstrated that IGF-1 and IGFBP-3 signaling pathways contribute to the pathogenesis of asthma [65]. The mechanism of action of corticosteroids in asthma therapy appears to be associated with inactivation of IGF-1/IGF-1R signaling [66, 67]. Due to the existing relationship between IGF-1 and Th2 and Th17 cells involved in asthma pathogenesis, the immunoregulatory role of IGF-1 in this pathology is being actively studied [68]. S.R. Kim et al. showed that IGFBP-3 reduces allergic inflammation and airway hyperresponsiveness in asthma by inhibiting IGF-1 activity [69]. The role of IGF-1/IGF-1R in the regulation of phagocytic activity of airway cells in asthma was revealed [70].

IGF-1 has an insulin-like metabolic effect and does not affect lipolysis or lipogenesis. In contrast to insulin, the biological activity of IGF is regulated by high-affinity binding proteins that influence the metabolic homeostasis and can directly participate in the molecular regulation of insulin signaling. Currently, IGFBP-1 and IGFBP-2 are considered as biomarkers and promising therapeutic targets in obesity and diabetes [71]. Therefore, this proinflammatory mediator can be a novel promising target for therapeutic measures in asthma associated with obesity.

CONCLUSION

Asthma and obesity are common diseases and a combination of these diseases is one of the pressing global problems because of the decrease in the patients' quality of life and the increase in the frequency and duration of hospitalization. Detailed mechanisms underlying the relationship between these diseases and a pathogenetic target for their effective therapy have been actively studied. In the last decade, along with the immune component of the inflammatory response, attention of researchers has been focused on studying the role of the neurogenic component in the pathophysiology of asthma associated with obesity.

Over the past 30 years, the role of neurotrophic growth factors has been to the largest extent studied in diseases of the nervous system, and a search for a potential therapeutic target is extremely urgent at present. At the same time, NTFs are expressed by many cells and involved in the pathogenesis of bronchopulmonary and metabolic diseases. Therefore, these factors can play a significant role in the development of asthma associated with obesity. However, there are few research works regarding the role of neurotrophic factors in the pathophysiology of asthma and obesity; moreover, there are no data on their contribution to the development of asthma associated with obesity. We hope that this review will draw attention to the complex relationship between neurotrophins, nerve, and immune cells in respiratory diseases, in particular, asthma associated with obesity.

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The possibility of using radiology modalities in the diagnosis of crystalline arthropathy

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ABSTRACT

A review of current techniques of instrumental and laboratory diagnosis of crystalline arthropathies is presented. Advantages and disadvantages of various methods of diagnostic radiology and diagnostic radiologic criteria employed in primary and differential diagnosis of crystal deposits are discussed in relation to their etiology and clinical peculiarities. It is proven from a wide pool of published studies that the method of ultrasonic diagnosis is the most available one, has no contraindications, and demonstrates the best sensitivity and specificity in the diagnosis of crystalline arthropathy.

Key words: diagnostic ultrasound, diagnostic radiology, crystalline arthropathies, gouty arthritis, hydroxyapatite deposition disease, calcium pyrophosphate-deposition disease.

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Возможности применения лучевых методов исследования в диагностике кристаллических артропатий

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РЕЗЮМЕ

Представлен обзор современных методов инструментальной и лабораторной диагностики кристаллических артропатий. Обсуждаются достоинства и недостатки лучевых методов исследования в выявлении критериев и построении дифференциального ряда среди различных по природе кристаллических депозитов.

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Показано, что метод ультразвуковой диагностики обладает высокой чувствительностью и специфичностью в диагностике кристаллических артропатий.

Введение в диагностическую практику ультразвукового метода исследования позволит значительно повысить точность диагностики кальцификатов с последующим своевременным назначением лечебной и профилактической тактики пациентами с данным типом артрита, а новый набор диагностических критериев применительно к ультразвуковому исследованию в артрологии может способствовать дальнейшему повышению точности диагностики кристаллических артропатий.

Ключевые слова: УЗИ, радиология, кристаллические артропатии, подагрический артрит, гидроксиапатитная артропатия, пирофосфатная артропатия.

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

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INTRODUCTION

Joint diseases are the most common pathology of the musculoskeletal system, leading to disability among all age groups of the population [1]. Rheumatic diseases with joint damage include such nosological forms as rheumatoid arthritis, psoriatic arthritis, and reactive arthritis. Lesions of the joints of the peripheral skeleton belong to a large group of spondyloarthritis. Most rheumatic diseases (RD) affect the structures of the human musculoskeletal system and, thus, significantly reduce the quality of life and limit opportunities for active social life [2].

According to the American Rheumatology Society, the distinction of up to 200 nosological units of rheumatic pathology is justified [3]. Older persons are most susceptible to metabolic and degenerative rheumatic diseases. About 40% of people over the age of 70 suffer from osteoarthritis, and 25% of them cannot tolerate daily physical activity. More than half of patients with rheumatoid arthritis are unable to work within 10 years after the onset of the disease, and 80% of them constantly experience severe pain, significantly impairing the quality of their life [1].

Timely diagnosis of early arthritis is a serious problem of modern rheumatology. Among patients with rheumatic diseases who sought medical care for the first time, 41% of cases were incorrectly diagnosed with rheumatic diseases at the outpatient stage. Only in 49% of patients, the diagnosis was confirmed after a specialist examination [2].

Another problem is high cost of managing patients with RD. This is determined by the complexity and high cost of research methods, as well as the need for long-term and sometimes lifelong treatment of many, especially young, patients using expensive conservative, surgical methods, sanatorium-resort, and social and psychological rehabilitation.

The nature of pathological changes, their localization and prevalence, and the patterns of involvement of different groups of joints provide a differential diagnosis between various rheumatic diseases.

A special place in the list of joint diseases is occupied by crystalline arthropathies due to the complexity of diagnosis and the peculiarities of the clinical presentation. Arthropathies include a group of diseases characterized by deposition of microcrystals of various chemical composition in the joints and periarticular tissues. The main nosological forms of inflammatory polyarthritis associated with the deposition of crystal structures are gout (deposition of urate crystals), pyrophosphate arthropathy (deposition of calcium pyrophosphate crystals), and hydroxyapatite arthropathy (deposition of hydroxyapatite crystals) [1, 3].

THE MAIN NOSOLOGIES OF INFLAMMATORY POLYARTHROPATHIES

Gout (gouty arthritis) is a chronic progressive joint disease characterized by disruption of purine metabolism and increased content of uric acid in the blood, followed by monosodium urate depo-

sition in the tissues and formation of gouty tophi surrounded by fibrovascular tissue. Gouty tophi are painless nodular formations ranging from 5 mm to 10 cm. The period of their formation from the onset of the disease is 5–6 years. The prevalence of gout is 3.9% in the US, 10.9% in France, 1.4–2.5% in the UK, 1.4% in Germany, and 3.2–6.1% in New Zealand [3].

The formation of gouty tophi in the articular and periarticular tissues is accompanied by the development of gouty arthritis (gouty arthropathy). Recurrent acute mono- or oligoarthritis of the joints of mostly the lower extremities – the metatarsophalangeal joint of the 1st finger and the interphalangeal joint of the 3rd finger, dominates in the clinical presentation of gouty arthritis. This does not exclude the involvement of other small and large joints of the peripheral (hand, knee, shoulder, elbow, ankle, and hip joints) and axial (sacroiliac joints, spine joints) skeleton in the process, with rapid development of acute pain syndrome, reaching its maximum within 6–12 hours, and subsequent chronic pain syndrome.

With a chronic course, damage to target organs, in particular, kidneys, progresses with the development of chronic renal failure. In the absence of treatment, the attack-free periods are shortened, arthritis attacks become more frequent, their intensity and duration increase, and "new" joints get involved.

During visual examination, attention is drawn to hyperemia and hyperthermia of the skin in the joint area, pain upon palpation, and formation of protruding areas at the tophus level [4]. Prolonged exposure of the underlying structures to tophi leads to damage to the soft tissue structures of the joint, articular hyaline cartilage, and the underlying bone tissue. Periarticular and intraarticular tophi affect the formation of erosions – marginal destructions, usually with clear external contours.

A standardized and fundamental method of detecting erosions is radiography, which has limitations in early diagnosis of diseases of the joints. Radiographic signs of gout appear in the late chronic period, usu ally no earlier than 6 years [5] after the onset of the disease, and are almost always present in patients with subcutaneous tophi.

Hydroxyapatite crystal deposition disease (HADD) is a disease of unknown origin, characterized by joint pain syndrome in combination with the deposition of hydroxyapatite crystals in the periarticular tissues, mainly in the tendons, joint capsules, sacs, and synovial membrane.

There are primary (idiopathic) and secondary calcium-hydroxyapatite arthritis. The latter develops in chronic renal failure, diffuse diseases of the connective tissue, and after traumatic injuries. Calcinosis of individual tumors is also hydroxyapatite crystal deposition.

The most common localization of the deposition of hydroxyapatite crystals is the shoulder joint, less often the femur (large trochanter), the elbow joint, the wrist and knee joints, as well as the joints of the hand, foot, and lumbar spine. Hydroxyapatite arthropathy can affect the tendons of other muscles, such as the gluteus medius or the thigh muscles. The deposition of crystals often occurs at a distance of 1 cm from the place where the tendons attach to the bones. Calcifications of various sizes and shapes can be deposited in articular sacs and capsules.

The disease is more common in middle age, more often in men, and proceeds according to both mono- and oligoarthritis pattern [6]. It may occur acutely or have a chronic recurrent character. It is characterized by soft tissue edema and limited mobility in the affected joints. An asymptomatic course of the disease is also possible.

Visualization or detection of calcifications is crucial in determining the cause of the pain syndrome. The most sensitive in detecting calcifications in the tendons and ligaments are radiological methods, such as plain radiography and computer tomography (CT).

Calcium pyrophosphate dihydrate crystal deposition disease (CPPD crystal deposition disease, pseudogout or pyrophosphate arthropathy) is an inflammatory joint disease of the mono-or oligoarthritis type with acute, subacute, and chronic course. It is the third most prevalent after rheumatoid arthritis and gout among inflammatory arthritis. The frequency of CPPD crystal deposition disease increases with age [7].

The development of CPPD crystal deposition disease is accompanied by the formation and deposition of calcium pyrophosphate dihydrate crystals (CPPD) in the joints and resulting inflammation [8], pseudogout forms of inflammatory arthritis, [9] and a possible asymptomatic course of the disease [10]. The most commonly affected joints are the knee joints, followed by the joints of the upper extremi-

ties, the hip joint, and the joints of the foot, as well as the pubic symphysis [11].

The clinical presentation of CPPD crystal deposition disease is often similar to that of gout (pseudogout) with a longer period of development and less pronounced pain syndrome. At the same time, hyperthermia, hyperemia, swelling, and concretion of soft tissues may occur in the area of the affected joint. An acute attack of CPPD crystal deposition disease may be accompanied by fever.

The chronic course is characterized by a less pronounced clinical picture, rare involvement of the metacarpophalangeal joints in the pathological process, the absence of extra-articular symptoms, and morning stiffness. Against the background of a chronic disease course, acute attacks may occur as well.

With a complicated course, a destructive (rheumatoid-like) form of the disease may appear, requiring differential diagnosis with all erosive arthropathies, as well as with pseudo-neuropathic arthropathy, characterized by an increased sensitivity to palpation and the absence of obvious neurological symptoms. Rheumatoid-like and pseudo-neuropathic forms present a significant diagnostic problem.

In an acute attack, especially with monoarthritis of a large joint, septic arthritis can develop in some cases. In these cases, it is important if the patient had similar attacks in the past. Besides, the absence of "septic" changes in the blood, the noticeable effect of antibiotics, and characteristic changes on X-ray images are essential. The appearance of acute synovitis should always lead to the consideration of septic arthritis as a differential diagnosis.

METHODS OF INSTRUMENTAL AND LABORATORY DIAGNOSTICS

The leading place in the diagnosis of crystalline arthropathies is occupied by radiation methods of investigation, which include radiography, tomographic methods (CT and magnetic resonance imaging (MRI)), and ultrasound. The features of visual manifestations of certain forms of crystalline arthropathies are determined by the chemical composition of the crystals.

Computed tomography. Urate crystals, which lead to the formation of gouty tophi, are non-radiopaque in *gouty arthritis*, so in radiography and CT, bone changes at the site of tophus deposition have the form of erosion – marginal lytic destruction with clear contours. In gouty arthropathy, erosions have a rounded or oval shape are of various sizes and are accompanied by a periosteal reaction in the form of a thin rim of bone density surrounding the soft-tissue structure of the tophus completely or partially.

Large expansion and severity of intra-articular tophi, as well as long duration of the process lead to widespread extensive destruction of the adjoining articular surfaces. With para-articular localization of the tophus, the usual width of the articular fissure is maintained for a long time, since its deposition at the level of the hyaline cartilage does not occur simultaneously over its entire surface. In case of violation of calcium metabolism, calcified inclusions can be visualized in the tophi.

Small bone defects may resemble erosions in rheumatoid arthritis, psoriatic arthritis, calcium pyrophosphate arthropathy, or cysts that do not have specific manifestations. When using single-energy CT, gouty tophi look like low-density bone defects with more accurate (compared to radiography) determination of the prevalence, localization, nature of the periosteal reaction, and calcified inclusions in disrupted calcium metabolism in tophi.

In the last decade, dual-energy CT (DECT), a modified computed tomography based on the use of low- and high-energy X-rays (80 and 140 kV) to obtain images of various types of tissues, has been used in clinical practice.

Registration of the difference in the attenuation of the X-ray beam by urates and trabeculae, supplemented by color coding of urates and calcium in the joints and surrounding tissues, allows for the identification of urate salts *in vivo* with high accuracy, regardless of their size and localization.

DECT provides a qualitative and quantitative assessment of monosodium urate crystals and shows good sensitivity and specificity in predicting gout compared to the synovial fluid analysis. The sensitivity of the method is 85–100% and the specificity is 83–92% [12, 13].

Deposits of hydroxyapatite crystals and calcium-pyrophosphate crystals in *hydroxyapatite arthritis and calcium-pyrophosphate arthritis* are accompanied by the formation of chondrocalcinosis, so X-ray methods are highly sensitive in their detection. In radiography, chondrocalcinosis (CC)

manifests itself in the form of point and linear areas of calcification in the projection of hyaline and fibrocartilage. Typical sites of calcification include the fibrous tissue of the the knee joint meniscus, the triangular fibrocartilage disc of the wrist joint, the temporomandibular joint disc, and the intervertebral disc.

Radiography is still a popular method in detection of chondrocalcinosis. At the same time, the prevalence and localization of chondrocalcinosis are being discussed in publications. Therefore, A. Abhishek et al. (2012) conducted an X-ray study of the knee, hip, and hand joints among a representative group of 3,170 volunteers [14]. Signs of CC were detected in 428 (13.7%) people. The most frequent localization of CC was the knee joint (8%). At the same time, its fibrocartilage was affected more often (88.5%) than hyaline cartilage (55%). It was more often affected in lateral (89.1%) than medial (74%) aspects.

The prevalence of CC in the wrist joint was 6.9%, in the hip joint – 5%, in the pubic symphysis – 3.6%, and in the meta carpophalangeal joint – 1.5%. The age of the patient did not correlate with the localization of CC in the hyaline cartilage compared to the fibrocartilage.

Radiologically, crystalline deposits are visualized as structureless areas of induration, varying in size and density. Periarticular calcifications have a linear, triangular, rounded, or oval shape and are localized according to the place of tendon attachment. Large tumor-like areas of calcification occur in patients with chronic renal failure or diffuse connective tissue diseases.

In *osteoarthritis*, the prevalence of calcification deposits in the tissues of the ankle joint is 51.3% [15], in the shoulder join – 98.9% [16], in the hip joint – 96.6% [17], and in the knee joint – 4.3–100% [17, 18]. Computed tomography has high sensitivity and specificity in the diagnosis of joint tissue calcification. In the work of M. Devyani et al. (2014) using single- and dual-energy CT, the presence of CPPD crystals in several structures of the knee joint was demonstrated [19].

In 2013, S. Touraine et al. performed a high-resolution CT on 68 knee joints (34 pairs of joints) from postmortem donors with an average age of 84 years. The results showed calcified fibrocartilage in 34% and calcified hyaline cartilage in 21% of the knee joints [20]. This study also revealed a high prevalence of CPPD deposits in the periarticular tissues of the tibia.

A broader CT study of the knee joints in a group of 608 patients conducted in Japan demonstrated a clear correlation between the presence of CPPD crystals and the depth of cartilage degeneration in the knee joint, confirming the opinion that crystal deposition is associated with cartilage thinning [21]. However, the reasonability of using this modality due to the presence of radiation exposure, its cost, and the issues of CT accuracy requires additional research aimed at justifying the availability of this method in the diagnosis of crystalline arthropathies (or in the CC detection) [22].

Magnetic resonance imaging. Among crystalline arthropathies, MRI is highly informative in the diagnosis of gout based on the visualization of both heterogeneous, mainly the hypo-intensive T2 WI and FSat tophus, and fibrovascular tissue surrounding tophus, clearly visualized after contrast enhancement. However, MRI is rarely used to visualize crystals in other crystalline arthropathies (hydroxyapatite and calcium-pyrophosophate arthropathies) due to the fact that the crystal structures do not generate a signal.

The use of MRI as an imaging method in conditions associated with the deposition of CPPD crystals often leads to misdiagnosis [23]. The insensitivity of MRI to the detection of calcified crystals in the cartilage tissue was confirmed by the study of B. Dirim et al. (2012). It showed that 75% of CPPD crystal deposits were missed when using MRI with a field strength of 1.5 T in the study of the cadaveric knee joint [24]. In this regard, it is justified to conduct a greater number of studies to determine the possibilities of MRI diagnostic methods in crystalline arthropathies for using the techniques in routine diagnostic practice.

Light methods of investigation (light microscopy, polarized light microscopy, phase-contrast microscopy) are currently the standard in the detection of CPPD crystals in the synovial fluid in the diagnosis of patients with CPPD [25]. In CPPD, crystals can be detected in the synovial fluid even in a previously non-inflamed joint, which is also a characteristic feature of gout. On the one hand, the phenomenon of the formation and persistence of crystals in the joints is extremely stable and does

not depend on the stage and period of the disease. On the other hand, CPPD crystals have weak refraction or do not refract the light at all [26]. This means that they are poorly visualized and require more experience and time (looking at a minimum of 30 fields of view), and sometimes special calcium coloring. A prerequisite for the visualization of CPPD crystals is their high concentration in the synovial fluid. A low concentration of CPPD crystals in the synovial fluid can lead to a negative result, due to the difficulty of detecting them in a single sample of synovial fluid.

Ultrasound diagnostics. To date, there is no doubt about the relevance of ultrasound in arthrology as a non-invasive and safe method, which hashigh informative value and is a valuable diagnostic tool for the accurate assessment of intra-articular and para-articular structures involved in a wide range of rheumatic diseases in adults and children [27]. In the modern literature, the issues of ultrasound diagnostics in the study of RA patients are widely covered and include assessment of the thickness and nature of synovial vascularization, the state of the state of synovial tissue in chronic arthropathies, and the activity of the inflammatory process [7, 23, 28–32].

Along with the indicated field of ultrasound use in arthrology, the literature data of the last decade indicate the increasing role of ultrasound in the diagnosis of crystalline arthropathies and high diagnostic effectiveness of the method in detecting small crystal deposits [7, 23, 28–32]. Ultrasound imaging of tissues is based on acoustic resistance of tissues with their reflection of ultrasound and formation of an image of the object under study as acoustic reflections. This makes it possible to better differentiate the crystalline bodies from the surrounding tissues (hyaline cartilage, synovial fluid, etc.) due to the greater number of acoustic signals reflected from them [32].

In *gouty arthropathy*, ultrasound is used not only to determine intra-articular exudate, which is noted in various phases of the disease, but also to visualize the tophi and clarify the condition of the hyaline cartilage. Tophi in ultrasound imaging are characterized as heterogeneous, mainly hyperechoic formations, which may be surrounded by a more hypoechoic surface rim. Additionally, the signs of gout include a hyperechoic surface of the hyaline cartilage, in contrast to the localization of the hyperechoic line in the thickness of the articular cartilage, which occurs in calcium-pyrophosphate arthropathy [33].

Ultrasound can visualize hyperechoic point and linear areas in the projection of soft tissues, the snowstorm sign in the synovial fluid due to the presence of small, rounded particles of different echogenicity, as well as bone erosion [33]. The fibrovascular tissue surrounding the inflammatory process results in the presence of color loci when using Doppler color flow mapping.

High accuracy of ultrasound in the detection of gouty tophi is described in the work of M. Gruber et al. (2013) [31]. A study of 21 patients with clinical suspicion of chronic or acute gout in 37 joints showed a comparable sensitivity of DECT (67.6%) and ultrasound (64.7%).

Different variants of the visual pattern of calcified intraarticular and para-articular structures according to ultrasound data in hydroxyapatite and calcium-pyrophosphate arthropathies were described [7, 28, 34–36].

Thus, in patients with *hydroxyapatite arthropathy*, four morphological forms of tendon calcification were identified: arc-shaped (a hyperechoic arc with clear acoustic shadowing), nodular (a single hyperechoic focus without an acoustic shadow), fragmentary (two or more hyperechoic foci with or without acoustic shadowing), fragmented and cystic (hyperechoic band with anechoic or low-echogenic content) [36]. The nodular, fragmented, and cystic forms are associated with the acute symptomatic phase of calcific tendinitis, while the arc-shaped form is more consistent with the chronic or asymptomatic phase [36].

For visualization of CPPD deposits in the study of patients with calcium-pyrophosphate arthropathy, A. S. Ellabban et al. (2011) proposed the following ultrasound criteria. Criterion I: thin hyperechoic bands parallel to the surface of the hyaline cartilage; criterion II: a “point pattern” consisting of several thin, glittering hyperechoic areas; criterion III: homogeneous hyperechoic nodular or oval deposits, often mobile, localized in the articular sacs and recesses [37].

In the same study, the results of ultrasound of 60 patients with exudate in the knee joint showed the following. Criterion II was detected in 30 pa-

tients (criterion II alone – in 21 patients, and in combination with criterion I and/or criterion III – in 9 patients), criterion III alone was detected separately in 2 patients. Criterion II was identified in all 18 patients with signs of CC of the wrist joint, which makes it the most common one. On ultrasound, CPPD deposits are represented by hyperechoic inclusions with clear acoustic shadowing with a diameter of >10 mm. However, the appearance of acoustic shadowing is possible at the early stage of the disease with a crystal diameter of up to 2–3 mm.

When visualizing CPPD deposits, criterion II is most often found inside the cartilage tissue or tendons of the joint [38]. Moreover, ultrasound has demonstrated successful detection of CPPD crystals in periarticular tissues that were not radiologically visualized [39].

In the course of the conducted studies, the ultrasound method showed fairly high sensitivity and specificity (from 60–85% to 90.6–100%, respectively [29, 30, 34, 37, 40], as well as successful identification of the criteria for CPPD deposit occurrence [34, 37]. However, the sensitivity of ultrasound to detect CPPD varied depending on the structure under study, ranging from 34% (tendon) to 80% (hyaline cartilage).

Low sensitivity in detecting calcifications at the tendon level is probably determined by late involvement of these structures in the pathological process. [35]. In addition, low sensitivity of ultrasound to the visualization of calcinates in tendon tissues may be associated with their high echogenicity or late involvement in the pathological process [35].

The sensitivity indicators for detecting chondrocalcinosis ranged from 55–81% for hyaline cartilage and up to 68–100% for fibrocartilage. The best diagnostic results were achieved when the cartilage tissue itself was visualized in more than one joint.

CPPD deposits on the surface of the hyaline cartilage can imitate the "double contour" (in the form of an additional band of increased echogenicity) of uric acid deposits in gout [41, 42]. Since this pattern is considered the most specific sign of gout, it is necessary to conduct more studies to improve the accuracy of ultrasound diagnostics in the visualization of crystal structures in gout in order to establish a specific nosological form of joint damage [43].

The values of sensitivity and specificity of ultrasound also depend on the reference method used in the study. Thus, the use of knee cartilage biopsy as a reference method resulted in lower sensitivity and specificity of ultrasound results, which may be explained by the presence of too small CPPD crystals in the tissue. [7].

In the last decade, **ultrasound elastography** has been introduced into clinical practice, particularly for cirrhotic liver lesions.

Quasi-static ultrasound elastography is based on the assessment of the elasticity of tissues by comparing images before and after their compression. The possibilities of the method vary depending on how the mechanical stress in the tissues is created (by static or dynamic compression), and on the method of result evaluation. The method shows high efficiency in the study of superficially located organs, especially the mammary and thyroid glands [44].

Shear wave elastography (dynamic elastography*)* is based on the use of transverse waves, as opposed to longitudinal waves emitted by sensors in traditional ultrasound diagnostics. The method is used in the diagnosis of cirrhosis and pronounced fibrotic changes in the liver, although it cannot be considered reliable for detecting the onset of the pathological process. A serious disadvantage of the method is the inability to obtain a two-dimensional picture with sufficient resolution [44].

Indications for elastography are gradually expanding, and in recent years, publications have begun to appear that provide data on the use of elastometry in the study of soft tissue structures of the peripheral and axial skeleton [45–47]. The findings of the studies by E.E Drakonaki et al.(2012) showed that this method may be even more sensitive than MRI or B-mode imaging with detection of subclinical changes in the muscles and tendons [40].

A common problem of most of the conducted studies is insufficient correctness of the methodological aspects of the research: a small number of studied patients, incorrect material selection for comparative characteristics of the obtained results, and a relatively small number of studies performed in general [7].

CONCLUSION

The literature data indicate a current indicate the current demand for ultrasound examination for the identification of crystal structures in joint diseases,

in particular, in crystalline arthropathies. This is determined by the physical capabilities of the method, which provides visualization of the echogenicity of crystal structures, the presence of acoustic shadowing typical of calcified structures, as well as determination of the exact localization of calcinates. All of the above-mentioned justifies the use of ultrasound as a valuable tool in assessing the state of joint structures in various types of joint dam- age, including crystalline arthropathies.

However, to date, contradictory data on the results of ultrasound in the study of patients with crystalline arthropathies have been published. Thus, there is relatively high variability in the sensitivity of the method, depending on the structure under study: cartilage or tendon. There is no definite clarity in the differentiation of hyperechoic deposits, similar in structure (urate crystals, pyrophosphate and calcium hydroxyapatite, crystals of other etiologies).

At the same time, the possibilities of ultrasound are not fully applied. Currently, the B-mode is mainly used in diagnostics, while additional introduction of shear wave elastography can significantly expand the diagnostic potential of this modality. There is no clarity on ultimate possibil- ities of ultrasound in the diagnosis of crystal- line arthropathies due to the insufficient number of studies on this topic with the use of the same criteria and the same groups of joints.

Radiography, computed tomography, and polar- ized light microscopy continue to provide important information in the diagnosis of CPPD crystals. In combination with ultrasound, these methods emphasize the polyarticular and systemic nature of CPPD crystals, high rate of calcification of not only cartilage, but also ligaments and tendons, as well as inflammation and destruction of tissues associated with the crystal deposition. Introduction of ultrasound into diagnostic practice will significantly improve the accuracy of calcification diagnosis, followed by the timely administration of therapeutic and preventive tactics in patients with this type of arthritis.

Further research is required to determine the possible potential of the method. A new set of diagnostic criteria applied to ultrasound in arthrology may further improve the accuracy of examination. It is also necessary to study the reliability of the method and bring the research results to a consensus.

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Modern scintigraphic methods for assessing myocardial blood flow and reserve

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ABSTRACT

Background. Today, myocardial perfusion scintigraphy is an informative and accessible method for evaluating ischemic changes in the heart. However, this method has limitations, which are more connected with a semi-quantitative assessment of the study results. Currently, there is a class of specialized gamma cameras with cadmium zinc telluride detectors, which allow for quantitative analysis of scintigraphic data on coronary hemodynamics, i.e. evaluate indicators of coronary blood flow and reserve.

The aim of the review was to present and summarize the information about the coronary circulation under physiological and pathological conditions, as well as the possibilities of modern radionuclide methods in assessing coronary blood flow and reserve.

Materials and methods. In the process of preparing the review article, "PubMed", "Web of Science", "ScienceDirect", and "Elibrary" research databases were used. Search requests included such key words as: coronary artery disease, myocardial blood flow, coronary (myocardial) flow reserve, single photon emission computed tomography, cadmium-zinc-telluride, positron emission tomography.

Results. The review includes information on the state and methods of regulating coronary hemodynamics under normal conditions and against the background of pathological changes. It also includes information about radionuclide methods for assessing coronary hemodynamics which were used in the past, are currently being used, and promising ones,including dynamic single photon emission computed tomography.

Conclusion. The potential of dynamicsingle photon emission computed tomography as a method for quantification of coronary blood flow and reserve is high. This technique can become a simple and affordable alternative to the existing methods for assessing coronary (myocardial) blood flow and reserve. This will increase the information content of radionuclide diagnostics in assessing the severity of coronary insufficiency for more accurate risk stratification and determination of appropriate treatment strategy for cardiac patients.

Key words: dynamic single photon emission computed tomography, myocardial blood flow, coronary (myocardial) flow reserve, coronary artery disease, coronary artery atherosclerosis.

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Современные сцинтиграфические методы оценки миокардиального кровотока и резерва

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РЕЗЮМЕ

Введение. Перфузионная сцинтиграфия миокарда на сегодняшний день является информативным и доступными методом оценки ишемических изменений сердца. Однако эта методика, как и любая другая, имеет ряд определенных ограничений, которые в большей степени связаны с полуколичественной оценкой результатов исследования. В настоящее время существует класс специализированных гамма-камер с детекторами на основе кадмий-цинк-теллура, позволяющих проводить количественный анализ сцинтиграфических данных о состоянии коронарной гемодинамики, т.е. оценивать показатели коронарного кровотока и резерва.

Цель обзора – представить и обобщить сведения о физиологии кровообращения сердца в норме и при патологии, а также возможностях современных радионуклидных методов в оценке коронарного кровотока и резерва.

Материалы и методы. В процессе подготовки обзорной статьи использовались научные базы данных PubMed, Web of Science, ScienceDirect, Elibrary. Поисковый запросы включали ключевые слова: coronary artery disease, myocardial blood flow, coronary (myocardial) flow reserve, single-photon emission computed tomography, cadmium-zinc-telluride, positron emission tomography, ишемическая болезнь сердца, миокардиальный кровоток, однофотонная эмиссионная компьютерная томография, позитронная эмиссионная томография, резерв коронарного (миокардиального) кровотока.

Результаты. Обзор включает в себя сведения о состоянии и способах регуляции коронарной гемодинамики в условиях нормы и на фоне патологических изменений, радионуклидных методах оценки состояния коронарного русла, имеющих историческое значение, использующихся в настоящее время и перспективных, в том числе динамической однофотонной эмиссионной компьютерной томографии.

Заключение. Потенциальные возможности динамической однофотонной эмиссионной компьютерной томографии как метода количественной оценки коронарного кровотока и резерва высоки. Эта методика может стать простой и доступной альтернативой существующим способам оценки коронарного (миокардиального) кровотока и резерва, что позволит повысить информативность радионуклидной диагностики в оценке тяжести коронарной недостаточности, а значит будет способствовать более точной стратификации риска и определению походящей тактики лечения кардиологических пациентов.

Ключевые слова: динамическая однофотонная эмиссионная компьютерная томография, миокардиальный кровоток, резерв коронарного кровотока, ишемическая болезнь сердца, атеросклероз коронарных артерий.

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

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INTRODUCTION

Today, quantitative analysis of myocardial perfusion is believed to be the most informative method in assessing ischemic changes in the heart [1]. The main quantitative indicators reflecting the state of coronary hemodynamics are myocardial blood flow (MBF) and coronary flow reserve (CFR) [2]. However, methods for determining MBF and CFR are complex, expensive, and, therefore, are prac- tically not used in clinical practice. A new type of cadmium zinc tellurium (CZT) gamma cameras allows to perform dynamic single photon emission computed tomography, which was previously unavailable. This technology makes it possible to assess the MBF and CFR scintigraphic parameters.

The aim of this review was to present the normal and pathological physiology of cardiac circulation, as well as capabilities of advanced radionuclide methods in assessing parameters of coronary hemodynamics, i.e. assessment of MBF and CFR.

"PubMed", "Web of Science", "ScienceDirect", and "Elibrary" research databases were used in preparing as coronary artery disease (CAD), myocardial blood flow, coronary (myocardial) flow reserve, single photon emission computed tomography (SPECT), cadmium-zinc-telluride, positron emission tomography (PET); acronyms were also used, such as CAD, CFR, MBF, MFR, SPECT, CZT, PET.

The review includes information on the state and methods of regulating coronary hemodynamics under normal conditions and against the background of pathological changes, as well as on radionuclide methods for assessing myocardial perfusion which are of historical significance, are currently being used and promising ones, including dynamic single photon emission computed tomography (SPECT).

PHYSIOLOGICAL AND PATHOLOGICAL FOUNDATIONS OF CORONARY CIRCULATION

Understanding the physiology and pathophysiology of coronary circulation and such terms as coronary autoregulation, MBF, CFR (relative and absolute), and microvascular resistance is necessary for a correct clinical interpretation of a quantitative analysis of MBF and CFR.

Anatomy and physiology of coronary circulation

Approximately 5% of the circulatory minute volume flows into coronary arteries (CA) during the diastole phase. This is approximately 250 ml / min for a 300 gram heart muscle at functional rest. Thus, myocardial blood flow may vary from 0.3 to 0.8 ml / min / g.

Epicardial arteries, having low resistance, determine only ~5% of vascular resistance at rest [3, 4]. Arterioles outnumber arteries and determine 60% of vascular resistance [4]. Coronary capillaries account for ~25%, while venules and veins account for the remaining 10% [4].

According to the Hagen – Poiseuille equation, the pressure gradient that provides blood flow is inversely proportional to the vessel diameter in the fourth power. Thus, it means that even minimal reduction of vessel internal diameter leads to a significant decrease in the pressure gradient. Considering that proximal (i.e. epicardial) coronary arteries have a diameter of 3–4 mm [5], they represent lower resistance to blood flow than arterioles, the diameter of which is 20–200 μm [6].

Coronary arterioles are the main resistant vessels and determine myocardial blood flow conditions. [7]. The arteriole muscular wall allows for coronary autoregulation and metabolic vasodilation. Coronary autoregulation describes the capacity of the heart to maintain steady myocardial perfusion across a range of perfusion pressure [8, 9].

Capillaries are the smallest components of the heart vasculature (5–10 μm). However, this vascular structure provides 25% of vascular resistance, and at any given time it may contain up to 90% of the total blood volume of the heart muscle. Therefore, the functional condition of the capillaries determines myocardial blood flow to a greater extent than the tone of arterioles.

MECHANISMS REGULATING CORONARY VASCULAR TONE

The main mechanisms that regulate vascular tone include: 1) metabolic; 2) myogenic; 3) endothelium-dependent [8]. These three groups of factors affect arterioles depending on their diameter. [6].

Metabolic factors affect small arterioles (< 40 μm) [10, 11]. An increase in myocardial metabolism leads to an increase in the concentration of adenos-

ine [12], carbon dioxide [11], as well as in the level of acidosis [13, 14]. These metabolites penetrate into the interstitial space and interact with smooth muscle cells [15]. This interaction leads to arteriole vasodilation and increased myocardial perfusion.

The myogenic mechanism prevails in regulating the tone of arterioles with medium diameters (40–100 μm) [16, 17]. Calcium channels of smooth muscle cells (SMC) open in response to increased distension. Increasing intravascular blood pressure leads to vasoconstriction, and vice versa, when the intravascular blood pressure decreases, the intracellular Ca^{2+} concentration falls, leading to relaxation of the SMC and vasodilation. This form of myogenic control maintains stable arterial tension and is one of the mechanisms of vascular tone control [16].

The tone of large arterioles (more than 100 μm), as well as coronary arteries, is regulated mainly by the endothelium. The interaction between the blood flow and endothelial cells triggers the process of NO synthesis from L-arginine via endothelial NO synthase [18]. Then, NO diffuses into the underlying layer of smooth muscle cells of the vascular wall and activates soluble guanylate cyclase, which, in turn, converts guanosine triphosphate into cyclic guanosine monophosphate (cGMP). As a result, cGMP gives a signal to the smooth muscle cells to relax, thus vasodilation occurs.

The implementation of these mechanisms leads to the fact that the coronary blood flow increases by 4–5 times at stress and is equal to 5–6 ml / min / g. Therefore, an adequate level of myocardial blood supply is maintained [19].

MBF at rest depends on cardiac oxygen demand and myocardial contractility. Cardiac oxygen demand is determined by the heart rate and blood pressure, as well as myocardial contractility. It has been proven that women have higher MBF. In addition, it is known that taking medications, such as beta-blockers, can affect MBF, even at functional rest. The myocardial blood flow value also depends on age, the presence of endothelial dysfunction, left ventricular fibrotic changes, and anemia. [20].

The main factors which affect the magnitude of stress-induced MBF include submaximal coronary dilation, anatomical remodeling of the macro- and microcirculatory bed, increased microvascular resistance, fibrotic changes, heart denervation, systemic inflammation, and risk factors (diabetes, arterial hypertension, smoking, hypercholesterolemia). In addition, the use of caffeinated products can reduce coronary vasodilation [21].

AUTOREGULATION IN CORONARY ARTERY STENOSIS

Experimental studies by K.L. Gould and L. Lipscomb showed that myocardial blood flow remained stable until the narrowing of the coronary artery to ~85% of the diameter [22]. This was determined by great possibilities of coronary autoregulation. Further narrowing of the coronary artery could lead to a decrease in MBF at rest. One of the drawbacks of this study was the inability to assess the effect of microcirculatory dysfunction. This is explained by fact that the animals used are, as a rule, young and do not yet have disorders at the microvascular bed level.

The results obtained by K.L. Gould and K. Lipscomb were confirmed in a clinical study evaluating the impact of coronary autoregulation mechanisms in patients with coronary artery disease [23, 24]. The IDEAL study showed that patients with more severe atherosclerotic lesions had an increase in the transtenotic pressure gradient, but the MBF did not change. This was due to a decrease in microvascular resistance.

These experimental and clinical data indicate that in moderate stenosis, it is coronary autoregulation that ensures the stability of myocardial perfusion due to processes occurring at the microvasculature level. For this reason, these kinds of stenosis do not cause myocardial ischemia at rest and such patients remain asymptomatic. However, under conditions of increased loads on the cardiac muscle, compensatory mechanisms at the microcirculation level are depleted, and the epicardial artery stenoses become a limiting factor that prevents adequate MBF.

CORONARY BLOOD FLOW RESERVE

The ratio of stress-induced MBF to blood flow under functional resting is described as coronary flow reserve (CFR) [20]. CFR depends on the following factors: 1) MBF at rest; 2) perfusion pressure in arterioles; 3) extravascular coronary resistance; 4) cross-sectional area of arterioles per unit volume of myocardium [20].

CFR is a relative index and is determined by the total capacity of the coronary arteries in hyperemia

and at physiological rest and reflects the hemodynamics of the micro- and macrocirculation [20]. This distinguishes CFR from the fractional flow reserve (FFR), which is defined during invasive coronary angiography using a specialized transducer as the more distal to stenosis / more proximal to stenosis pressure ratio against the background of pharmacologically induced hyperemia. Thus, using FFR, it is possible to assess only the blood flow decrease in large epicardial arteries.

RADIONUCLIDE METHODS FOR ASSESSING CORONARY BLOOD FLOW AND CORONARY FLOW RESERVE

One of the first methods for assessing MBF and CFR was myocardial scintigraphy with ^{131}I-labeled macroaggregated albumin (^{131}I–MAA) [25]. The method consists in injecting a radiopharmaceutical (RP) with ^{131}I–MAA directly into the left ventricle and temporary embolization of the capillary bed of the coronary arteries [25]. Heymann et al. played the most significant role in the development, validation, and implementation of myocardial scintigraphy with ^{131}I-MAA [25]. In the experimental work of these authors, the scintigraphic data were compared with results of invasive measurement of MBF. A strong correlation between MBF velocity and the distribution of microspheres was shown [25].

Further studies demonstrated reliability of the ^{131}I-MAA method and recommendations for its clinical use [26–28]. Additionally, the safety of myocardial scintigraphy with ^{131}I-MAA was demonstrated in a clinical study by W.L. Ashburn et al. [29].

There are very few works by Russian authors devoted to the assessment of MBF using ^{131}I-MAA. The main contribution to the study of this quantitative assessment method of left ventricular myocardial perfusion was the research of a scientific group led by A.Z. Eventov [30]. In the works of this group, the fundamental possibility of using ^{99m}Tc for the labeling of human serum albumin microspheres was demonstrated. The method was validated, and its diagnostic capabilities were shown.

Positron emission tomography (PET) has been named the "gold standard" for evaluating MBF and CFR [31]. The fundamental possibility and validation of this method for MBF and CFR evaluation have been shown in a large number of experimental and clinical studies [1, 2, 32–34]. In particular, it was shown that only $[^{15}O]H_2O$ has a direct linear correlation between the extraction fraction value and MBF. Based on this, we can conclude that PET with $[^{15}O]H_2O$ is the most accurate method for estimating MBF and CFR. Other PET tracers (Rb, NH_3), due to the peculiarities of pharmacokinetics, describe the change in the dynamics of the MBF value worse, and the results of studies using these radionuclides are approximate [33].

However, due to the greater availability of radiopharmaceuticals, PET with Rb or ^{13}N is more commonly used in clinical practice. In addition, it should be noted that $[^{15}O]H_2O$ is not available for the practical use in the USA.

Wide diagnostic capabilities in the MBF and CFR assessment in patients with various cardiac pathologies were also demonstrated in a large number of different types of studies. The article by A. Kaufmann et al. proves the advantage of quantitative data analysis over visual evaluation of PET results. A large number of studies [34–37] demonstrated the prognostic significance of quantitative myocardial PET perfusion in assessing the risk of unfavorable cardiac events. A decrease in CFR < 1.5 is associated with a 16-fold increase in the cardiac death risk. [38].

However, the use of PET in cardiology practice is limited by such factors as the unavailability of PET scanners, radiopharmaceutical synthesis systems, as well as injection equipment. According to the European Commission for Health and Consumer Protection, PET accounts for about 6–7% of all radioisotope studies [39]. Therefore, SPECT is still the most common method of radionuclide diagnostics.

The first scientific research in MBF and CFR determination using SPECT was based on collected data of the first RP bolus passage through the cardiac cavities and ventricular myocardium and the calculation of the global retention index in the planar mode. This method made it possible to determine the global retention index of the tracer. Therefore, the CFR value was calculated based on the retention index, taking into account the activity of the RP in the pulmonary artery [40].

A strong correlation of the obtained indicator with the FFR value was demonstrated (r = 0.85, p < 0.001) [40, 41]. Yoshinori et al. [42] compared the CFR evaluated by SPECT with ^{99m}Tc-Sestami-

bi and PET with [^{15}O]H_2O. However, the SPECT method lowered CFR values in comparison with PET [42].

Thus, a tomographic mode was used to estimate the regional values of MBF and CFR by fast rotating the gamma-camera detectors. Using this recording technique, Cuocolo et al. [43] showed a strong correlation ($r = 0.85$, $p < 0.001$) between the scintigraphic CFR index and intracoronary Doppler data: 1.36 ± 0.43 vs. 1.39 ± 0.42, respectively.

Similar results were obtained by Hsu et al. [44]. The authors did not find significant differences between MBF and CFR determined by SPECT with ^{99m}Tc-Sestamibi and PET with ^{13}N-ammonium. There were no significant differences in the studied parameters: MBF at rest was 0.78 ± 0.14 ml / min / g vs. 0.78 ± 0.22 ml / min / g ($p = 0.929$), MBF against the background of the stress test was 2.80 ± 0.39 ml / min / g vs. 2.83 ± 0.54 ml / min / g ($p = 0.766$), CFR was 3.58 ± 0.47 vs. 3.67 ± 0.47 ($p = 0.472$) in the group of healthy volunteers; CFR at rest was 0.83 ± 0.24 ml / min / g vs. 0.74 ± 0.31 ml / min / g ($p = 0.088$), CFR against the background of the stress test – 1.95 ± 0.66 ml/min/g vs. 1.93 ± 0.78 ml/min/g ($p = 0.813$), CFR was 2.4 ± 0.78 vs. 2.53 ± 0.72 ($p = 0.601$) in coronary artery disease (CAD) patients for PET and SPECT, respectively. As in the previous work, there was an insignificant downward shift in the MBF and CFR indices determined by SPECT compared to PET. In addition, the authors demonstrated high inter-and intra-operative reproducibility of quantitative SPECT results.

A group of authors from Japan, led by T. Tsukamoto [45], compared the CFR values determined by dynamic SPECT with ^{99m}Tc-MIBI and PET with [^{15}O]H_2O. As a result, a strong correlation between MBF and CFR values estimated by these methods was shown ($r = 0.84$, $p < 0.0001$). However, the MBF values according to the SPECT data were significantly lower compared to the PET results. The authors pointed out that the modification of the formula for calculating MBF values could improve the accuracy of the SPECT method for quantitative analysis of myocardial perfusion.

However, it must be noted that a significant disadvantage of the conventional SPECT is the inability to perform dynamic data collection in the tomographic mode. In addition, Anger-type gamma cameras are significantly inferior to PET in terms of temporal and spatial resolution [46].

Today, there is a new generation of gamma cameras with cadmium zinc telluride (CZT) detectors, as well as a subclass of specialized cardiac devices. Such gamma cameras have high sensitivity and resolution [48]. Tomographic three-dimensional images are generated during data collection. This, in combination with new algorithms for reconstruction of scintigraphic images, makes it possible to perform dynamic SPECT and evaluate the MBF and CFR indicators [49].

One of the fundamental works on assessing the capabilities of CZT gamma cameras to evaluate MBF and CFR is a study by Ruddy et al. [50]. In the experiment on large animals, the authors compared the MBF and CFR values determined by SPECT with three radiopharmaceuticals: ^{201}Tl, ^{99m}Tc-Tetrofosmin, and ^{99m}Tc-Sestamibi. Scintigraphy with ^{99m}Tc-MAA was chosen as a reference method. A strong correlation between tracers and the reference method was found. Thus, MBF values correlated better with ^{201}Tl ($r = 0.81$) and to a lesser extent with ^{99m}Tc preparations with 0.56 (Tetrofosmin) and 0.38 (Sestamibi). However, according to the RCC indicator, a strong correlation was found with all investigated RPs: ^{201}Tl ($r = 0.81$), ^{99m}Tc-Tetrofosmin ($r = 0.82$), ^{99m}Tc-Sestamibi ($r = 0.8$) [50].

One of the first clinical studies was a work of a scientific group led by S. Ben-Haim from the Institute of Nuclear Medicine, University College London [51]. The authors demonstrated the practical possibility of evaluating MBF and CFR using dynamic SPECT and showed high reproducibility of the results. Additionally, it was shown that the CFR value was statistically significantly lower in the group of patients with angiographically significant coronary artery stenoses, compared to patients without them. In addition, CFR significantly decreased as the degree of coronary artery stenosis increased. However, the authors emphasize the need for further clinical validation of this method.

A study by B. Bouallègue et al. [52] compared the global and regional CFR values determined by dynamic SPECT with the results of invasive coronary angiography and FFR in patients with severe multivessel coronary artery disease. According to the data obtained, the global CFR determined

for the entire left ventricle significantly correlated with the number of vessels with stenosis ($r = 0.70$, $p < 0.001$); and its regional value (determined for the coronary artery pool) was associated with both the degree of stenosis and the FFR value. At the same time, ROC analysis showed that the sensitivity, specificity, and diagnostic accuracy of this indicator for assessing the hemodynamic significance of coronary artery stenoses were 89%, 82%, and 85%, respectively.

It should be noted that, in contrast to MBF, the FFR value does not reflect the microcircular conditions. Comparison of the two methods does not contradict logic; however, certain inaccuracies will inevitably arise in the results of such an analysis. Based on the foregoing, a direct comparison of PET and dynamic SPECT data is more correct from the point of view of the coronary microcirculation physiology.

Nikoulou et al. performed such kind of comparative analysis between the results of dynamic SPECT with ^{99m}Tc-tetrofosmin and PET with ^{13}N-ammonium. The authors did not find differences for MBF at rest, but dynamic SPECT lowered the stress-induced MBF values compared to PET [53]. The sensitivity, specificity, and diagnostic accuracy of dynamic SPECT in identifying ischemia with a cut-off CFR value of 1.26 were 70, 78, and 75%, respectively. The authors emphasize that the CFR determination on CZT cameras can be used in clinical practice as an alternative to PET.

A comparative analysis of the results of dynamic SPECT and cardiac magnetic resonance imaging in patients with known or suspected CAD was carried out in the work of Fang et al. According to the results obtained, stress-induced MBF, assessed using the above-described methods, showed a strong correlation ($r = 0.76$). The ROC analysis showed that, with a stress-induced MBF of 1.32 ml / g / min, the sensitivity, specificity, and diagnostic accuracy of dynamic SPECT in identifying obstructive coronary artery disease were 94%, 90%, and 93%, respectively. Scintigraphic assessment of CFR was not performed in this study.

A study by Miyagawa et al. [55] was devoted to the assessment of the coronary hemodynamics in patients with multivessel CAD. It showed that CFR correlated with left ventricular ejection fraction, FFR, and SYNTAX Score. The sensitivity and specificity of dynamic SPECT in the identification of multivessel coronary artery disease were 93.3% and 75.9%, respectively. The sensitivity and specificity of dynamic SPECT in the identification of multivessel coronary artery disease with a cut-off CFR value of 1.3 was 93.3 and 75.9%, respectively.

Significant results considering the dynamic myocardial SPECT technique were obtained by Wells et al. [56]. The authors investigated the effect of such factors as the attenuation correction, motion correction and binding of the tracer to red blood cells on CFR and MBF. According to the data obtained, corrections for the displacement of the patient's body and for the residual activity of RP in the blood pool increased the accuracy of the MC and RCC assessment using dynamic SPECT compared to PET. However, the correction of attenuation did not increase the accuracy of the scintigraphic technique. Additionally, the authors showed a correlation between the scintigraphic values of MBF and CFR and the PET data with $[^{15}O]H_2O$.

In 2018, the WATERDAY study [57] was carried out, aimed at assessing the MBF and CFR values based on dynamic SPECT data. The study included patients with stable ischemic heart disease. All patients underwent dynamic SPECT, PET with $[^{15}O]H_2O$, and invasive coronary angiography with FFR assessment. The authors showed high interoperable reproducibility of the scintigraphic method of MBF and CFR assessment. Also, a strong correlation between dynamic SPECT and PET data and the FFR value was found. Additionally, the sensitivity, specificity, accuracy, and positive and negative predictive values of the scintigraphic CFR were calculated for the identification of ischemia: 83.3%, 95.8%, 93.3%, 100% and 85.7%, respectively, and for the detection of hemodynamically significant stenosis (FFR $\leq$ 0.8): 58.3%, 84.6%, 81.1%, 36.8% and 93%, respectively.

In the work in the nonselective group of patients with an established diagnosis and suspicion of coronary artery disease, Zavadovsky et al. [58] showed a strong positive correlation between MBF, absolute and relative CFR, and FFR value: $p = 0.63$ ($p < 0.001$), $p = 0.66$ ($p < 0.01$), and $p = 0.73$ ($p < 0.01$), respectively (a cut-off CFR value $\leq$ 1.48). The sensitivity and specificity of dynamic SPECT with the assessment of quantitative myocardial perfusion indices for identifying

the hemodynamic significance of coronary artery stenosis were 69.2% and 93.3%, respectively. In another study, the same group of authors [59] showed a decrease in global CFR in patients with multivessel CAD compared to the control group: 1.39 (1.12; 1.69) and 1.86 (1.59; 2.2), $p < 0.001$. Thus, the sensitivity and specificity of dynamic SPECT in the identification of multivessel CAD were 81.8% and 66.7%, respectively.

CONCLUSION

Despite the technical differences between PET and SPECT, the methods used to evaluate MBF and CFR are largely similar. Moreover, these methods practically do not differ from one of the first methods for determining MBF and CFR – scintigraphy with 131I and ^{99m}Tc-MAA. The basic principle of the above-described methods is to evaluate the retention index of the radionuclide tracer and convert it to the myocardial blood flow value, using various mathematical algorithms and models. In the studies analyzed, various techniques were used for both dynamic SPECT and the processing of the results obtained (Table).

Most studies (62.5%) used a common radiopharmaceutical, ^{99m}Tc-MIBI. The bolus principle of RP injections was used in 75%. Therefore, short frames were more often used to construct the "activity-time" curve. In half of the studies, a one-compartment model was used to evaluate the dynamic myocardial SPECT data. This is determined by the fact that this model is more common and is the base model in processing PET data. At the same time, the Net Retention model has been used more recently.

Table

Analysis of various techniques for conducting and processing the results of dynamic SPECT

Author	Gamma camera	Type/administration method of RP	Stress agent	Algorithm of reconstruction	Model of reconstruction	MC/AC	Research time	Protocol
Agostini D. et al., 2018	D-SPECT	MIBI/injector (bolus)	Regadenoson, 400 mg	32 frames 21 × 3 sec 1 × 9 sec 1 × 15 sec 1 × 21 sec 1 × 27 sec 7 × 30 sec	Net Retention	?/–	6 min	One-day
Fang Y.D. et al., 2017	GE Discovery NM 530c	MIBI/? (bolus)	Dipyridamole, 0.124 mg/kg/min	48 frames 48 × 30 sec	2-compartment model	?/–	5 min	One-day
Bouallègue F.B. et al., 2015	GE Discovery NM 530c	Tetrofosmin/? (bolus)	Dipyridamole, 0.75 mg/kg, 4 min	48 frames 30 × 3 sec 18 × 15 sec	1-compartment model	?/–	6 min	One-day
Nikoulou R. et al., 2016	GE Discovery CT/NM 570c	Tetrofosmin/? (bolus)	Adenosine, 140 mcg/kg/min	12 frames 6 × 10 sec 6 × 30 sec	1-compartment model	?/–	4 min	One-day
Miyagawa M. et al., 2017	GE Discovery NM 530c	MIBI, Tetrofosmin/? (bolus)	ATP, 160 mcg/kg/min, 5 min	200 frames 200 × 3 sec	1-compartment model	?/–	10 min	One-day
Ben-Haim S. et al., 2013	D-SPECT	MIBI/injector (bolus)	Adenosine, 140 mcg/kg/min, 6 min; Dipyridamole, 0.142 mg/kg/min, 4 min	60–70 frames	2-compartment model	?/–	6 min?	One-day, two-day
Wells R.G. et al., 2017	GE Discovery NM 530c	Tetrofosmin/ injector, 9 ml in 30 sec	Dipyridamole, 0.142 mg/kg/min, 5 min	19 frames 9 × 10 sec 6 × 15 sec 4 × 120 sec	1-compartment model	+/+	11 min	One-day
Zavadovsky K.V. et al., 2019	GE Discovery NM/CT 570 c	MIBI (bolus)	Adenosine, 140 mcg/kg/min, 4 min.	44 frames 40 × 4.5 sec 4 × 45 sec	Net Retention	+/+	6 min	One-day, two-day

Note. The study was carried out with correction of scintigraphy images – "+", without correction of scintigraphy images – "–"; data were not presented in the study – "?".

This model is less dependent on the pharmacokinetics of the radionuclide indicator as compared to one- and two-compartment models. This model is appropriate for tracers with a nonlinear dependence of retention and the blood flow, such as ^{99m}Tc-MIBI or ^{99m}Tc-tetrofosmin. It should be noted that most authors used a one-day study protocol for dynamic SPECT. This requires a correction parameter for the second study and complicates the mathematical processing of results.

Methods for improving the quality of scintigraphic images, such as attenuation correction (AC), motion correction (MC), and correction of heartbeat artifacts [60], were not used in most of the reviewed studies. Currently, there is no common opinion about the need to include such tools for processing dynamic SPECT data. However, the work of Ruddy et al. [56], devoted to the influence of various correcting factors on scintigraphic parameters of MBF and CFR, showed the need to use motion correction, while the use of attenuation correction alone did not significantly affect these indicators.

Dynamic SPECT is performed according to basic principles; however, there is a large number of technical differences that are listed above. Thus, the evolution of the dynamic SPECT method with MBF and CFR assessment is not complete, although it is at the final stage of conceptual and methodological research. This is confirmed by similar research results presented in this review and validation of the scintigraphic method with "gold standards" – PET and FFR [57, 58].

Currently there is an insufficient number of clinical trials in this area. However, the potential of dynamic SPECT as a method for quantification of coronary blood flow and reserve is high. This technique can become a simple and affordable alternative to existing methods for assessing MBF and CFR. This will increase the informative value of radionuclide diagnostics in assessing the severity of coronary insufficiency for more accurate risk stratification and determination of appropriate treatment strategy for cardiac patients.

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The role of metabolic syndrome in the pathogenesis of knee osteoarthritis: a new view on the problem

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ABSTRACT

Currently, numerous studies undeniably prove the influence of metabolic syndrome on osteoarthritis (OA) progression.

In hyperlipidemia, free fatty acids abnormally accumulate in the cartilage tissue and provoke cell dysfunction and necrosis. Studies show that palmitate and stearate have a pronounced proapoptotic effect on chondrocytes of the articular cartilage.

Mediators of the systemic inflammatory response produced by the adipose tissue act as a significant link in the pathogenesis of metabolic OA in the knee joint. Metabolic disorders, insulin resistance, and dyslipidemia boost production of inflammatory mediators and glycosylated compounds and formation of free oxygen radicals provoking endothelial dysfunction.

A relationship between intra-articular structures (articular cartilage, synovial membrane, subchondral bone, and synovial fluid) and the intra-articular infrapatellar fat pad is a local pathogenetic factor in the metabolic OA of the knee. It is proven that the intra-articular infrapatellar fat pad increases significantly in obese patients. Due to proximity to the articular cartilage and synovial membrane, the adipose tissue is in close contact with them. The influence of systemic metabolites activates the growth of adipocytes, preadipocytes, macrophages, fibroblasts, and other fat body cells which enhance the production and release of adipokines, such as leptin, adiponectin, visfatin, and cytokines, that, in turn, stimulate aseptic inflammation resulting in development of synovitis, cartilage degeneration, and gonarthrosis progression.

Therefore, the metabolic syndrome has a negative impact on the condition of the joint tissues, contributing to the development of gonarthrosis or its progression. It manifests itself both through systemic effects and the local impact of the hypertrophied infrapatellar fat pad on the components of the synovial joint environment.

Key words: metabolic syndrome, osteoarthritis, dyslipidemia, adipokines, oxidative stress, infrapatellar fat pad.

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Роль метаболического синдрома в патогенезе гонартроза. Новый взгляд на проблему.

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РЕЗЮМЕ

На сегодняшний день получены многочисленные данные, неоспоримо доказывающие взаимосвязь остеоартроза (ОА) с метаболическим синдромом.

При гиперлипидемических состояниях свободные жирные кислоты аномально накапливаются в тканях суставов. Это оказывает липотоксическое действие, что приводит к дисфункции клеток и их гибели. Пальмитаты и стеараты вызывают выраженное проапоптотическое действие на хондроциты суставного хряща.

Факторы системной воспалительной реакции, продуцируемые жировой тканью, выступают в качестве значимого звена в патогенезе метаболического ОА коленного сустава. Метаболические нарушения, инсулинорезистентность, дислипидемия способствуют увеличению продукции медиаторов воспаления, гликированных соединений, образованию кислородных радикалов, провоцирующих эндотелиальную дисфункцию.

Локальным звеном в патогенезе метаболического гонартроза является взаимосвязь внутрисуставных структур (суставного хряща, синовиальной оболочки, субхондральной кости и синовиальной жидкости) с внутрисуставной инфрапателлярной жировой клетчаткой. Доказано, что инфрапателлярная жировая клетчатка значительно увеличивается у пациентов с ожирением. Из-за близкого расположения с суставным хрящом и синовиальной оболочкой, жировая ткань находится с ними в тесном контакте. Под влиянием системных метаболитов, разрастаясь, адипоциты, преадипоциты, макрофаги, фибробласты и другие клетки жировых тел усиливают продукцию и высвобождение адипокинов, таких как лептин, адипонектин, висфатин, цитокинов, которые стимулируют асептическое воспаление, приводящее к развитию синовита, дегенерации хряща и прогрессированию гонартроза.

Таким образом, метаболический синдром оказывает негативное влияние на состояние тканей сустава, способствуя возникновению гонартроза или его прогрессированию, реализуясь как через системные эффекты, так и за счет локального воздействия гипертрофируемой инфрапателлярной жировой клетчатки на компоненты синовиальной среды сустава.

Ключевые слова: метаболический синдром, остеоартроз, дислипидемия, адипокины, оксидативный стресс, жировые тела Гоффа.

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INTRODUCTION

Metabolic syndrome (MS) is a complex of metabolic and hormonal disorders that are risk factors for development of cardiovascular diseases. The syndrome classically includes abdominal obesity, insulin resistance, arterial hypertension, impaired carbohydrate metabolism, increased triglycerides, and reduced high-density lipoprotein cholesterol. All these components are predictors of the adverse course of osteoarthritis (OA) [1].

Statistics shows a clear dependence of joint remodeling on metabolic disorders [2]. Each newly manifested component of MS makes the course of OA more severe. Therefore, in on a sample of 482 patients, among individuals with a mono-factor dis-

order, 12.8% had knee OA, while in people with 2 or more MS components, knee OA was registered in 23.2% [3]. Comorbid metabolic and vascular pathologies cause an early progressive course of OA with pronounced clinical and functional disorders. Increased duration of relapses, predominance of generalized forms, frequent development of synovitis and periarthritis, and more intense pain are observed [4].

The combination of MS and OA is more pronounced in young people and decreases with age [5]. Gender predisposition to OA in MS showed a prevailing risk in women of all age groups, which should be associated with peculiarities of the function of reproductive hormones [6].

Development of osteoarthritis, especially in the knee joints, leads to limited physical activity and subsequent progression of obesity. Therefore, a vicious circle forms, where, on the one hand, MS contributes to the development of OA, and on the other hand, the mobility is limited due to degenerative and dystrophic processes in the joint, which contributes to the progression of obesity and MS.

All pathogenetic factors of gonarthrosis in MS can be divided into systemic ones (such as lipotoxicity and endothelial dysfunction), developing as a result of dyslipidemia; activation of oxidative stress against the background of hyperglycemia and insulin resistance; and increased production of active metabolites by adipokines in the conditions of obesity, that induce and promote inflammation in the joints. A number of authors prove the importance of local factors in the pathogenesis of gonarthritis and, in particular, the role of infrapatellar fat pad, which is in direct contact with the synovial membrane of the knee joint, is hypertrophied in the metabolic syndrome, and participates in the implementation of destructive processes in the intra-articular structures.

SIGNIFICANCE OF THE MECHANICAL FACTOR

According to the original concept of OA development in MS, it is the mechanical factor (overweightness) that contributes to hyperextension of the ligamentous apparatus and increases the mobility of the intra-articular structures and the load on the articular surfaces. Therefore, friction increases, which implies constant irritation and development of aseptic inflammation [7]. In addition, obese patients often have big bone mass, which increases the pressure in the subchondral bone and contributes to trophic disturbances, formation of subchondral cysts, and destruction of the cartilage [8]. In this case, the knee joint is the primary target affected by significant biomechanical factors [9]. Numerous studies of women with MS showed that an average weight loss of 5 kg reduced the risk of developing knee OA by 50% [10, 11].

However, overload of the joints cannot explain convincing epidemiological data demonstrating a link between obesity and OA of the upper limb joints that do not carry a significant load. At the same time, patients with obesity and MS have a higher risk of gonarthrosis than patients with obesity without the metabolic syndrome [12].

According to modern data, the mechanical factor in MS plays an aggravating role in progression of OA, but this role is not the primary one. The main systemic pathological processes are dyslipidemia and hypersecretion of proinflammatory mediators and cytokines by the adipose tissue [13]. Accumulation of lipids in chondrocytes and low-grade systemic inflammation (metainflammation) involving adipokines and cytokines disrupt homeostasis, leading to lipotoxicity and degenerative changes in the joint tissues [14].

DYSLIPIDEMIA

Lipid imbalance is a key metabolic disorder associated with metabolic syndrome and obesity. A high-calorie diet affects adversely the barrier characteristics of the gastrointestinal mucosa, contributes to the violation of its integrity, and reduces the production of antimicrobial peptides and mucins. In addition, the intestinal microbial flora changes, and the production of proinflammatory metabolites, in particular, lipopolysaccharides (LPS), increases [15]. The increased permeability of the intestinal wall provides excess supply of LPS to the systemic circulation, which supports low-grade chronic inflammation in the body with the activation of innate immune components via Toll-like receptors (TLR) 4 [16].

Preclinical studies showed that LPSs suppress cartilage matrix synthesis, increasing the production of IL-1β by the upregulation mechanism via TLRs present in human articular cartilage [17]. In addition, indirectly through activation of circulating monocytes, synthesis of osteopontin increases,

which is responsible for activation of matrix metalloproteinases (MMR) and regulation of cell migration, has proinflammatory effect, and is able to participate in vascular remodeling, contributing to ischemization of the joints [18]. Accumulation of LPS due to activation of transforming growth factor (TGF)β leads to an increase in ectopic bone formation and enhances inflammation in the synovial membrane due to activation of macrophages [17].

Dyslipidemia in MS leads to abnormal accumulation of lipids in tissues, and hyperinsulinemia occurring against the background of insulin resistance with a compensatory purpose is both direct alternative and mediated in nature [13].

Articular chondrocytes, unlike most other cells, are characterized by significant lipid deposits. Infiltration of excessively high lipid levels in chondrocytes in hyperlipidemia leads to lipotoxicity. Free fatty acids are considered the main factors that have a damaging effect. The obtained data prove that the main mechanism underlying the damaging effect of toxic concentrations of oleate and palmitate in articular chondrocytes is implementation of apoptosis. A quantitative assessment of DNA hypoploidia using flow cytometry revealed accumulation of apoptotic cells with a subdiploid DNA content, and an assessment of nuclear morphology showed that nuclear condensation and fragmentation were significantly increased. In addition, Western blotting showed that oleate induced caspase-3 and -7, and the pan-caspase inhibitor zVAD-fmk completely prevented oleate-induced cytotoxicity [13].

Experiments on a mouse model using a high-fat diet showed that the incidence of OA in the group with the addition of polyunsaturated fatty acids (PUFA) was lower, both for idiopathic and post-traumatic OA. PUFA inhibits apoptosis caused by hyperlipidemia by redirecting saturated fatty acids to triglyceride pools, which are stored as neutral fats [19].

The two central categories of lipid mediators of inflammation are omega-6 and omega - 3 PUFAs. Omega-6 PUFAs, such as arachidonic acid, are precursors of proinflammatory eicosanoids, including prostaglandins, thromboxanes, and leukotrienes. Omega-3 PUFAs, such as eicosapentaenoic and docosahexaenoic acids, on the contrary, inhibit the intensity of inflammation. The experimental model proved that the decrease in the omega-6 / omega-3 PUFA ratio, either through diet or genetically by introducing the fat-1 transgen, which endogenously converts omega-6 to omega-3 PUFA, leads to a reduced risk of developing knee OA [20, 21].

Mediated damaging factors of dyslipidemia are manifested in disturbances of the microcirculatory supply in the cartilage. Increased infiltration of the synovial and subchondral vascular intima by lipids contributes to the activation of lipid peroxidation (LPO), development of endothelial dysfunction, plasmorrhagia, progression of atherosclerosis, and vascular remodeling [13].

HYPERGLYCEMIA AND INSULIN RESISTANCE

Hyperglycemia and glucose toxicity are significant triggers of damage in gonarthrosis. Changes in the function of glucose carriers on the surface of chondrocytes contribute to maintaining the inflammatory and degenerative and dystrophic processes, inhibiting the anabolic effects. Neurotoxic effects of hyperglycemia lead to neuromuscular damage, which worsens the course of OA and leads to destabilization of the joints [22].

Hyperinsulinemia contributes to activation of the sympathetic system; Na retention in the body increases, which leads to additional occlusion of the vascular lumen, development of arterial hypertension, and deterioration of microcirculation [23]. Impaired joint perfusion under vascular remodeling increases the risk of oxidative stress, which is especially unfavorable for the cartilage tissue due to the absence of blood vessels and its initially reduced antioxidant potential [24].

CHRONIC METABOLIC INFLAMMATION

Taking into account the developing hyperlipidemia and constantly increasing adipose tissue mass in the metabolic syndrome, as well as its good vascularization, inflammatory mediators, free fatty acids from the adipose tissue, enter the systemic circulation in a significant amount, subsequently affecting the intra-articular structures. The spectrum of signaling molecules secreted by adipocytes is very diverse. They can be divided into specific molecules, such as leptin, and non-specific ones, such as cytokines, in particular, IL-1β, IL-4, IL-6, IL-8, IL-13, IL-17, IL-18, chemokines (CCL2, MIP-1α), and growth factors (TGFβ, IGF-1, VEGF, TNFα) [25].

There is evidence that leptin is able to act through insulin receptors and components of the insulin cascade, becoming one of the causes of insulin resistance in pathological concentrations [26]. Leptin has a dose-dependent effect on the joints. In the physiological state, small doses of it stimulate formation of the extracellular matrix in the cartilage and expression of TGFβ and IGF-1 by osteoblasts [27]. The concentration is maintained by the relationship between the articular cartilage and the infrapatellar fat pad. The metabolic syndrome is characterized by hyperleptinemia. In addition, most cells of the immune system have receptors for leptin and its antagonist, ghrelin. Leptin is involved not only in metabolic correction, but also in immune processes [28]. In particular, in innate immunity, it activates macrophages and natural killers (NK) and causes neutrophil chemotaxis. In adaptive immunity, leptin affects proliferation and differentiation of T cells, stimulates the formation of Th-1 lymphocytes, and suppresses the concentration of Th-reg [29].

In an experimental mouse model, leptin inhibition led to suppression of inflammatory responses, including symptoms of arthritis. Intraperitoneal injection of leptin resumed the inflammatory process. Therefore, leptin indirectly induced the release of IL-6 via synovial macrophages, which can lead to degradation of proteoglycans, inhibit cartilage regeneration, and enhance MMP13 expression [30]. Under the influence of leptin in chondrocytes, the production of IL-1, one of the key proinflammatory agents that has a catabolic effect through cascade activation of other interleukins, such as MMP9 and MMP13, and attraction of inducible NO-synthetase, increases. Such effects also lead to chondrocyte apoptosis, including p53-dependent apoptosis, and activation of subchondral bone osteoclasts, which causes bone remodeling and lysis with formation of cysts [31]. In addition to inducing proinflammatory cytokines, leptin also contributed to expression of other cartilage catabolic factors, such as IL8, MMP2, cathepsin, and calpain [32]. Moreover, leptin mediated the dose-dependent expression of vascular cell adhesion molecules (VCAM) in the synovial membrane, correlating with severe OA [33].

Adiponectin is considered to be a mediator that has a protective effect on the joint in metabolic OA. However, all new studies characterize it as a proinflammatory agent, the effects of which are largely similar to those of leptin [34]. In progressive obesity, it is a mediator of insulin resistance and tissue inflammation, contributing to the formation and progression of OA [35].

Visfatin is produced constitutively not only by adipocytes, but also by almost all local tissues that are involved in the pathogenesis of OA, to a greater extent by the synovial membrane and chondrocytes. Like other adipokines, it has pleiotropic effects and performs immune, proinflammatory, and enzymatic functions [36]. The concentration of visfatin in the blood plasma in OA significantly increases. Besides, its high content was determined immunohistochemically in the synovial membrane, especially around blood vessels. In the experiment, osteoblasts were as sensitive to visfatin as chondrocytes, since their stimulation induced the expression and production of the same proinflammatory cytokines and chemokines (disintegrin, prostaglandin E2, IL-6, CCL2, and MCP-1), and the prodegradative effects were determined by the release of MMP-3, MMP-13, and involvement of immunocompetent cells. The use of the visfatin inhibitor APO866 showed a decrease in the production of proinflammatory cytokines in various cells from 63 to 94% [37]. A special role in the formation of insulin resistance belongs to recombinant visfatin, which acts through the insulin receptor IR3 [38].

OXIDATIVE STRESS

Chronic inflammation affects metabolism in the joint by activating oxidative stress, which is one of the most significant factors of genomic and mitochondrial damage that initiates the processes of cellular aging. The synergistic effect of systemic inflammatory factors on chondrocytes and synovial fibroblasts stimulates synthesis of cytokines and degrading enzymes that induce destruction of proteoglycans and type II collagen (Col2A1), the main structural protein of the cartilage tissue. In addition, impaired regulation of Col2a1 genes is observed, which indicates a decrease in the reparative potential. Adipokines perform their functions by binding to Toll-like receptors (TLRs) on target cell membranes and initiating phosphorylation of the ERK/p38/mitogen-activated protein kinase (MAPK) cascade, which primarily causes changes in the intracellular homeostasis, in particular, increasing the

activity of NADP oxidase (NOX), the main source of ROS generation.

The damaging effect of ROS in physiological conditions is inhibited by the antioxidant system, which is controlled by the transcription factor NRF2 (nuclear related factor 2). Receiving signals, its inactive cytoplasm form KEAP1 (Kelch-like ECH associated protein 1) undergoes hydrolysis with cleavage of the active NF-E2-dependent factor 2 (nuclear factor erythroid 2) and translocation to the nucleus, where it launches biosynthesis of cytoprotective enzymes, such as superoxide dismutase (SOD) and catalase (CAT). During experimental sensitization of chondrocytes and synovial fibroblasts with visfatin, leptin, and resistin, a significant increase in the endogenous superoxide anion, as well as NRF2, catalase, and superoxide dismutase was proved. The latter is explained by an acute adaptive compensatory response. However, given that the potential of antioxidant protection in the cartilage tissue is relatively reduced due to the peculiarities of its histophysiology, the membrane-protective effect is suppressed under conditions of considerable amounts of ROS, which affect the phospholipid bilayer both endogenously, forming inside cells, and exogenously, appearing mainly from targets undergoing apoptosis [39].

Apoptosis is also facilitated by reduced expression of genes of the BCL-2 family proteins, which are some of the regulators of this process intended to increase cell survival. In addition, there is significant positive modulation of the expression of some micro interfering RNA (miRNA) genes, leading to a decrease in the proliferative potential, stoppage of the cell cycle and cellular aging, implementation of apoptosis, and violation of the oxidative balance. The effects of miRNA are enhanced by the synergistic signaling of the NF-kB pathway, which is a family of transcription proteins involved in proinflammatory, immune, and stress responses and activated by the MAP-kinase cascade [40]. Experimental modeling of oxidative and inflammatory stress on synovial fibroblasts and chondrocytes through constant impact of proinflammatory cytokines, in particular, TNFα and H_2O_2, significantly increased the proportion of aging cells among the young population and limited it among the old population. This indicated special susceptibility of young cells to this type of alteration, while the introduction of the antioxidant N-acetylcysteine or fenofibrate suppressed aging and slowed down the progression of OA. Aging cells are characterized by irreversible stoppage of the cell cycle with a shift of the phenotype towards a proinflammatory one, therefore being pathological factors that can independently maintain inflammation in the joint for a long time [41].

VIOLATIONS OF ANGIOGENESIS

Activation of angiogenesis in chronic systemic inflammation is of great importance in metabolic OA. The balance between angiogenic and antiangiogenic factors in the joint regulates the growth of blood vessels, while proinflammatory factors that increase with the metabolic status lead to a balance shift. Inflammation in the joint can promote angiogenesis directly by releasing growth factors from cells, such as macrophages, as well as by stimulating or sensitizing other cells, such as chondrocytes and osteoblasts, which, in turn, release additional angiogenic factors.

What is more, hypoxia in the inflamed tissues is a powerful stimulant of angiogenesis. Due to hypoxia, a compensatory increase in the expression of the *VEGF* (vascular endothelial growth factor) gene is observed. Recent studies confirm the significant role of TNFα, which induces accumulation of leucine-rich alpha-2-glycoprotein 1 (LRG1) in the articular cartilage and subchondral bone, as a powerful stimulator of pathological angiogenesis and mesenchymal cell migration, which contribute to aberrant osteogenesis [42]. In turn, progressive angiogenesis aggravates the course of chronic inflammation and leads to endochondral ossification and formation of osteophytes in the region of the bone-cartilage junction. Increased permeability of newly formed blood vessels contributes to the development of edema [43]. Adhesion molecules, such as E-selectin, are highly expressed by new vessels, facilitating inflammatory cell infiltration [44].

SIGNIFICANCE OF INFRAPATELLAR FAT PAD

All systemic processes in the metabolic syndrome, directly or indirectly affecting the joint, change the relationship between its structures, such as cartilage plates, synovial membrane, and intra-articular adipose tissue. Lately, special attention has been paid

to the infrapatellar fat pad (Hoffa's fat pad), which is intracapsular but extrasynovial, performs the cushioning function, and acts as a local paracrine apparatus. It is well supplied with blood and innervated, like subcutaneous fat, and also has a strong connective tissue framework [45]. Due to its location between the articular cartilage and the meniscal surface, the Hoffa's fat pad reduces the load on the knee joint and protects it in physiological conditions or at an early stage of OA [46]. The infrapatellar fat pad improves distribution of fluid in the joint by increasing synovial membrane area and reducing friction, thus enhancing stability in the joint [47].

The main functional unit of this structure is the adipocyte which determines the ability to secrete specific adipocytokines (such as leptin and adiponectin), that have anti-catabolic effects on the cartilage tissue (increase the production of proteoglycans, type II collagen, expression of TGFβ and IGF-1) in physiological concentrations or the ones slightly exceeding them, and, therefore, prevent the development of OA at early stages [48]. Consequently, the infrapatellar fat pad can be defined as an independent formation that regulates the metabolic processes in the joint and counteracts the pathogenesis of OA at initial stages.

However, against the background of a gradual increase in proinflammatory systemic factors in the body in the metabolic syndrome, the first response is usually given by the synovial membrane. Findings of knee joint MRI in patients with gonarthritis demonstrated thickening of the synovial membrane in 73% of patients with early OA, which corresponds to development of chronic synovitis. Histological changes were characterized by massive lymphohistiocytic infiltration. Chronic inflammation in the synovial membrane activated proliferation of fibroblasts and blood vessels and migration of macrophages. At the same time, the inability of synovial macrophages to switch from the proinflammatory M1 subtype to the anti-inflammatory M2 subtypes was observed, which can contribute to the initiation and maintenance of synovitis in OA, and cellular apoptosis is enhanced. M1 macrophages contribute to inflammatory microenvironment and OA progression by interacting with synovial fibroblasts and chondrocytes, thereby increasing MMP secretion [49].

Many authors believe that the Hoffa's fat pad is involved in the catabolic processes with a change in their proinflammatory profile [50]. In this case, the infrapatellar fat pad is able to produce the same proinflammatory mediators and growth factors directly into the synovial fluid as distantly located adipocytes in the blood [51]. In MS, the infrapatellar fat pad secretes higher levels of inflammatory factors and adipokines than subcutaneous fat [52].

On the other hand, chronic hypertrophy of the Hoffa's fat pad and concomitant damage to the soft tissues of the joint lead to ischemia, inducing abnormal distribution of the neurotransmitter SP in the afferent fibers of nerve endings inside the adipose tissue, which ultimately leads to chronic neurogenic tissue inflammation, associated with increased paraarticular pain by some authors [53].

The progression of gonarthrosis in MS leads to changes in the infrapatellar fat pad, that increases in size and becomes denser, which is confirmed by MRI studies [54]. At initial stages of the disease, this change is associated with the development of edema, and at later stages – with hyperplasia caused by the growth of the connective tissue, in which Hoffa's fat pad is a complex, well vascularized, layered structure with fat lobules and foci of lymphohistiocytic infiltration [55].

CONCLUSION

The metabolic syndrome is characterized by low-grade systemic inflammation with the development of obesity, dyslipidemia, insulin resistance, hyperglycemia, and oxidative stress. Each component of MS is involved in the pathogenesis of osteoarthritis (Figure).

Systemic exposure to inflammatory mediators produced in MS, such as adipokines, cytokines, adiponectin, and visfatin, stimulates aseptic inflammation in the joint, leading to the development of synovitis and cartilage degeneration.

Hyperlipidemia and oxidative stress have lipotoxic and proapoptotic effects, which leads to chondrocyte dysfunction and death.

Of the local factors in the pathogenesis of metabolic gonarthrosis, an important role is assigned to the intra-articular infrapatellar fat pad. New studies demonstrated that the Hoffa's fat pad is a substrate that performs not only a cushioning function, but also produces proinflammatory factors directly into the synovial fluid and is significantly hypertrophied in the MS.

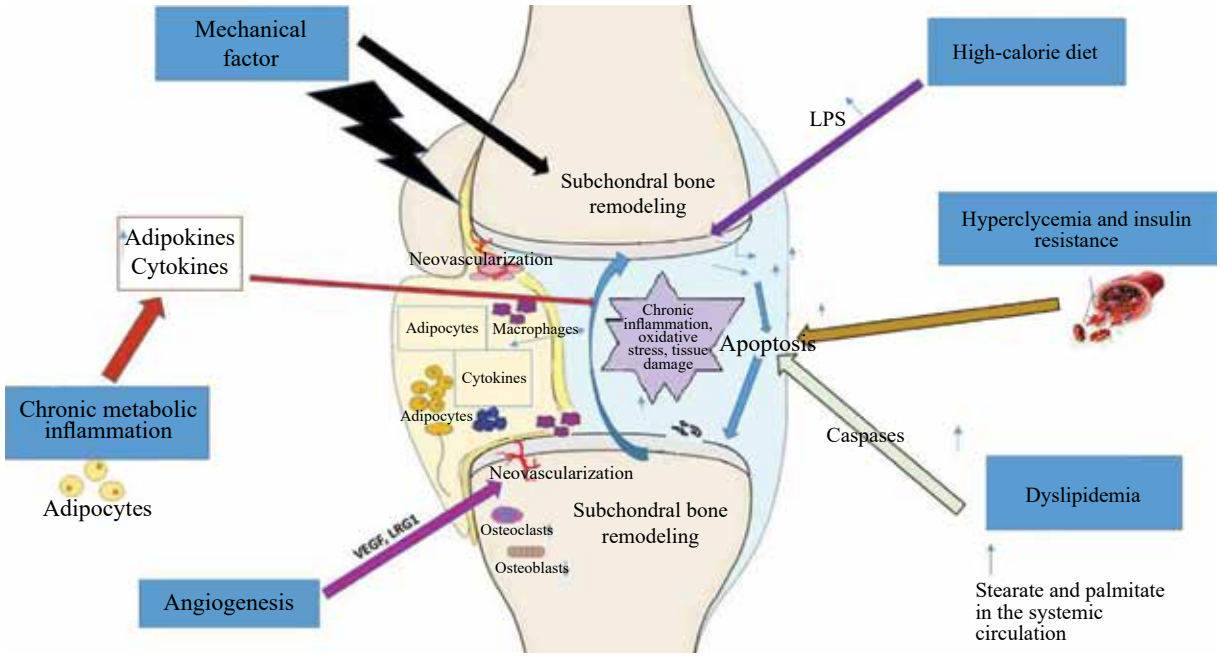

Figure. Components of the metabolic syndrome in the pathogenesis of osteoarthrosis

Therefore, MS leads to development of gonarthrosis or contributes to its progression and is manifested through systemic effects as well as through the induced local effect of hypertrophied infrapatellar fat pad on the components of the synovial environment of the joint. Products of tissue degradation and local and systemic inflammatory mediators form a vicious circle that supports chronic inflammation associated with impaired microcirculation, neovascularization, and vascular sprouting into the cartilage tissue with activation of foci of osteogenesis around the vascular channels, which causes irreversible changes in the joint and contributes to progression of gonarthritis.

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Coronary stent technology and the role of inflammation in the atherogenesis: problems and prospects

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ABSTRACT

Coronary artery disease (CAD) remains one of the leading causes of death in developed industrial countries. Timely and effective medical care for CAD patients depends on availability and application of endovascular methods for CAD treatment. Percutaneous coronary intervention (PCI) using drug-eluting stents allows to achieve good clinical results even in most severe patients. The issues of personalized invasive treatment for patients with chronic coronary syndrome and optimal prevention of recurrent clinical events in survivors of acute coronary syndrome and PCI remain relevant.

One of most important and unresolved problems in the pathophysiology of CAD is assessment of the nature of the inflammatory reaction that develops in the coronary vessels and myocardium in response to ischemic damage and PCI. Clinical studies focused on exploring a correlation between the proinflammatory parameters of the patient's status and the rate of secondary adverse events and aimed at revealing triggers of systemic and local inflammation are of great interest. Such a trigger could be the intestinal endotoxin (ET) which is capable of inducing systemic inflammation and, therefore, plays a significant role in the atherogenesis. A relationship between the endotoxin and cytokine system parameters should be investigated to develop a therapeutic concept for supporting CAD patients, including individuals after PCI. Parameters of systemic endotoxemia could be used as additional factors in developing the biomarker-based approach to identify patients with active inflammation or fibrosis. This could result in development of specific therapy aimed at suppressing proinflammatory mediators and protecting the heart from inflammation.

Key words: coronary artery disease, percutaneous coronary intervention, inflammation, endotoxin, cytokines, systemic endotoxinemia.

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Технология коронарного стентирования и роль воспаления в атерогенезе: проблемы и перспективы

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РЕЗЮМЕ

Ишемическая болезнь сердца (ИБС) остается одной из ведущих причин смертности в развитых индустриальных странах. Своевременная эффективная помощь данному контингенту больных зависит от доступности и эффективности применения эндоваскулярных методов лечения ИБС. Чрескожное коронарное вмешательство (ЧКВ) с применением стентов с лекарственным покрытием позволяет добиваться хороших клинических результатов даже у самых тяжелых групп пациентов. Остаются актуальными вопросы персонализации планового инвазивного лечения пациентов с хроническим коронарным синдромом, а также оптимальной вторичной профилактики повторных клинических событий у пациентов, благополучно пережившим острый коронарный синдром и ЧКВ.

Одной из важнейших и неразрешенных проблем в патофизиологии ИБС является оценка характера воспалительной реакции, развивающейся в венечных сосудах и миокарде в ответ на ишемическое повреждение и ЧКВ. Представляют интерес клинические исследования, направленные на изучение корреляции показателей провоспалительного статуса пациентов с частотой развития повторных неблагоприятных клинических событий с целью выявления индуктора системного и местного (в стенте) воспаления. Вероятным кандидатом является кишечный эндотоксин, способный индуцировать системное воспаление и таким образом играющий существенную роль в атерогенезе. Необходимы исследования взаимодействия параметров эндотоксиновой и цитокиновой систем для выработки терапевтической концепции поддержки больных ИБС, в том числе после проведения процедуры стентирования коронарных артерий. Использование показателей системной эндотоксинемии в прогнозе течения заболевания может быть дополнительным фактором для выработки подходов, основанных на биомаркерах для идентификации больных с активным воспалением

или фиброзом, что привело бы к разработке специфической терапии, направленной на подавление провоспалительных медиаторов и защиту сердца от воспалительного повреждения.

Ключевые слова: ишемическая болезнь сердца, чрескожное коронарное вмешательство, воспаление, эндотоксин, цитокины, системная эндотоксинемия.

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

Источник финансирования. Авторы заявляют об отсутствии финансирования при написании статьи.

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INTRODUCTION

Cardiovascular diseases (CVD) account for the largest share of overall mortality in European Society of Cardiology (ESC) member countries [1]. A common group of risk factors underlying the cardiovascular pathology are associated with unhealthy life style and may cause cancer, diabetes mellitus, and chronic lung diseases, that account for 80% of overall mortality. According to the last European registry, CVD risk factors remain prevalent in patients with chronic coronary syndrome, and prescription of secondary prevention medications is not reasonable. Elderly patients and to some extent female patients have a lower chance of receiving appropriate therapy than young male patients [2].

Coronary artery disease (CAD) accounts for more than 50 % of CVD mortality and 25.9 % of overall mortality in Russia [3]. Timely and effective medical care for CAD patients depends on availability and application of endovascular procedures for CAD treatment, especially in acute coronary syndrome (ACS), and on primary and secondary prevention.

In recent years, a rapid increase in the number of endovascular diagnostic and therapeutic interventions has been registered in Russia. More than 740,000 procedures were performed in 2018, of which more than 220,000 were percutaneous coronary interventions (PCI) [4]. It must be noted that implementation of invasive methods for ACS therapy allowed to achieve prominent results in several Russian clinics. Nevertheless, despite the application of antiplatelet and invasive reperfusion therapy, the incidence of ACS and its complications remains high [5, 6].

PCI is the most common invasive method to treat CAD due to high early procedural success and relief of symptoms. Technological development of ultrathin passivated and drug-eluting stents enables to achieve good clinical results even in most high-risk patients with ST-segment elevation myocardial infarction (STEMI) [7].

The prognostic value of invasive therapy in patients with chronic coronary syndrome has been investigated for quite a long time. Thus, the ISCHEMIA clinical trial [8] failed to demonstrate that PCI with stenting resulted in a lower number of serious ischemic complications (death, myocardial infarction (MI), cardiac arrest with return of spontaneous circulation (ROSC), hospitalization due to heart failure (HF) or congestive HF (CHF)) in patients with moderate CAD, as opposed to optimal drug therapy. Consequently, the issue of personalization of planned invasive treatment for patients with chronic coronary syndrome, namely selection of patients that will benefit from invasive therapy to the largest extent, remains relevant.

It is worth noting that along with improvement of the stent technology, the problem of coronary event recurrence has come to the forefront. It is obvious that even with optimal secondary prevention in survivors of ACS and PCI, intervention in chronic coronary syndrome does not provide zero residual risk of recurrent clinical events, such as angina pectoris, MI, HF, and sudden cardiac death.

Irrespective of the therapy success, researchers more often confirm the inadequacy of the infiltration theory of atherogenesis and its consequences [9]. Drug therapy optimization is associated to a large extent with the anti-inflammatory effect

of medications. Today, assessment of the nature of the inflammatory reaction that develops in the coronary arteries and myocardium in response to ischemic injury is one of the most important and unresolved problems in the pathophysiology of CAD. In this respect, an obvious gap in knowledge is observed that was obtained in the clinical trials [10, 11] studying a correlation of proinflammatory parameters of the patient's status with the incidence of recurrent adverse events, including the rate of in-stent restenosis.

The role of the immune system in the pathogenesis of CVD is well known: body immune protection is triggered by any stress effect [12], and acute myocardial ischemia is not an exception. There are several interdependent components of immune response that can be engaged in CAD and HF pathogenesis. Low-grade chronic systemic inflammation is essential that manifests through chronic nonspecific diseases involving the cytokine system. Hyperactivation of this system accompanies degradation of extracellular myocardial collagen matrix, ventricular dilatation, and cardiomyocyte hypertrophy (CMH).

There are many hypotheses on how and why the level of proinflammatory cytokines, especially tumor necrosis factor alpha (TNFα), increases and what causes immune response activation while common symptoms of inflammation are absent. Besides the assumption on myocardial TNFα production stimulated by elevated blood pressure proportional to myocardial wall tension and left ventricular end-diastolic pressure [13] and the hypothesis on extramyocardial cytokine production facilitated by tissue hypoxia and the excess of free radicals [14], there is an endotoxin (ET) concept of atherogenesis [15]. It is based on experimental results [16] and is confirmed by clinical trials [17], which allowed to formulate the endotoxin theory of atherosclerosis [18]. Apparently, the intestinal endotoxin may play a significant role in the atherogenesis, because it is capable of inducing systemic inflammation [19]. Therefore, further clinical trials are required to study the possibility of using systemic endotoxemia (SE) parameters for prognosing the course of postoperative (after coronary stenting) and follow-up periods to improve the patient's quality of life after endovascular interventions.

DEVELOPMENT OF THE CORONARY STENT TECHNOLOGY

As the PCI technology with stenting (stent technology) evolved, it became a common minimally invasive method to cure different CAD forms due to high procedural success, quality of life, and survival rate comparable to those coronary artery bypass grafting (CABG). Development of drug-eluting stents (DES) created on the basis of bare-metal stents (BMS) with addition of an anti-proliferative medication resolved the problem related to restenosis progression [20]. Release of the drug from the stent surface allows to regulate the intensity of inflammation occurring after coronary angioplasty and stent implantation and thereby inhibit neointimal hyperplasia in the region of blood vessel wall damage [21]. Clinical data confirm the long term (5-year follow-up) [22, 23] benefit of DES implanted in millions of CAD patients [24].

Technological progress led to development of DES with a unique hybrid coating combining passive and active components [25, 26]. The stent skeleton made of cobalt-chromium alloy with ultrathin 60 mm struts allows for perfect wall apposition, that is very important for regional blood flow [27] and stent endothelialization [28].

The metal body of the stent is completely covered with a thin passivation layer of amorphous silicon carbide (aSiC:H) that promotes stent endothelialization. The passivating effect of aSiC:H, which is a wide-bandgap semiconductor, consists in inhibition of electron transfer from the fibrinogen molecule with zero total electric charge in the non-excited state to the metal surface. Thereby, conversion of fibrinogen to fibrin (through electrostatic interaction of charged excited fibrinogen molecules) and its deposition on the stent surface are reduced [29]. Clinical studies [30] demonstrated that the passivation layer reduces adhesion and activation of blood platelets and leukocytes and significantly reduces release of potentially allergenic ions from the metal stent skeleton, the latter being especially important in long-term follow-up after stent implantation and total drug dilution.

Besides the silicon carbide layer, the stent body is completely covered by a biodegradable poly l-lactic acid (PLLA) polymer for limus delivery. PLLA has been approved for many medical applications since 1960s, and its advantages include high biocompati-

bility [31–33] and well-controlled solubility within 1–2 years, which contributes to gradual limus release, minimizing the inflammatory response over a longer period of time [34].

Sirolimus, a natural macrocyclic lactone isolated from *Streptomyces hygroscopicus* in the mid-1970s and approved by FDA for prevention of kidney transplant rejection in 1999, has immunosuppresive, anti-inflammatory, and strong anti-proliferative effects [35]. It inhibits activation of the rapamycin protein target and stops the cell cycle (progression from phase G1 to S). Therefore, sirolimus restricts proliferation of cells, including T-cells, and proliferation and migration of smooth muscle cells, thereby suppressing restenosis [36].

Sirolimus-eluting stents, as opposed to bare-metal stents, reduce neointimal hyperplasia [36]. Re-endothelialization of human coronary arteries occurs to the same extent in the BMS and DES groups [37, 38]. In various animal models and clinical studies [39–41], sirolimus-eluting stents, as opposed to BMS and polymer-coated stents, reduce neointimal hyperplasia. Additionally, early effective neointimal tissue maturation takes place [42, 43], and the risk of stent thrombosis drops by 25% in comparison to other new-generation DES [44].

According to the results of the BIOSTEMI trial, sirolimus-eluting stents with ultrathin struts showed excellent clinical results in most high-risk STEMI patients [7]. As the stent technology develops (approaches perfection) and related clinical outcomes improve, the problems of postoperative complications and optimization of drug therapy are coming to the forefront.

Myocardial remodeling in acute MI mediated by cytokines and inflammatory cells includes myocardial healing encompassing phagocytosis and resorption of necrotic tissue, hypertrophy of survived cardiomyocytes, degradation and synthesis of collagen, proliferation of myofibroblasts, angio- and vasculogenesis, and proliferation of progenitor cells. Death of cardiomyocytes and degradation of the extracellular matrix induce release of signals activating innate and adaptive immunity and determine the intensity of the inflammatory response. Inflammatory mediators are involved in adverse cardiac remodeling (dilatation) and HF progression. Timely suppression of proinflammatory mediators can protect the heart from excessive inflammation-induced damage. New approaches are required based on detection of biomarkers (first of all inductors) of systemic inflammation for identifying patients with high risk of restenosis. Drug treatment of these agents could improve clinical outcomes for patients after PCI during a long follow-up period [9, 45].

Identification of factors that increase the risk of in-stent restenosis, including cellular and inflammatory factors and blood markers, is a topical issue [10]. The role of SE (in its pathogenic form – ET aggression (EA)) in induction of atherogenesis is becoming more prominent [46, 47]. Therefore, studying the role of the lipopolysaccharide (LPS) factor in initiation of systemic inflammation, development of postoperative complications, and the rate of restenosis progression becomes more relevant.

INFLAMMATION AS A FACTOR OF CARDIAC PATHOLOGY

The immune aspect of CVD pathogenesis is well known: immune defense mechanisms are activated not only in any infection, but also in response to any stress impact [12], including ischemia, hemodynamic overload, intoxication, etc. There are several inter-dependent immune system components that can be involved in the pathogenesis. The main of them are proinflammatory cytokines, the durable effect of which leads to gradual destruction of myocardial extracellular collagen matrix, ventricular dilatation, and cardiomyocyte hypertrophy. These cardiac remodeling processes can become irreversible [48] and facilitate HF progression along with cytokine-induced enhancement of cardiomyocyte apoptosis.

Cytokines can be defined as a new autonomous system that regulates the main body functions, exists along with nervous and endocrine regulatory systems, and is primarily aimed at maintaining homeostasis upon penetration of pathogens and disruption of tissue integrity. Death of cardiomyocytes and degradation of the extracellular matrix in the infarcted myocardium induce signal release for activating innate and adaptive immunity and determine the intensity of the inflammatory response. The role of post-infarction inflammation in progression of ischemic inflammation is contradictory, and inflammatory mediators are involved in adverse cardiac remodeling (dilatation) and HF advancement.

The main cause of immunity activation in patients without commonly recognized attributes of inflam-

mation remains unclear. Along with neurohumoral factors, realizing their effect through activation of renin-angiotensin-aldosterone and sympathoadrenal systems, the key role in the pathogenesis belongs to proinflammatory cytokines, such as TNFα [49–52] and interleukins IL-1 and IL-6, that modulate cardiovascular system functions [12, 53]. The source of excessive cytokines can be "overstressed" cardiomyocytes [54] or peripheral muscle cells. However, data are available that cytokine release is provoked by endotoxins (ET), i.e. lipopolysaccharides (LPS) of Gram-negative bacteria, which are capable of penetrating into the systemic circulation through the impaired intestinal barrier.

Stagnation of venous circulation in the intestine, which is inevitable when the myocardium is damaged and the cardiac output drops, facilitates wall permeability for bacteria and/or their toxins, which, penetrating the circulation and interacting with the CD14-receptor (CD – cluster of differentiation) of immunocompetent cells, trigger synthesis of TNFα and other cytokines [13, 14]. The intestinal origin of ET and its transport into the circulation of patients without signs of active infection are confirmed by the fact that ET concentration in hepatic veins is significantly higher than in the left ventricle (LV) and pulmonary veins [55]. Absence of any difference between the TNFα levels in the pulmonary veins and in the LV presumably excludes the heart as the source of systematically increased cytokine level [56].

Endotoxin is considered as a fundamental trigger of cytokine storm, and chronic ET load is at least one of the reasons for immune response activation [57–59]. However, ET is also capable of causing a hypo-response to successive loads known as ET tolerance: repeated stimulation of monocytes leads to reduced production of TNFα, IL-1α, and IL-6 cytokines via the negative feedback mechanism [60–62]. The ET tolerance phenomenon, being a complex regulatory response of the body to inflammation, was studied at the level of changes in cellular membrane molecules, signaling proteins, pro- and anti-inflammatory cytokines, and other mediators [63, 64]. Therefore, the ET activity in the blood plasma, being a potential stimulator of immune activation, has pathogenic effects.

The ability of LPS to activate the immune response results from its interaction with TLR4, the key receptor of innate immunity, which is cardio-pathogenic in nature [65]. TLR4-mediated innate immune responses [66, 67] are capable of triggering myocardial defense after the ischemia-reperfusion sequence (I/R) [68, 69], but they are also involved in myocardial damage in the I/R sequence and HF advancement [66, 67, 70–72]. Lack of TLR4 [70, 71] or modulation of TLR4-mediated activation of the factor kappa-B (NFκB) [66] significantly reduces myocardial damage caused by I/R, improves restoration of the cardiac function, and reduces expression of inflammatory cytokines and adhesion molecule genes [72].

Inflammation in some form and with some severity grade is present almost in all main types of cardiac pathology. The pathogenetic and morphological patterns of inflammation are almost identical and independent of its localization, except for small variations in involvement of cellular elements in the process. It was shown that the risk of developing acute MI in CAD patients increases during outbreaks of influenza [18] and after surgical interventions. Recipients of transplanted hearts suffer from dramatically accelerated atherosclerotic damage to the coronary system.

Clinical studies demonstrate that low-grade inflammation is associated with the pathogenesis of serious chronic diseases, such as atherosclerosis [46], diabetes mellitus, and age-specific neurological diseases [73].

A correlation was discovered between the inflammation process and arrhythmias, in particular, paroxysmal atrial fibrillation (AF), often occurring after different interventions on the heart [74–76]. Increased levels of C-reactive protein (CRP) and proinflammatory TNFα and IL-6 cytokines in the blood plasma were found both in patients with paroxysmal and persistent AF [77–82], while higher CRP level was observed in persistent AF [77, 78]. Furthermore, the CRP level can be used to predict sinus rhythm restoration or AF recurrence in patients undergoing cardioversion [83, 84].

CRP and lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1), almost undetectable in healthy arteries, can form a cyclic mechanism with oxidized LDL (OxLDL) or L5 in proatherogenic conditions, while elevated LDL level induces CRP expression by endothelial cells [85]. In turn, that can increase LOX-1 expression, facilitating atherogenic

LDL capture by endothelial cells and appearing as a key phagocytic receptor (macrophage receptor) to bind OxLDL in atherosclerosis [86]. LOX-1 receptors mediate proatherosclerotic effects of OxLDL that lead to endothelial dysfunction, proinflammatory monocyte recruitment to the arterial intima, formation of foam cells, apoptosis of endothelial cells and vascular smooth muscle cells (VSMC), and destabilization and rupture of plaques [86].

Atherosclerosis and vascular restenosis develop with VSMC proliferation. Recent studies have demonstrated that VSMC proliferation is stimulated by LDL via TLR4 receptors, however, the signaling pathways are not completely studied [87]. It is necessary to understand their role and molecular mechanisms involved into control over VSMC proliferation stimulated by LDL via the signaling pathways of TLR4 receptors. ET can stimulate different signaling pathways, such as PI3K/Akt, MAPKs, and IRAK1/4, which then facilitate NF-kB expression for VSMC proliferation. Studying potential TLR4 signaling pathways of VSMC proliferation remains relevant, which can be a new therapeutic target for proliferative vascular diseases.

For further increase in the efficacy of CAD treatment, especially its severe forms, such as acute MI, new approaches based on biomarkers to identify patients with active inflammation or fibrosis are required, that could result in development of specific therapy. Timely suppression of proinflammatory mediators can protect the heart from excessive inflammation that can be a direct cause of plaque destabilization.

PROBLEMS AND PROSPECTS

Development of the stent technology has enabled to solve one of the problems of PCI, namely prevention of in-stent thrombosis and restenosis in early postoperative period. The problem of preserving the patient's quality of life in the longer term apparently cannot be solved only by improving the quality of the stent and the implantation procedure, for example, by applying control and visualization methods like optical coherence tomography [43]. Further efforts are needed to study the mechanisms of stenosis and restenosis, find inducers of atherogenesis, and search for measures to prevent or at least slow down the process. Identification of the role of systemic inflammation in atherogenesis is relevant, from the mechanism of its induction through SE (and the role of LPS and antibodies to it in this process) [18, 46, 47] to inclusion of the cytokine system in the process.

This topic is investigated in the Russian national prospective multi-center non-randomized non-interventional clinical study "BIOFLOW-III VIP Registry" organized for clinical evaluation of DES implantation efficacy in daily clinical practice [11]. One of the secondary endpoints of the study which is of great scientific interest is a working hypothesis on whether patient's inflammation status correlates with clinical outcomes – serious adverse events (SAE).

To prove the hypothesis, besides standard clinical and biochemical blood parameters, vulnerable inflammation parameters (VIP) are measured in the blood serum of patients upon inclusion in the study: IL-1, IL-6, CRP, cortisol, ET (LPS), antibodies (AB) to the hydrophobic region of the ET molecule (AB-LPS-PHOB), AB to the hydrophilic region of the ET molecule (AB-LPS-PHIL). These laboratory measurements are performed twice: during PCI and if the patient has serious adverse events in the course of 36-month follow-up.

The primary endpoint of the study is identification of target lesion failure (TLF) within 12-month follow-up due to cardiac death, target vessel Q-wave or non-Q wave MI, emergency CABG, and clinically driven target lesion revascularization (TLR).

Secondary endpoints also include TLF at 6 and 36 months of follow-up; target vessel revascularization (TVR) at 6, 12, and 36 months; target lesion revascularization (TLR) at 6, 12, and 36 months; stent thrombosis at 6, 12, and 36 months; clinical device success; clinical procedural (PCI) success; VIP registered during inclusion in the study; VIP registered during SAE.

Acquisition of data on the correlation between blood parameters and the frequency of SAE in patients will be relevant for optimizing dynamic FU of CAD patients after PCI. Along with the possibility to resolve the problem for a definite patient cohort, the study results might be promising due to general pathological meaning of VIP for developing a dynamic predictive algorithm of life- and health-threatening adverse events (including vascular catastrophes, HF decompensation, and different life-threatening forms of arrhythmia) with

the use of integrative data bases and digital health platforms [88]. The "BIOFLOW-III VIP Registry" findings will allow to gain insight into the problem of stent lifespan extension and could provide the basis for developing a follow-up algorithm for PCI patients using methods to normalize integrated SE parameters.

MEASURES OF ENDOTOXIN AGGRESSION PREVENTION AND ELIMINATION

Systemic inflammation is an attribute of life itself and an obligatory factor of homeostasis (intestinal LPS activates adaptive body systems, including the immune system) [19]. Its pathogenic form resulting from excessive LPS in the circulation is considered as a pre-disease or a universal pathogenic factor of human and animal diseases [89]. This approach is confirmed by clinical observations of patients with allergic disorders, autoimmune diseases, female infertility, idiopathic and viral uveitis, anorexia, obesity, type 1 and 2 diabetes, chronic viral pathology (including AIDS), and physical and psycho-emotional stress [19, 90–98].

The list of drugs, foods, food additives, and procedures that are able to lower the ET concentration in the systemic circulation is quite long [99–101]. It includes enterosorbents and foods rich in fiber; Bifidobacterium-containing products (live cultures) and foods (starch drinks, etc.) that envelope the intestinal mucosa; choleretic medications, products (garlic, etc.), and procedures (gallbladder cleanse etc.); antiviral medications with rectal administration; antibiotics (gentamicin, etc.) binding LPS in the circulation; bacteriophages and foods selectively eliminating various Gram-negative bacteria, which cause EA development; moderate physical and aquatic exercises; intravenous laser blood irradiation as a method to increase anti-endotoxin immunity; selective hemoadsorption (LPS filters) and immunodrugs – concentrate human AB to LPS (in critical states). Furthermore, development of new selective hemo- and enterosorbents on the basis of oligonucleotides appears to be promising [102].

CONCLUSION

Advances in endovascular methods of CAD therapy and prevention of acute cardiac pathology using coronary stents are evident. Achievements in stent technology refinement based on development and application of new materials allowing for a significant decrease in the rate of inflammatory complications (primarily thrombosis) in early postoperative period are impressive. This has permitted to decrease mortality and preserve work capacity of a large population cohort.

Further progress in vascular surgery can be achieved in extending stent lifespan. A clear understanding of the key role of systemic inflammation in development of restenosis in the stented coronary artery has appeared, that apparently develops according to the mechanisms similar to those in atherogenesis. Systematic understanding of the role of microbiota in the homeostasis and general pathology and the contribution of intestinal ET to adaptation and atherogenesis induction was shaped.

Methodological and methodical basis for studying the role of endotoxemia in the pathogenesis was developed. We identified the range of drugs, foods, additives, and procedures capable of preventing and/or stopping EA and systemic inflammation induced by it by affecting the inflammation inductor. The relevance of creating a new generation of anti-endotoxin agents using selective hemo- and enterosorbents on the basis of oligonucleotides was substantiated.

Due to the general pathological significance of the inflammatory process, further studies on the relationship between ET and cytokine system parameters are promising for developing a therapeutic concept of CAD patient management, including individuals after coronary stenting.

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Clinical genetic description and analysis of the case of chromosomal mosaicism mos47,XY,+8/46,XY

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ABSTRACT

The article describes a clinical case of chromosomal mosaicism in a boy, 4 months and 3 weeks old. Cytogenetic analysis of peripheral blood lymphocytes of the child made it possible to establish the karyotype mos47,XY,+8/46,XY with an approximately equal ratio of normal and abnormal cells. The pathogenetic effects of the mosaic form of trisomy 8 are discussed. The authors discussed the results of examination of the patient's mother during pregnancy as part of a combined prenatal screening for congenital and hereditary diseases. The difficulty in prenatal diagnosis of chromosomal mosaicism is noted and explained by the lack of specific biochemical and ultrasound markers. However, in the late pregnancy period, ultrasound signs of impaired development of the brain, heart, and kidneys associated with a chromosomal abnormality can be detected.

Key words: prenatal diagnosis, trisomy 8, chromosomal mosaicism, cytogenetic analysis

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Клинико-генетическое описание и анализ случая хромосомного мозаицизма mos47,XY,+8/46,XY

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РЕЗЮМЕ

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Описан клинический случай хромосомного мозаицизма у мальчика в возрасте 4 мес и 3 нед. Цитогенетический анализ лимфоцитов периферической крови ребенка позволил установить кариотип mos47,XY,+8/46,XY с приблизительно равным соотношением нормальных и аномальных клеток. Обсуждаются патогенетические эффекты мозаичной формы трисомии 8. Приводятся результаты обследования матери пациента во время беременности в рамках комбинированного пренатального скрининга врожденных и наследственных болезней. Отмечается сложность пренатальной диагностики хромосомного мозаицизма в связи с отсутствием специфических биохимических и ультразвуковых (УЗ) маркеров. Однако на поздних сроках беременности могут быть обнаружены УЗ-признаки нарушения развития головного мозга, сердца и почек, ассоциированные с хромосомной аномалией.

Ключевые слова: пренатальная диагностика, трисомия 8, хромосомный мозаицизм, цитогенетический анализ.

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

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INTRODUCTION

Complete trisomies of autosomes in humans usually lead to early intrauterine fetal death and abortion before 7–8 weeks of gestation. Trisomies on chromosomes 13, 18, and 21 are exceptions, as they are not fatal, but are accompanied by congenital malformations. Biochemical and ultrasound markers of a these aneuploidies, as a rule, are quite clearly detected from 11–14 weeks of gestation, which allows for performing prenatal screening of genetically abnormal fetuses [1].

On the contrary, mosaic variants of trisomies are not often associated with pronounced developmental anomalies and may be unrecognized during prenatal screening. Health status of such patients at birth and in the future depends on the type of chromosome involved in the abnormality and the ratio of normal and mutant cells in the body. The clinical presentation of chromosomal mosaicism is unstable, which complicates the establishment of the cause of the pathology and the final diagnosis [2].

Trisomy on chromosome 8 in newborns is a rare event as only its mosaic variant is compatible with life and currently considered an independent syndrome (constitutional trisomy 8 mosaicism syndrome, T8MS). Single studies of sufficiently large population samples give an approximate estimated frequency of the anomaly ranging from 1 / 25000 to less than 1 / 50000 [3]. Among patients, there is a heterogeneity of clinical manifestations associated, apparently, with the proportion of mutant cells in the body and their predominant localization. To date, few newborns without serious abnormalities were identified. They had demonstrated satisfactory physical and intellectual development in the future, normal life expectancy reproductive problems in adulthood [4] and even had children [5]. On the other hand, the majority of patients have pronounced malformations that are already noticeable at birth [2, 6–10].

Characteristic symptoms of T8MS with a significant proportion of mutant cells are anomalies of the axial skeleton and limbs: a short wide neck, skull deformities, scoliosis, and camptodactyly. Deep transverse folds on the palms and feet are almost always revealed. Craniofacial dysmorphisms may include protruding forehead, hypertelorism, flattened nasal bridge, upturned nose, and ear deformities and misalignment. Internal organs are usually without lethal lesions; often there is agenesis of the corpus callosum, ventriculomegaly, heart defects of varying severity, and hydronephrosis. Disorders of psychomotor development are rather mild: delayed development of speech and motor skills and moderate mental retardation [6–8, 10].

Currently, a positive association has been established between the presence of an additional 8th chromosome in the karyotype and the likelihood of myelodysplastic syndrome [11–14]. According to some data, trisomy 8 as a somatic mutation is found in 5–7% of cases of this pathology [11, 12]. It is recommended to refer patients with an identified clone of bone marrow mu- tant cells to the oncological risk group [13]. Obviously, patients with a mosaic variant of trisomy 8, established on the basis of a cytogenetic analysis of lymphocytes, automatically fall into this cohort and should be under the supervision of an oncologist.

Registration and description of all new cases of T8MS are necessary not only to assess the frequency and spectrum of chromosomal abnormalities in the population, but also to determine the correlation between the

proportion of abnormal cells and severity of clinical symptoms in a patient. The search for prenatal pathology markers by retrospective analysis of the pregnancy course in mothers with children having mosaic variant of trisomy 8 is also relevant.

CLINICAL CASE

A boy S., 4 months and 3 weeks old, was referred to the Medical-Genetic Consultation Department of Kemerovo Regional Clinical Hospital for examination by a geneticist. The reason for the referral was a disorder in development of the external genital organs (hypospadias). At the examination, numerous craniofacial dysmorphisms were additionally revealed, such as hydrocephalic form of the skull, sunken nasal bridge, Asian-like eyes, upturned nose, abnormal auricles, and a short neck. In addition, partial cutaneous syndactyly of the 2nd and 3rd toes of both feet was revealed.

The child was born from the 1st pregnancy. The mother was 22 years old, had been overweight since childhood, was not registered at the dispensary, her work was not associated with harmful conditions, and poor health habits were absent. Intrauterine development of S. proceeded against the background of chronic placental and isthmic-cervical insufficiency, gestational hypertension, and polyhydramnios. S.'s weight at birth was 3,780 g and the Apgar score was 7/7. The child was bottle-fed, weight at the age of 4 months is 6,700 g (slightly underweight). In the maternity hospital, the following was established: moderate asphyxia, cephalohematomas over the right and left parietal bones, glanular hypospadias, and hypoconjugational jaundice.

From the maternity hospital, the child was transferred to the neonatal pathology department due to the diagnosed heart defect. Detailed examination revealed perimembranous defect of the interventricular septum, moderate stenosis of the pulmonary artery, and minor anomalies in heart development, such as open oval window and anomalies of the chordal apparatus. According to the ultrasound examination of the brain, S. had partial agenesis of the corpus callosum, moderate deformation of the ventricular system, and hydrocephalus. Cerebral ischemia of the 2nd degree and excitability syndrome were established. Psychomotor reactions with slight deviations included holding the head unconfidently, not always reacting to sounds, turning over on his side, following objects with eyes, reacting to toys, or smiling. Concomitant pathologies included dacryocystitis, bilateral focal pneumonia, and chronic tubo-otitis on both sides. Sensorineural hearing loss was suspected. The bilirubin level remained high and reached up to 143.9 μmol / L due to the indirect fraction.

To exclude chromosomal pathology, the child was referred for karyotyping. Cytogenetic analysis of 100 metaphase plates from peripheral blood lymphocytes revealed two clones of cells with a normal male karyotype 46,XY and trisomy on the 8th chromosome 47,XY,+8 in almost equal proportions. Thus, we can state that S. had trisomy 8 in a mosaic form with the karyotype mos47,XY,+8[52]/46,XY[48] (Figure).

This clinical case raises the question of the possibility of prenatal diagnosis of chromosomal mosaicism of the established type. We performed a retrospective analysis of the results of ultrasound and biochemical studies of S.'s mother during pregnancy. The first examination was carried out at 13 weeks of pregnancy. Fetal heart rate – 157 beats / min, crown-rump length (CRL) value – 67.4 mm, nuchal translucency (NT) – 1.1 mm. All indicators were within normal limits. The content of the free β-subunit of hCG in the blood serum of the woman was 49.5 IU / L (1.458 MoM), PAPP-A – 0.703 IU/L (0.214 MoM). Taking into account the age of the mother, the individual risk of trisomy 21 was 1:124, the risk of trisomies 18 and 13 was 1:1902 and 1:3848, respectively.

Since the risk of common chromosomal abnormalities of fetus was calculated to be low (less than 1:100), the woman was not referred for invasive prenatal testing. Further ultrasound examination of the fetus was carried out in the II trimester of pregnancy. No developmental defects associated with chromosomal abnormalities were identified.

The difficulty in prenatal diagnosis of the mosaic form of trisomy 8 is also confirmed by the previously published data. Comparison of the results of combined screening of 28 pregnancies that ended in birth of children with T8MS demonstrated that the most common (50% of cases) reason for invasive diagnosis is the age of the woman. In 18% of cases, deviations in the biochemical parameters of the pregnant women's blood were found. Finally, 21% of fetuses had ultrasound signs of developmental disorders. At the same time, the spectrum of ultrasound indicators was quite wide, which does not allow for identification of specific prenatal markers for T8MS [2].

Surprisingly, in most of the previously described cases, immediately after the birth of a child, a complex of malformations was detected, comparable to the one found in S. Three groups of anomalies were noted: those associated with the development of the brain, heart, and kidneys [2, 4, 6, 9, 10]. Obviously, with qualified ultrasound diagnostic doctors and proper equipment of medical institutions, these T8MS markers might have been detected at least in the II or III trimester of pregnancy.

CONCLUSION

Thus, prenatal detection of mosaicism on chromosome 8 in the framework of the current screening of pregnant women is difficult. Biochemical and ultrasound indicators of an increased risk of trisomies on chromosome 13, 18, and 21 may not reflect the health status of a fetus with T8MS. Ultrasound markers of pathology become noticeable in late gestation. Malformations the heart and kidneys are noted. At the same time, the prognosis for the life and health of newborns is relatively favorable, and the severity of developmental defects, apparently, is associated with the proportion and predominant localization of abnormal cells in the body.

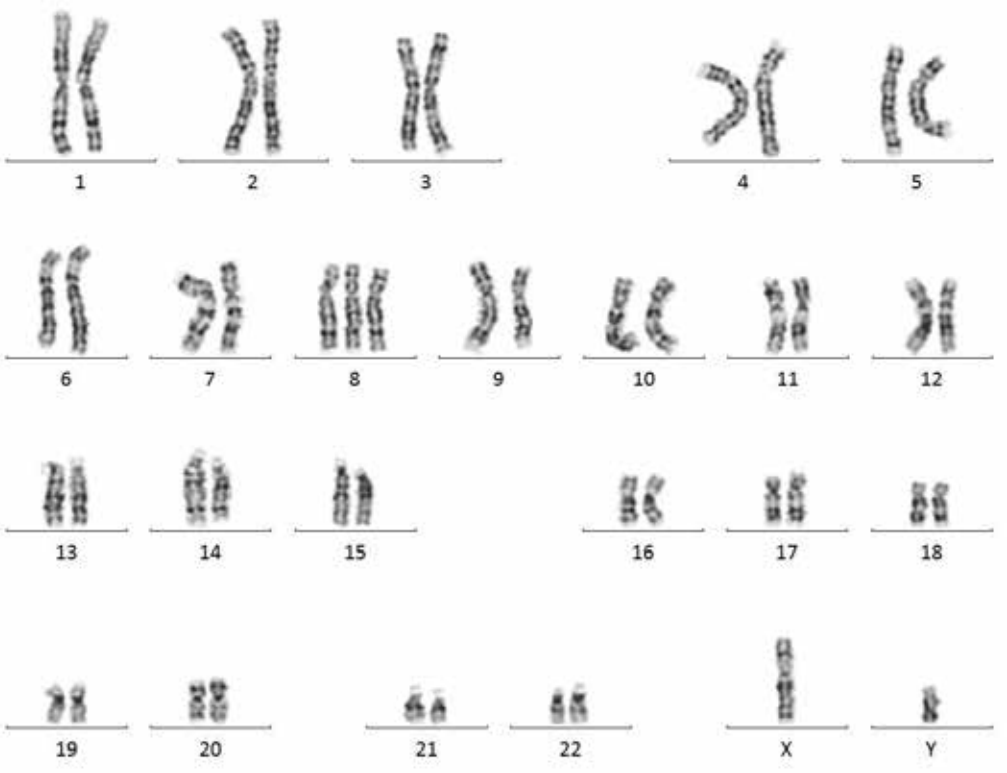

Figure. Abnormal karyotype 47,XY,+8, found in 52% of the patient's lymphocytes

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Clinical case of ataxia of toxic origin

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ABSTRACT

A clinical case of ataxia development associated with accidental phenobarbital overdose is presented. Clinical manifestations and differential diagnosis are described; the results of laboratory examinations for this pathology are presented. In conclusion, the importance of collecting anamnestic data is emphasized.

Key words: ataxia, clinical neurology, toxicology.

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

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Conformity with the principles of ethics. The patient's written consent was obtained for the publication of this clinical case. The description of the clinical case was approved by the local Ethics Committee of the Research Center of Neurology (Moscow).

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Клинический случай атаксии токсического генеза

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РЕЗЮМЕ

Представлен клинический случай развития атаксии на фоне случайно передозировки фенобарбитал-содержащего препарата. Описаны клинические проявления, дифференциальная диагностика, приведены результаты лабораторных обследований при данной патологии. В заключение делается акцент на важность сбора анамнестических сведений.

Ключевые слова: атаксия, клиническая неврология, токсикология.

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

Ataxia is a clinical syndrome of incoordination, which may result from damage to the cerebellum and related pathways [1]. Among the main manifestations of ataxia are gait disturbances and dysmetria, often associated with dysarthria and nystagmus. According to the classification [2], three major groups of ataxia can be defined: acquired, hereditary, and non-hereditary degenerative. The first group is comprised of a vast array of disorders including toxic, paraneoplastic, immune-mediated, and vitamin deficiency disorders. This article presents an unusual case of subacute ataxia of toxic origin due to phenobarbital exposure.

CLINICAL CASE

Patient D., a 32-year-old male, a dentist, had an appointment with a neurologist at the Research Center of Neurology where he complained of slurred speech, dizziness, unsteadiness and "staggering" gait (the patient himself described it "as if I were drunk"). Three days prior to the visit, he was admitted to one of the city hospitals with the specified symptoms. Upon admission, a computed tomography of the brain was performed. A CT scan with no pathological changes was obtained, and the patient was diagnosed with a transient ischemic attack in the vertebrobasilar basin. Subsequently, the patient left the medical facilityon his own. The symptoms developed gradually in the course of several weeks with no obvious etiological factor.

On examination, the patient was conscious, though drowsy. Systolic blood pressure was 100 mm Hg, diastolic – 60 mm Hg; heart rate was 64 bpm. A neurologic examination revealed moderate dysarthria, severe horizontal and vertical nystagmus, dysmetria during finger-to-nose test, and dysdiadochokinesia. Kinetic tremor, muscle hypotonia, and hyporeflexia were also noted. The gait was unsteady, wide-based.

Magnetic resonance imaging of the brain showed no signs of ischemic or degenerative pathology; the results of ultrasound examination were within normal values.

The patient denied the use of alcohol or any narcotic substances. However, when clarifying the anamnesis, it turned out that during the previous month the patient had problems with sleep, for which he was taking the drug "Valocordin" (Table 1).

Table 1

Composition of the "Valocordin" drug

Substance	Content, mg per 1 ml of the drug
Phenobarbital	18.40
Ethyl bromisovalerianate	18.40
Menthol oil	1.29
Hop oil	0.18
Ethanol	469.75

Due to its sedative properties, this drug is frequently used as a mild sleeping medicine with a standard dose amounting to 10–20 drops (0.25–0.5 ml). In the described case, the patient used 20–25 ml of "Valocordin" every other night for 4 weeks.

Subsequent blood and urine tests were performed (Table 2), which demonstrated a significant increase in phenobarbital excretion. On this basis, it was suggested that the patient had phenobarbital intoxication with ataxia being the main clinical manifestation.

The patient was prescribed intravenous saline infusions, as well as oral activated charcoal for the next 10 days. On the 2nd day of the therapy, a significant improvement was observed, on the 8th day the patient had made a complete recovery.

Table 2

Laboratory findings in patient D., 32 years old

Parameter	Value	Reference
Complete blood count		
Hemoglobin, g/L	151	131–173
Erythrocytes, × 10^{12}/L	5.06	4.3–5.7
Hematocrit, %	44.3	39–49
Platelets, × 10^{9}/L	386	180–320
Leukocytes, × 10^{9}/L	7.29	4.5–11.3

Table 2 (continued)

Parameter	Value	Reference
ESR, mm/h	17	0–15
Hemostasis		
APTT, s	31.9	25.1–36.5
PTI, %	98	70–130
Fibrinogen, g/L	5.11	1.8–4.0
Antithrombin III, %	153	75–125
Blood chemistry		
Creatinine, mcmol/L	68.0	62–106
Glucose, mmol/L	5.0	4.1–5.9
ALT, U/L	18.6	< 41
AST, U/L	28.3	< 40
CPK, U/L	211	< 190
Bilirubin, mcmol/L	3.1	< 24
C-reactive protein, mg/L	83.97	< 5
Uric acid, mcmol/L	468.0	202.3–416.5
Excretion of barbiturates in urine		
Amobarbital, ng/mL	0.0	0–200
Butalbarbital, ng/mL	0.0	0–200
Pentobarbital, ng/mL	0.0	0–200
Secobarbital, ng/mL	0.0	0–200
Phenobarbital, ng/mL	501,538.9	0–200

Note. ESR – erythrocyte sedimentation rate, APTT – activated partial thromboplastin time, PTI – prothrombin index, ALT - alanine aminotransferase, AST – aspartate aminotransferase, CPK – creatine phosphokinase.

CONCLUSION

Ataxia is a common neurological syndrome and a frequent reason for seeking medical care. Nevertheless, a broad spectrum of underlying pathologies is associated with this condition, which often presents difficulties in differential diagnosis. The described case underlines the necessity of a detailed and thorough patient interview, which was proven to be of vital importance in this particular patient. Although ataxia is listed in the "Side effects" section of the phenobarbital instructions for use, a search for relevant publications in the PubMed database did not reveal any reports of ataxia resulting from accidental overdose of phenobarbital drugs.

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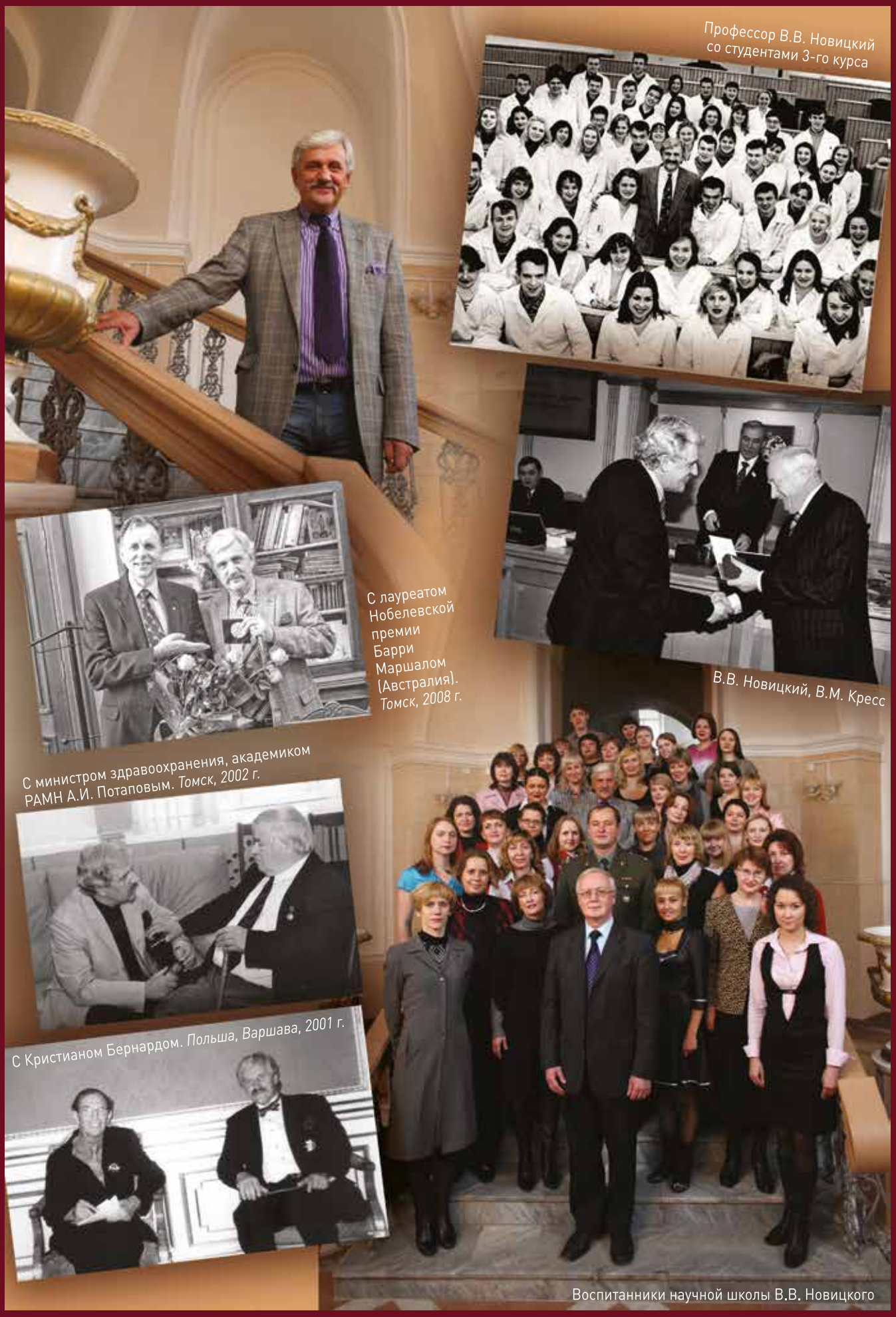

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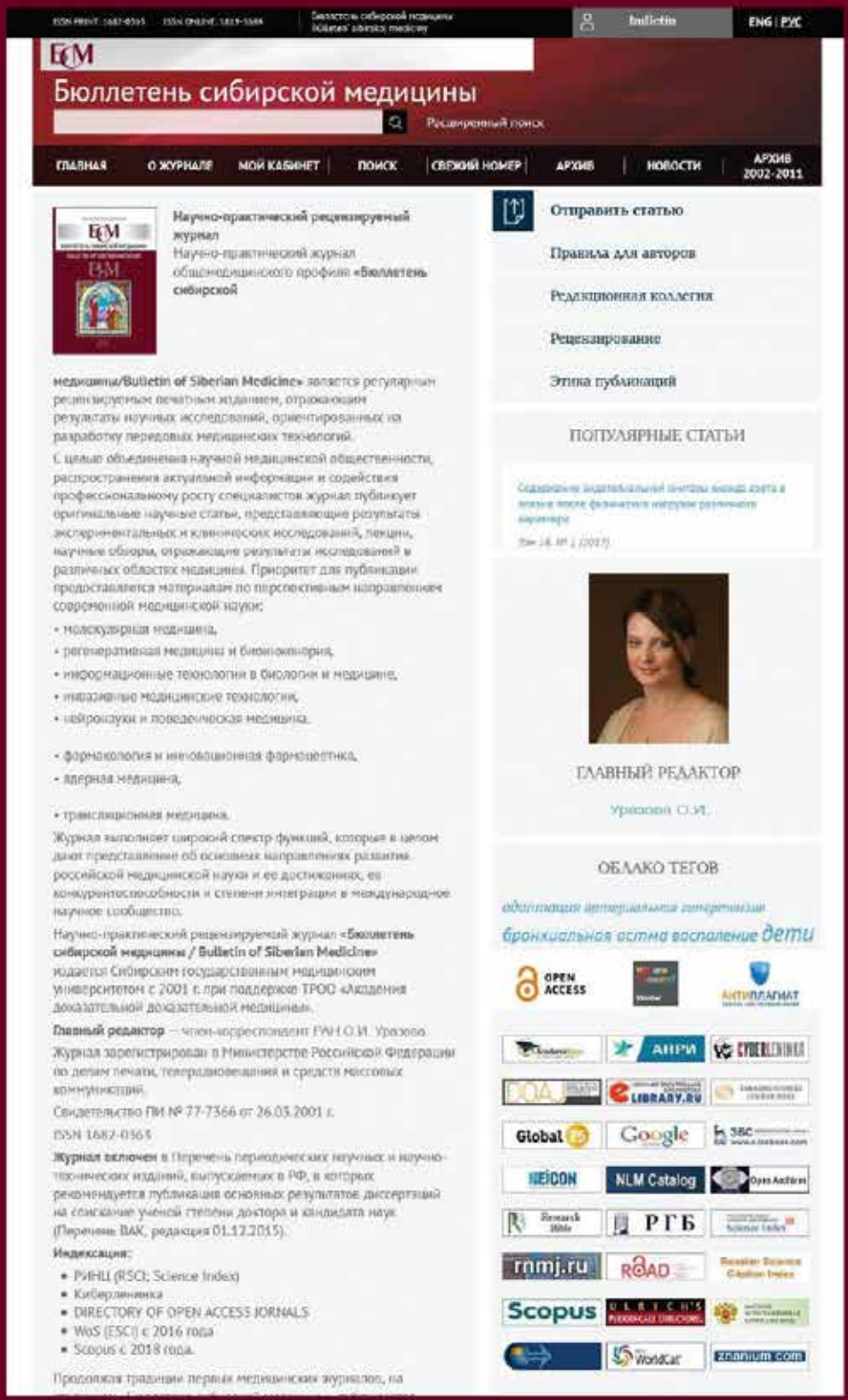

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