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EDITOR-IN-CHIEF'S PREFACE

EDITOR-IN-CHIEF'S PREFACE TO ISSUE 3, 2024

Sergey I. Kolesnikov

Member of the RAS

When scanning through the content of current issue of our journal I would like to highlight the works that are directly related to the priorities and critical technologies which were identified by the President of the Russian Federation in his Decree No. 529 dated June 18, 2024. These are "Preventive and personalized medicine, ensuring healthy longevity" and "Biomedical and cognitive technologies for healthy and active longevity".

The first study is a joint work of Russian and Belarusian scientists from Moscow and Grodno (Krivolapchuk I.A. et al.), who analyzed **the factors and level of physical performance of schoolchildren aged 13–14 years**. It was revealed that the transition to higher stages of puberty is accompanied with progressive dynamics of most indicators of anaerobic performance, while indicators of aerobic power and capacity change in different directions. This is important to take into account when regulating physical activity in adolescents during the critical period of ontogenesis.

The second work is a review by I.E. Pleshchev et al. (Yaroslavl, Moscow) with an analysis of the prevalence of **sarcopenic obesity**, the causes of its occurrence, modern methods of its prevention and physical rehabilitation, age-related changes in adipose and muscle tissue, effect of calorie restriction and physical exercise complexes that have a positive effect. The authors also analyzed gaps in clinical practice recommendations.

This "age series" is completed with an article of L.V. Poskotinova et al. (Arkhangelsk) on the possibility of using the parameters of auditory evoked potentials as a criterion for reduced cognitive reserve and increased **risk of developing cognitive** impairments.

Studies on **cardiovascular pathology**, which makes the main contribution to the mortality and the reduction in life expectancy, is represented an extensive study by A.Yu. Lazutkina (Khabarovsk), on the assessment of the quality **of the screening test for coronary heart disease predictors**. It is proposed to expand the number of detected markers for assessing the risk of coronary heart disease and other pathologies. The work of A.N. Sumin et al. (Kemerovo) included analysis of diagnostic tactics in patients with suspected obstructive coronary heart disease which, and a conclusion was made about the necessity in wider using non-invasive imaging tests. R.E. Kalinin et al. (Ryazan) revealed that indicators of spectral analysis of electroencephalogram and P300 cognitive evoked potential are the predictors of cognitive status 6 months after carotid endarterectomy.

Other works published in this issue also correspond to the mentioned Decree and the Strategy for Scientific and Technological Development of the Russian Federation. This applies to the studies on **socially significant diseases**: hepatitis, tuberculosis, HIV infection, etc. This is the subject of articles by S.S. Sleptsov and S.S. Sleptsova on chronic viral hepatitis in the Arctic zone of the Republic of Sakha (Yakutia), R.Yu. Abdullaev et al. (Moscow), who revealed the development of hypercoagulation in TB patients with diabetes mellitus after moderate and severe COVID-19, A.Yu. Sambyalova et al. (Irkutsk), who analyzed the antiretroviral drugs concentrations in children with perinatal HIV infection.

Several works are devoted to **reproductive health and children's health** – the problems that are also under magnifying glass of modern

health care and state leaders. One of them is a review by S.V. Zotov et al. (Novosibirsk, Novokuznetsk) with an analysis of the impact of environmental pollution, lifestyle, surgical history, bad habits and obesity, psychological and social factors that reduce ovarian reserve. K.D. Ievleva et al. (Irkutsk) studied the diagnostic value of interleukins in women with chronic endometritis and overweight, which will give an opportunity to develop a minimally invasive method for determining the risk of this disease. E.D. Kazantseva et al. (Irkutsk) revealed a higher level of lipid peroxidation products, a lack of fat-soluble vitamins and increased values of oxidized glutathione in children with influenza compared to the healthy children. The series is concluded by the work by V.V. Kocherova et al. (Chita), who determined the risk factors for the development of intraventricular hemorrhages in extremely premature newborns, which is very important for the providing medical care to such children.

Two original articles and a review are devoted to the **surgical problems**. V.A. Zaika, T.N. Iureva and D.B. Danzandorzhieva (Irkutsk) proved that episcleral methods of **treating rhegmatogenous retinal detachment** are characterized by the best anatomical, reconstructive and functional effect. L.V. Lyubimova et al. (Cheboksary) revealed a change in the spectrum of leading pathogens **of implant-associated infection** in the pre- and post-COVID period and a change in their antibiotic resistance, and recommend vancomycin for empirical therapy, but limited use of fluoroquinolones. The review by L.M. Tibekina et al. (St. Petersburg) proved that **the surgical method of treatment of drug-resistant epilepsy** is a priority.

This issue contains a larger number than ever of interesting experimental studies. This concerns the **modeling of various pathologies, such as: cataract** – by A.D. Chuprov et al. (Orenburg), who showed a decrease in stearyl-coenzyme-A-desaturase and melatonin concentration in the lens tissue; **non-alcoholic fatty liver disease** – by T.V. Brus and A.G. Vasilyev (St. Petersburg), offering additional tests to assess the severity of the process; scopolamine **cholinergic insufficiency** – by Ya.G. Razuvaeva et al. (Ulan-Ude), who studied the neuroprotective effect of *Orostachys spinosa* Sweet extract.

Three works are dedicated to the studying **new and potential substances and compositions** for the creation of vaccines, drugs and probiotics. For example, A.B. Pyatidesynikova et al. (Irkutsk) showed stimulation of *TLR2* and *TLR4* gene expression by organoselenium compound 2,6-dipyridinium-9-selenabicyclo[3.3.1]nonan dibromide, i. e. increase in the immunogenic properties of the vaccinal strain Y. *pestis* EV. S.M. Miroshnichenko et al. (Novosibirsk) found that using enterosorbent based on aluminum oxide and polydimethylsiloxane has protective effect for thymus functional activity in modeling continuous lighting in rats. A.S. Pendyukhova et al. (Irkutsk, Ulan-Ude) proved that both the biocompatibility of probiotic strains and the antagonistic activity of the consortium against pathogenic strains are important for the creation of a probiotic consortium with effective potential, which makes it possible to determine effective strategies for the use of probiotics.

The article by E.V. Saidakova et al. (Perm) proposes to modify the cultivation protocol by introducing interleukin 2 into the culture medium in order to increase the viability and mitotic activity of T lymphocytes thawed after cryopreservation.

As always, the issue contains a description of unique clinical observations. T.V. Sorokovikova et al. (Tver, Moscow) describe a rare prion disease – fatal familial insomnia caused by an autosomal dominant mutation D178N of the *PRNP* gene.

In conclusion, I would recommend paying attention to a very interesting review not only for theorists and clinicians, but also for teachers. This is

the review by E.T. Ablyakimov and M.A. Kriventsov (Simferopol) discussing ligand-associated activation of vitamin D receptors in the morphogenesis of immune inflammation. A better understanding of the intercellular relationships between vitamin D receptors, D3-VDR complex, and immune checkpoint receptors (PD-1, PD-L, CTLA) in inflammation could form the basis for the development of new strategies for the diagnosis, prognosis, and treatment of various diseases.

We wish you a good summer and new interesting ideas!

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ПРЕДИСЛОВИЕ ГЛАВНОГО РЕДАКТОРА К № 3 (2024)

Колесников Сергей Иванович

Академик РАН

Рассматривая содержание данного номера нашего журнала, я бы выделил работы, которые связаны непосредственно с теми приоритетами и критическими технологиями, которые определил Президент Российской Федерации в своём указе от 18.06.2024 № 529. Это «Превентивная и персонализированная медицина, обеспечение здорового долголетия» и «Биомедицинские и когнитивные технологии здорового и активного долголетия».

Первое исследование – это совместная работа российских и белорусских учёных из Москвы и Гродно И.А. Криволапчука и соавт., проанализировавших **факторы и уровень физической работоспособности школьников 13–14 лет**. Выявлено, что с переходом на более высокие стадии полового созревания наблюдается прогрессивная динамика большинства показателей анаэробной работоспособности, тогда как показатели аэробной мощности и ёмкости изменяются разнонаправленно. Это важно учитывать при нормировании физических нагрузок у подростков в критический период онтогенеза.

Вторая работа – обзор И.Е. Плещёва и соавт. (Ярославль, Москва) с анализом распространённости **саркопенического ожирения**, причин возникновения, современных методов профилактики и физической реабилитации, возрастных изменений жировой и мышечной ткани, данных о влиянии ограничения калорий и комплексов физических упражнений, которые оказывают положительное влияние на физические показатели. Проанализированы пробелы в рекомендациях по клинической практике.

Дополняет этот «возрастной ряд» сообщение Л.В. Поскотиновой и соавт. из Архангельска о возможности использования параметров слуховых вызванных потенциалов как критерия сниженного когнитивного резерва и повышенного **риска развития когнитивных нарушений**.

В русле работ по **сердечно-сосудистой патологии,** вносящей основной вклад в формирование смертности и сокращение продолжительности жизни, лежит обширное исследование А.Ю. Лазуткиной (Хабаровск), посвящённое оценке качества **скрининг-теста предикторов ишемической болезни сердца**. Предлагается расширить количество выявляемых маркеров для оценки риска ишемической болезни сердца и иных заболеваний. В работе А.Н. Сумина и соавт. (Кемерово) проведён анализ диагностической тактики у пациентов с подозрением на **обструктивную ишемическую болезнь сердца** и сделан вывод о необходимости более широкого использования неинвазивных визуализирующих тестов. Р.Е. Калинин и соавт. (Рязань) выявили, что показатели спектрального анализа электроэнцефалограммы и **когнитивный вызванный потенциал P300** являются предикторами когнитивного статуса через 6 месяцев после каротидной эндартериоэктомии.

Соответствуют упомянутому Указу и Стратегии научно-технологического развития России и другие работы, опубликованные в журнале. Это касается исследований, посвящённых **социально значимым заболеваниям:** гепатиты, туберкулёз, ВИЧ-инфекция и др. Это статьи С.С. Слепцова и С.С. Слепцовой (Якутск) о хронических вирусных гепатитах в арктической зоне Республики Саха (Якутия); Р.Ю. Абдуллаева и соавт. (Москва), выявивших формирование гиперкоагуляции у больных туберкулёзом с сахарным диабетом после перенесённого COVID-19 средней и тяжёлой степени; А.Ю. Самбяловой и соавт. (Иркутск), проанализиро-

вавших концентрации антиретровирусных препаратов у детей с перинатальной ВИЧ-инфекцией.

Несколько работ посвящены **репродуктивному здоровью и здоровью детей** – проблемам, которые также находятся в фокусе внимания современного здравоохранения и руководителей государства. Это обзор С.В. Зотова и соавт. (Новосибирск, Новокузнецк) с анализом влияния загрязнения окружающей среды, образа жизни, перенесённых операций, вредных привычек, ожирения, психологических и социальных факторов, сокращающих овариальный резерв. К.Д. Иевлева и соавт. (Иркутск) изучили диагностическую значимость интерлейкинов у женщин с хроническим эндометритом и избыточной массой тела, что позволит разработать малоинвазивный метод определения риска наличия данного заболевания. Е.Д. Казанцева и соавт. (Иркутск) выявили у больных гриппом детей более высокий, чем у здоровых, уровень продуктов липопероксидации, недостаток жирорастворимых витаминов и повышенные значения окисленного глутатиона. Завершает серию работа В.В. Кочеровой и соавт. из Читы, определивших факторы риска развития интравентрикулярных кровоизлияний у глубоко недоношенных новорождённых, что очень важно для организации помощи таким детям.

Хирургическое направление в данном выпуске представлено двумя оригинальными статьями и обзором. В.А. Зайка, Т.Н. Юрьева и Д.Б. Данзандоржиева (Иркутск) доказали, что эписклеральные методики **лечения регматогенной отслойки сетчатки** характеризуются лучшим анатомо-реконструктивным и функциональным эффектом. Л.В. Любимова и соавт. (Чебоксары) обнаружили *изменение* спектра ведущих возбудителей **имплантат-ассоциированной инфекции** в до- и постковидном периоде и изменение их антибиотикорезистентности и рекомендуют применение в эмпирической терапии ванкомицина, но ограниченное использование фторхинолонов. Обзор Л.М. Тибекиной и соавт. (Санкт-Петербург) доказывает, что **хирургический метод лечения фармакорезистентной эпилепсии** является приоритетным.

В данном выпуске как никогда большое количество интересных экспериментальных исследований. Это касается **моделирования различных патологий**: **катаракты** – А.Д. Чупровым и соавт. (Оренбург), показавшими снижение стеарил-коэнзим-А-десатуразы и мелатонина в ткани хрусталика; **неалкогольной жировой болезни печени** – Т.В. Брус, А.Г. Васильевым (Санкт-Петербург), предлагающими дополнительные тесты для оценки тяжести процесса; скополаминовой **холинергической недостаточности** – Я.Г. Разуваевой и соавт. (Улан-Удэ), изучавших нейропротективное действие экстракта *Orostachys spinosa* Sweet.

Три работы посвящены исследованию **новых и потенциальных веществ и композиций** для создания вакцинных, лекарственных препаратов и пробиотиков. Так, А.Б. Пятидесятникова и соавт. (Иркутск) показали стимулирование экспрессии генов *TLR2* и *TLR4* селенорганическим соединением 2,6-дипиридиний-9-селенабицикло[3.3.1]нонан дибромид (974zh), т. е. повышение иммуногенных свойств вакцинного штамма *Y. pestis* EV. С.М. Мирошниченко и соавт. (Новосибирск) доказали, что применение энтеросорбента на основе оксида алюминия и полидиметилсилоксана при моделировании непрерывного освещения способствует сохранению функциональной активности тимуса. А.С. Пеньдюхова и соавт. (Иркутск, Улан-Удэ) доказали, что важными для создания пробиотического консорциума с эффективным потенциалом являются как биосовместимость пробиотических штаммов, так и антагонистическая активность консорциума против болезнетворных штаммов, что позволяет определить эффективные стратегии применения пробиотиков.

В статье Е.В. Сайдаковой и соавт. (Пермь) для увеличения жизнеспособности и митотической активности размороженных после криоконсервации Т-лимфоцитов предлагается модифицировать протокол культивирования внесением интерлейкина 2 в культуральную среду.

Как всегда, в журнале есть описание уникальных клинических наблюдений. В этот раз Т.В. Сороковиковой и соавт. (Тверь, Москва) описывается редкое прионное заболевание – фатальная семейная бессонница, вызванная аутосомно-доминантной мутацией D178N гена *PRNP*.

В заключение я бы рекомендовал обратить внимание на очень интересный не только для теоретиков и клиницистов, но и для преподавателей обзор Э.Т. Аблякимова и М.А. Кривенцова (Симферополь), обсуждающий лиганд-ассоциированную активацию рецепторов витамина D в морфогенезе иммунного воспаления. Более глубокое понимание межклеточных взаимосвязей рецепторов витамина D, комплекса D_3-VDR и рецепторов иммунных контрольных точек (PD-1, PD-L, CTLA) в воспалении может стать основой для разработки новых стратегий диагностики, прогноза и лечения различных заболеваний.

Хорошего Вам лета и новых интересных идей!

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DISCUSSION PAPERS, LECTURES, NEW TRENDS IN MEDICAL SCIENCE

THE EFFECT OF EXERCISE AND NUTRITIONAL SUPPORT ON ELDERLY AND SENILE PATIENTS WITH SARCOPENIC OBESITY

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ABSTRACT

The aim of the review. *To analyze the prevalence of sarcopenic obesity among elderly and senile people, to assess its causes, and to present modern methods for its prevention and physical rehabilitation.*
This review article discusses the most recent evidence on age-related changes in fat and muscle tissue, and on calorie restriction and exercise that have positive effect on physical performance in older people with sarcopenic obesity. In addition, potential gaps in clinical practice guidelines that merit attention in future research are identified and analyzed.
Search strategy. *We used the following key words to define participation in the review: "sarcopenic obesity", "sarcopenia with obesity", "sarcopenia", "elderly/old age".*
Inclusion and exclusion criteria. *The review included original research results (reviews, meta-analyses). Editorials, proceeding of the conferences, and research protocols were excluded. The study sample included women and men of any race aged ≥ 60 years with a diagnosis of sarcopenic obesity and with preserved locomotion function. Articles involving hospital patients were also excluded. Non-human studies and studies that did not report precise intervention criteria (e. g., nutrition, exercise, duration, etc.) were excluded.*
The literature search was conducted in four electronic databases: PubMed, Cochrane Library, Springer, Scopus, for the period from 2013 to August 1, 2023. There were no restrictions on the language of the publication.

Key words*: sarcopenia, physical activity, aging, obesity, muscle strength, lean body mass, old age, morbidity*

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ВЛИЯНИЕ ФИЗИЧЕСКИХ УПРАЖНЕНИЙ И НУТРИТИВНОЙ ПОДДЕРЖКИ НА ПАЦИЕНТОВ ПОЖИЛОГО И СТАРЧЕСКОГО ВОЗРАСТА С САРКОПЕНИЧЕСКИМ ОЖИРЕНИЕМ

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РЕЗЮМЕ

Цель обзора. *Проанализировать распространённость саркопенического ожирения среди людей пожилого и старческого возраста, оценить причины его возникновения, представить современные методы профилактики и физической реабилитации.*
В данной обзорной статье обсуждаются самые последние данные о возрастных изменениях в состоянии жировой и мышечной ткани, а также об ограничении калорий и комплексах физических упражнений, которые оказывают положительное влияние на физические показатели пожилых людей с саркопеническим ожирением. Кроме того, выявлены и проанализированы потенциальные пробелы в рекомендациях по клинической практике, которые заслуживают внимания в будущих исследованиях.
Стратегия поиска. *Ключевые слова, используемые для определения условий участия в обзоре: «саркопеническое ожирение», «саркопения с ожирением», «саркопения», «пожилой/преклонный возраст».*
Критерии включения и исключения. *В обзор включались оригинальные результаты исследований (обзоры, метаанализы). Редакционные статьи, тезисы конференций, протоколы исследований были исключены. Выборка для исследования включала женщин и мужчин любой расы в возрасте ≥ 60 лет с диагнозом «саркопеническое ожирение» и сохранённой функцией локомоции. Также исключены статьи с участием госпитализированных пациентов. Исследования, не связанные с людьми, и исследования, в которых не сообщалось точных критериев вмешательства (например, питание, упражнения, продолжительность и т. д.), были исключены.*
Поиск литературы был проведён в четырёх электронных базах данных: PubMed, Cochrane Library, Springer, Scopus, – за период с 2013 г. по 1 августа 2023 г. Ограничений на языковой уклон публикации введено не было.

Ключевые слова: *саркопения, физическая активность, старение, ожирение, мышечная сила, мышечная масса тела, пожилой возраст, заболеваемость*

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INTRODUCTION

The number of elderly and senile people is growing worldwide. In 2017, the number of elderly people accounted for 13 % of the world population and is expected to increase to 20 % of the population by 2030, and to reach 2.1 billion by 2050 [1]. Most chronic diseases worsen with age, which is associated with profound changes in body composition, i.e., an increase and redistribution of fat mass and a loss of muscle and bone mass [2].

Among the most common conditions is obesity, which is a complex, multifactorial and relapsing disease that has become a pandemic worldwide [3]. In several countries, the prevalence of obesity reaches 30–40 % of the population, and a further increase in incidence is expected over the next decades [4]. Obesity is characterized by excessive accumulation of white adipose tissue, not only in fat depots, but also ectopically, which significantly reduces physical function [2, 3]. Thus, it is not surprising that obesity is associated with more than 200 medical complications and an increased risk of morbidity and mortality, being the fifth leading cause of death worldwide [3, 4]. Decreased muscle mass and strength, known as sarcopenia, is very common among older adults with obesity (sarcopenic obesity) and is closely associated with frailty, which is a state of impaired homeostatic reserve and stress tolerance that leads to increased vulnerability to adverse health outcomes [5]. Thus, sarcopenic obesity is closely associated not only with cardiometabolic dysfunctions, but also with physical disability [2].

Although we clearly see a significant increase in overall life expectancy, chronic diseases associated with aging, which are exacerbated by obesity, seriously impair quality of life during these "gained years." Therefore, there is increasing effort to identify effective strategies that can curb the obesity pandemic and support the process of healthy aging. Among them, lifestyle interventions, including dietary and training protocols, have been widely studied [6]. In this context, it is important to highlight that diet-induced weight loss involves not only a loss of fat, but also of muscle and bone mass and may further exacerbate age-related sarcopenia and frailty in older adults. Successful approaches that can induce fat loss while preserving muscle and bone mass are critical to reducing the cardiometabolic risks associated with aging and obesity, while preventing or mitigating frailty [2, 7, 8].

Obesity is a chronic metabolic disorder characterized by increased body fat stores, which consequently increases the risk of metabolic diseases, cardiovascular diseases, and mortality. As with sarcopenia, there is currently no consensus on appropriate cutoff values for obesity. The World Health Organization (WHO) uses body mass index (BMI) to define obesity (≥ 30 kg/m2) and overweight (25–29.9 kg/m2) [3]. The American Association of Clinical Endocrinology [9] recommends using cutoff values for body fat percentage to diagnose obesity (> 25% in men and > 35% in women). The amount of abdominal fat is easily estimated using waist circumference (WC), which is highly correlated with intra-abdominal fat content. WHO has also used WC cutoff values (≥ 102 cm for men, ≥ 88 cm for women) as a surrogate for visceral fat. Lower cutoff values for central obesity are required for different ethnic groups, including Asians (2, 8). The Korean Society for the Study of Obesity defines abdominal obesity as WC ≥ 90 cm and ≥ 85 cm in men and women, respectively, based on the results of an epidemiological study [10].

Sarcopenia is a predominantly geriatric disease with a gradual loss of muscle strength, skeletal muscle mass and muscle function. The term was officially adopted at the 2010 meeting of the European Working Group on Sarcopenia in Older People (EWGSOP), and in September 2016, sarcopenia was included in the International Classification of Diseases of the 10th revision (ICD-10) under the code M62.84. In 2018, the updated consensus EWGSOP-2 was aimed at improving the effectiveness of early detection and treatment of sarcopenia and its risk in clinical practice [11]. The group adopted low muscle strength as the main determinant of sarcopenia, since muscle strength is considered to be better than muscle mass in predicting adverse outcomes [11, 12]. EWGSOP-2 focused on low muscle strength (grip strength) as the main parameter of sarcopenia (hand dynamometry); low muscle quantity and quality were also used to confirm the diagnosis of sarcopenia (dual-energy X-ray absorptiometry (DXA), bioelectrical impedance analysis (BIA), ultrasound, etc.), and the assessment of the severity of sarcopenia relied on physical performance indicators based on a series of physical functioning tests (SPPB, Short Physical Performance Battery) [5, 11].

A related disorder is sarcopenic obesity, a term coined by R.N. Baumgartner and used for a specific phenotype of low muscle mass and high body fat [13].

Sarcopenic obesity (SO) is a multifactorial disease characterized by the simultaneous presence of sarcopenia and obesity. For patients, SO poses a greater health risk than either sarcopenia or obesity alone [2, 13]. Therefore, studying sarcopenic obesity and finding effective treatment are important due to the constant increase in the elderly population.

Sarcopenia and obesity lead to decreased physical performance. The hallmark of sarcopenia is slower gait speed. In addition to a higher risk of falls [14], obese older adults have decreased physical performance, as assessed by self-assessment questionnaires or tests such as the SPPB [13]. Sarcopenic obesity is thought to have a synergistic effect on health deterioration compared to sarcopenia or obesity alone. It causes more health problems than sarcopenia or obesity [15] and is a major cause of metabolic disorders, disability, cardiovascular disease, and mortality [16]. Currently, there is no common definition, making it difficult to establish standardized diagnosis and management. Despite progress in defining sarcopenic obesity, according to the recent

Consensus of the European Society for Clinical Nutrition and Metabolism (ESPEN) and the European Association for the Study of Obesity (EASO) [17], the discussion on the treatment of this condition is still open. Currently, there is no common definition, making it difficult to establish standardized diagnosis and management. Despite progress in defining sarcopenic obesity, according to the recent Consensus of the European Society for Clinical Nutrition and Metabolism (ESPEN) and the European Association for the Study of Obesity (EASO) [17], the discussion on the treatment of this condition is still open.

Sarcopenic obesity is a global health phenomenon driven by both the rapid increase in the elderly population and the obesity epidemic. Aging-associated increase in visceral fat and decrease in muscle mass are associated with numerous adverse cardiometabolic effects and contribute to poor health outcomes [18]. Several biological pathways lead to age-related sarcopenic obesity. Aging reduces resting metabolic rate and metabolic adaptations, including adaptive thermogenesis, maintaining low muscle mass and increasing body fat [13]. Reduced resting metabolic rate, physical activity, mitochondrial volume, and oxidative capacity with age contribute to age-related declines in muscle mass and strength. Furthermore, age-related changes in body fat distribution include loss of subcutaneous fat and accumulation of visceral fat [19]. Also, age-related changes accompanied by a decrease in bone mineral density can lead to the development of osteosarcopenic obesity, the main criterion of which is the deterioration of bone condition and loss of muscle mass, coupled with the presence of sarcopenia and obesity [10, 20].

Sex-specific hormonal changes are an important factor associated with sarcopenic obesity. In women, declining estrogen levels after menopause lead to increases in body weight and fat mass, as well as shifts in fat deposition from subcutaneous to visceral [13, 18]. In older men, total testosterone levels decline by approximately 1% per year, with lower levels associated with sarcopenia, decreased muscle strength, deterioration in physical performance, and increased risk of falls [21].

PREVALENCE AND MORTALITY RISK

As the population ages, the prevalence of sarcopenic obesity increases, as the prevalence of obesity and sarcopenia also increases, especially among adults aged 65 years and older [2]. This is associated with decreased physical activity and energy expenditure, as well as increased body weight [10, 13].

A study of sarcopenic obesity in South Korea, comprising healthy volunteers aged 40–80 years, found the prevalence of sarcopenic obesity ranging from 0.8% to 22.3% in women and from 1.3% to 15.4% in men [22]. Data from individuals aged 18–90 years from the Dutch Lifelines cohort study showed a global prevalence of sarcopenic obesity of 1.4% and 0.9% in women and men, respectively, with a rise in prevalence at 50 years and the prevalence reaching 16.7% in the 80–89 age group [23]. A meta-analysis of 50 studies including 86,285 individuals reported a global prevalence of sarcopenic obesity in adults aged 60 years to be 11% [24]. A meta-analysis of 50 studies including 86,285 participants found a global prevalence of sarcopenic obesity of 11% in adults aged 60 years [24].

In a study of older adults (n = 4652) aged over 60 years, conducted as part of the Third National Health and Nutrition Examination Survey (NHANES III), the prevalence of sarcopenic obesity was 18.1% in women and 42.9% in men. The study defined sarcopenia using the BIA-derived sex-specific cutoffs [25]. Another study conducted among Koreans (n = 2221) aged over 60 years, using the same SD criteria, found that the prevalence of sarcopenic obesity was 6.1% and 7.3% in men and women, respectively [10]. The rapidly increasing prevalence of obesity suggests a likely corresponding increase in sarcopenic obesity in elderly and senile people.

Multiple studies have assessed the association between sarcopenic obesity and mortality risk. NHANES III also conducted a mortality risk analysis in 4652 individuals aged 60 years and older with a 14-year follow-up, and found a significantly higher risk of all-cause mortality among women with sarcopenic obesity compared to women without obesity or sarcopenia. However, there was no statistically significant difference in mortality risk between men with and without sarcopenic obesity [25]. On the other hand, the British Regional Heart Study examined mortality risk in 4107 men aged 60 to 79 years. During an 11-year follow-up period, men with sarcopenic obesity had the highest mortality risk compared to non-obese and non-sarcopenic subjects [26]. A Swedish study of 809 people assessed mortality risk in sarcopenic obesity. Women aged 75 years with SO had a higher 10-year mortality risk compared to women without sarcopenia or obesity. Among men aged 75 years, a similar association with mortality was observed, although it did not reach statistical significance [27]. A cohort study using the UK Biobank (n = 452,931) showed a significantly increased mortality risk in individuals with sarcopenic obesity compared with control subjects with pre-existing cardiovascular disease [28]. Finally, a meta-analysis of 23 studies including 50,866 individuals found that sarcopenic obesity was significantly associated with a higher mortality risk in older adults. Multiple subgroup analyses showed that this higher mortality risk was significant among adults living alone and hospitalized patients. Furthermore, this indicator was consistent across studies that used different criteria to define obesity and sarcopenia [29].

A long-term study of 2,309 elderly Japanese American men in the Honolulu Heart Program in the United States reported a significantly higher mortality risk in the sarcopenic group (measured using DXA) than in the non-sarcopenic, non-obese group [30].

DIAGNOSIS OF SARCOPENIC OBESITY

Since sarcopenic obesity is a subclinical disease and there is no universal consensus on diagnostic criteria and their implementation in clinical practice, the identification of the disease and diagnosis depend on the sum of its components, specifically obesity and sarcopenia.

Recently (2022), ESPEN and EASO published a joint consensus statement on the definition and diagnostic criteria for sarcopenic obesity [31] (Fig. 1). The proposed diagnostic process is as follows: 1) screening of patients by high BMI or increased waist circumference and surrogate parameters for sarcopenia; 2) diagnosis of patients by testing muscle function followed by body composition analysis; 3) making a diagnosis in case of a positive result for sarcopenic obesity [31]. In practice, a person who screens positive for both conditions should consider undergoing diagnostic testing to confirm sarcopenic obesity, which first evaluates the decline in skeletal muscle function and then measures altered body composition, including increased fat mass and decreased muscle mass. In the future, these diagnostic criteria for sarcopenic obesity will need to be validated to establish universal reference values according to the measurement method and ethnic group.

There is currently no ICD code for sarcopenic obesity, and the working definition/differential diagnosis is constantly evolving. Age is a strong risk factor for the onset and severity of sarcopenic obesity, but the disease is not unique to the elderly [32]. Therefore, diagnostic consideration is given to the manifestation of symptoms, with age being a component of risk management [33].

Current definitions of sarcopenic obesity are based on individual definitions of sarcopenia and obesity. However, these definitions vary significantly, making it difficult to accurately diagnose, conduct epidemiological studies, and develop treatment strategies for the disease.

THE EFFECT OF EXERCISE ON PHYSICAL PERFORMANCE

Exercise can affect hormonal balance, reduce oxidative stress, induce mitochondrial synthesis, alter immunological and motor functions, and improve muscle oxidative capacity [34, 35]. Increased muscle protein synthesis with exercise sensitizes muscle sensitivity to insulin and promotes anabolism [6]. Sarcopenia is associated with reduced muscle protein synthesis, partly due to decreased anabolic stimulation (which can result from a lack of regular exercise). Aerobic training, strength training, and a combination of both increase muscle protein synthesis in older adults despite age-related declines in anabolic signaling [7, 10]. Aerobic activity can improve muscle oxidative capacity by counteracting the negative effect of intracellular lipids and accelerating lipolysis, leading to increased capillary density. Mitochondrial synthesis in myocytes is enhanced to meet the demands of increased capillary density, which in turn leads to increased oxygen extraction and metabolism through the induction of calcium and metabolic signaling pathways [34].

Myocyte apoptosis can be abolished by physical activity, while cell quality control mechanisms including autophagy, mitophagy and mitochondriogenesis contribute to the development of sarcopenic obesity and may be potential targets for therapy [36]. Reduction in cytokine production may lead to improvements in glucose metabolism, insulin sensitivity and muscle protein synthesis, which may slow the progression of sarcopenic obesity [2].

Resistance training increases the number and size of fast-twitch muscle fibers (IIA and IIX), which improve muscle glucose metabolism and muscle protein synthesis [37]. Muscle protein synthesis is also improved through nutrient-stimulated vasodilation and nutrient transport to local muscle myofibrils [7]. Muscle fascicle length and muscle tendon stiffness have been reported to increase after resistance training (leg press and extension) for 14 weeks in a group of men and women over the age of 65 [2]. Resistance training has also been shown to decrease levels of cytokines such as resistin, leptin, and IL-6 (REF142) [38].

TREATMENT OF SARCOPENIC OBESITY

Resistance, aerobic, and combined training programs reduce body fat and improve muscle function in older men and women with sarcopenic obesity [39, 40]. Notably, resistance training alone was more effective in improving muscle function. Resistance training improves physical function while reducing body fat in older women with sarcopenic obesity [40, 41]. Similarly, dietary modification to ensure adequate and/or high protein intake prevents muscle loss and in some cases improves muscle function [42]. Dietary restriction is the gold standard first-line treatment for obesity and to some extent counteracts the detrimental effects of aging on skeletal muscle function in mammals [43]. However, evidence for the safety and effectiveness of dietary restriction in patients with sarcopenic obesity is very limited. In a recent study, a very low calorie diet improved muscle performance in patients with sarcopenic obesity, although at the expense of muscle mass [44]. Patients receiving a very low calorie diet combined with physical training showed similar improvements in muscle function while maintaining muscle mass, indicating a synergistic effect of combination therapy in the treatment of the disease. Micronutrients and minerals such as amino acids, vitamin D, selenium, and magnesium can be added to the diet to correct pre-existing deficiencies or to establish supraphysiological concentrations that elicit a biological response

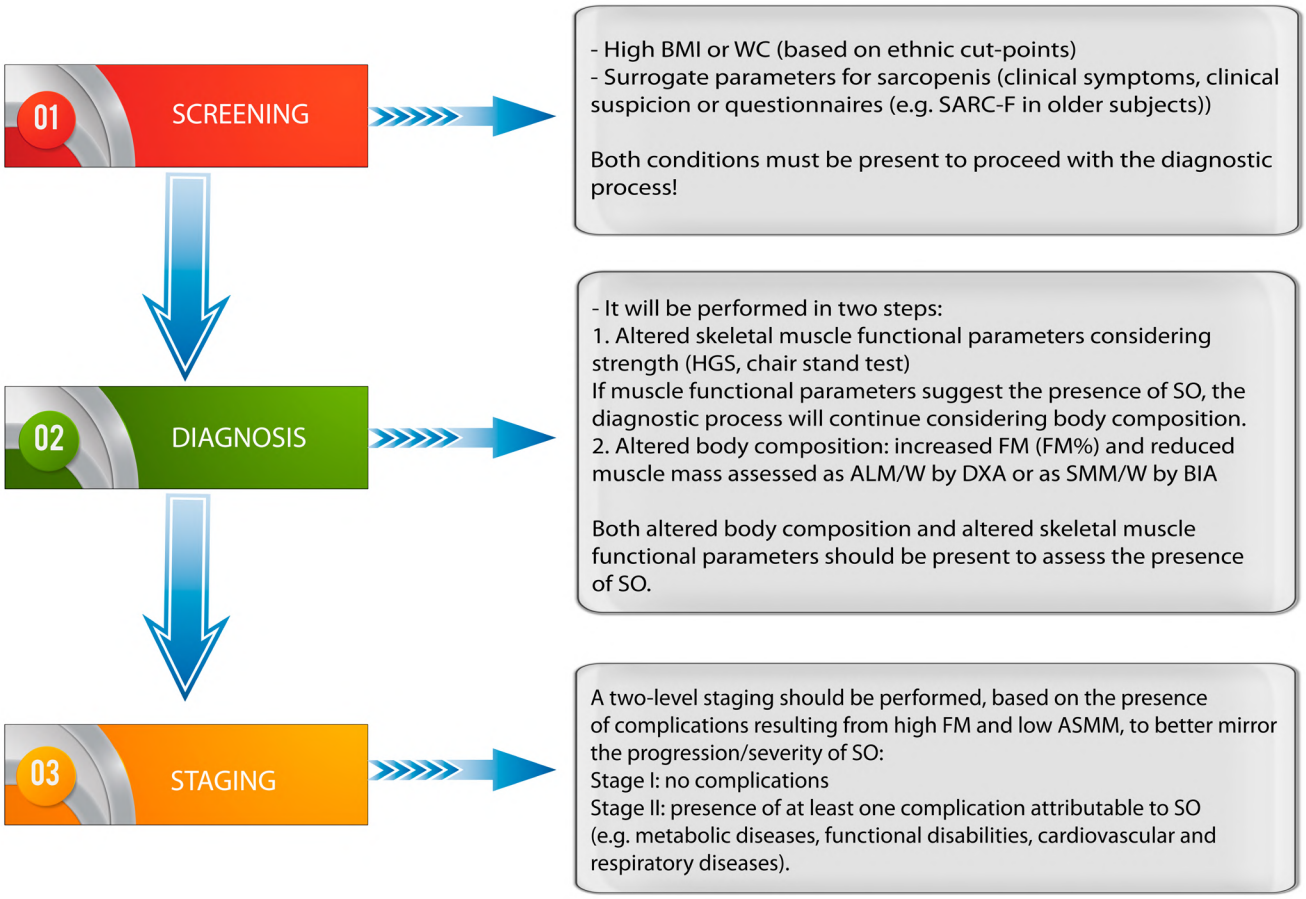

FIG. 1.
Diagnostic procedure for the assessment of sarcopenic obesity SARC-F – Strength, Assistance with walking, Rising from a chair, Climbing stairs and Falls; HGS – handgrip strength; FM – fat mass; ASMM – absolute skeletal muscle mass

[45]. Whey protein supplementation in combination with exercise improves muscle function in adults with sarcopenic obesity [46]. In men, testosterone replacement therapy can be used alone or in combination with diet, exercise, or vitamin supplements to restore androgen balance. Use in elderly and senile patients is somewhat limited due to the high risk of cardiovascular complications [47]. Similarly, in postmenopausal women, estrogen replacement therapy can be used alone or in combination with lifestyle modification with generally positive results in preserving muscle mass [48]. More recently, oxytocin therapy has been used as a trial therapy for sarcopenic obesity to restore the relative decline in oxytocin production with age. Intranasal oxytocin is well tolerated and improves muscle mass in older adults with sarcopenic obesity [49]. However, it remains unclear whether intranasal oxytocin improves muscle function and physical performance. A summary of the prevailing clinical interventions is provided in Table 1. Taken together, treatment options are insufficient and complicated by the competing needs underlying sarcopenia and obesity [33].

The approach to exercise prescription in sarcopenic obesity should be individualized. A program that includes a combination of resistance and aerobic training may be more beneficial than either intervention alone [2, 50]. Aerobic training should be aimed at achieving approximately 65% of peak heart rate, with the goal of increasing to a maximum of 75%. Resistance training should focus on only one or two muscle groups, with the initial 8–12 reps being approximately 65% of the maximum strength a person could develop in one repetition. Progression should be aimed at using 2–3 muscle groups and 75% of maximum intensity [2, 22].

In the sarcopenic obese population, the effects of resistance training on body composition and muscle function are less clear. K.S. Vasconcelos et al. [51] showed that a 10-week resistance training program was not effective in improving physical function in older women with sarcopenic obesity compared to a non-training control group: the SPPB score increased by 0.40 ± 1.3 points ($p > 0.05$), strength increased by 2.36 ± 12.0 N/kg ($p > 0.05$), and power increased by 15.87 ± 13.8 W/kg ($p < 0.05$). The small sample size and short duration of treatment may have

contributed to this result. The study by A.B. Gadelha et al. [52] demonstrated improvements in both strength (12.42 ± 1.5 N/kg; $p < 0.001$) and skeletal muscle mass (0.29 ± 0.4 kg; $p < 0.001$) compared to the control group after 24 weeks of traditional resistance training. Leg press and bench press performance increased by 78% and 70%, respectively. Furthermore, a 12-week study examined the effects of elastic band resistance training in older women with SO and found that skeletal muscle mass (0.73 kg; 95% confidence interval (95% CI): 0.08–1.39; $p < 0.05$) and physical performance (8.58 points; 95% CI: 4.79–12.36; $p < 0.001$) were significantly improved compared to the non-training group [40]. Also, another recent study reported that 8 weeks of resistance training in 60 elderly individuals with sarcopenic obesity resulted in a slight increase in skeletal muscle mass (0.1 kg; $p < 0.05$), a decrease in fat mass (≈ 1.0 kg; $p < 0.05$), and an increase in handgrip strength (3.5 N/kg; $p < 0.05$) compared to a group that did not engage in physical training [38]. W. Kemmler et al. (Germany) conducted a study on men aged ≥ 70 years ($n = 100$) using whole-body electromyostimulation (WBEMS): fat mass loss was 2.1%, handgrip strength increased by 6.3% [53]. A year earlier in Brazil, K. Wittmann et al., using WBEMS, achieved similar results [54]. The advantages of the treatment are its time-effectiveness and accessibility to populations with high levels of frailty. Although electrical myostimulation favorably improves body composition and muscle function compared to noninvasive control [53, 54], the effect sizes are small and may be ineffective in adults with more severe sarcopenic obesity [32, 55].

TABLE 1

CLINICAL TRIALS AIMED AT THE TREATMENT OF SARCOPENIC OBESITY

Sex	Age, number of observations	Study protocol	Duration, frequency	Result	Author, year of publication
Men/women	50-70 years, 24 observations	VLCKD in combination with IT	6 weeks, 30-35 min, 2 days a week	↑ HGS ↓ FM	Camajani E. et al. (2022)
Women	65-80 years, 28 observations	RT	10 weeks, 35 min, 3 days a week	= SPPB = SMM ↑ power	Vasconcelos K.C. et al. (2016)
Women	≥ 60 years, 26 observations	RT with WP	12 weeks, 35-40 min, 3 days a week	↓ FM ↑ LBM	Nabuco H.C.G. et al. (2019)
Women	≥ 70 years, 75 observations	WBEMS and WP + vitamin D	26 weeks, 20 min, 1 session a week	↑ SMM ↓ WT = BP	Wittmann K. et al. (2016)
Men	≥ 70 years, 100 observations	WBEMS and WP	16 weeks, 14-20 min, 2 sessions a week	↑ HGS ↓ FM ↓ BMI	Kemmler W. et al. (2017)
Men/women	65-75 years, 60 observations	RT, AT and CT	8 weeks, 40 min, 3 times a week	↑ SMM ↑ HGS ↓ FM	Chen H.T. et al. (2017)
Women	60-80 years, 56 observations	RT with elastic band	12 weeks	↑ SMM ↑ SPPB	Liao C.D. et al. (2018)
Women	≥ 60 years, 49 observations	RT	16 weeks, 40-45 min, 2 days a week	↑ SPPB ↑ walking speed	de Oliveira Silva A. et al. (2018)
Women	≥ 60 years, 69 observations	RT	24 weeks, 40 min, 3 days a week	↑ LBM ↑ HGS = FM	Gadelha A.B. et al. (2016)

Note. ↑ – increase; ↓ – decrease; = – no changes; VLCKD – very low-calorie ketogenic diet; IT – interval training; HGS – handgrip strength; FM – fat mass; RT – resistance training; SPPB – short physical performance battery; SMM – skeletal muscle mass; WP – whey protein; LBM – lean body mass; WBEMS – whole-body electromyostimulation; BP – blood pressure; WT – waist circumference; BMI – body mass index; AT – aerobic training; CT – combined resistance and aerobic training.

Overall, most of the studies mentioned showed that resistance training is an effective strategy to improve body composition in sarcopenic obesity and that it has the potential to improve physical performance [46, 54, 56].

NUTRITION: CALORIE RESTRICTION AND PROTEIN SUPPLEMENTS

Lifestyle approaches such as calorie restriction and physical training are considered the cornerstone of sarcopenic obesity treatment [15, 24]. With regard to nutrition, the optimal therapeutic approach for sarcopenic obesity remains to be determined due to the limited number of clinical trials conducted in this area [31, 43, 57].

Indeed, weight loss in obese older adults remains controversial as it is a double-edged strategy that has beneficial effects by reducing obesity-related complications and potential negative consequences. Currently, very low calorie intake should be avoided in older adults with sarcopenic obesity as this strategy may compromise overall health and lead to micronutrient and electrolyte deficiencies in the body, which will have a detrimental effect on skeletal muscle mass and reduce bone mineral density [13, 57].

In a pilot study by R. Sammarco et al. (Italy), participants with sarcopenic obesity who underwent a weight loss program supplemented with a high-protein diet showed improvement in muscle strength, and the SF-36 (Short Form 36) questionnaire for assessing the patient's quality of life showed a significant change in general health after 4 months of the study [58].

Although the exact amount of kilocalories (kcal) per day has not yet been determined, it should be less than 750 kcal per day [42]. Generally, high-quality protein intake (1–1.2 g/kg per day), especially those containing sources of leucine, is recommended and can be consumed in conjunction with a calorie-restricted diet [43, 44]. However, caution is needed when consuming high-protein diets due to the risk of renal impairment. Medical and dietary management is important to design a nutritional program that allows for moderate calorie restriction while optimizing protein intake [42, 46].

To minimize the risk of weight loss-induced decrease in bone turnover, generally accepted strategies to minimize the impact of weight loss on bone turnover are needed, including supplemental calcium intake at a dosage of 1200 mg per day and 800–1000 international units (IU) of vitamin D3 per day [59]. Oral calcium should be combined with vitamin D to reduce potential risks associated with over-the-counter supplements [60].

Vitamin D supplementation in patients with sarcopenic obesity has the potential to improve muscle function (25-hydroxyvitamin D3 and 1,25-dihydroxyvitamin D3 have differential effects on human skeletal muscle function and gene expression) and reduce proximal muscle weakness via the action of vitamin D metabolites [61]. Vitamin D deficiency is associated with an increased risk of falls and fractures, as well as decreased muscle mass and strength independent of obesity [62].

CONCLUSION

Physical performance declines with age, and the decline is steeper in sedentary adults with sarcopenic obesity. Physical training, and progressive resistance training in particular, is the most commonly used training method in adults aged 60–80 years [46, 51, 63]. It should be noted that no previous studies have examined differences in exercise prescription by classifying participants in subgroups based on age-level. This is a key aspect to develop in the future.

Sarcopenic obesity is a multifaceted disease with limited treatment options. Systemic energy load due to obesity in the context of aging serves as a pathophysiological cause of the disease, increasing the rate of muscle loss [10, 15, 64]. To this end, reducing energy load and improving muscle function are necessary components of a successful therapeutic intervention.

The lack of a universally used diagnostic method and definition criteria does not allow a clear assessment of the prevalence, which currently remains underestimated [23, 24]. Thus, the diagnosis of sarcopenic obesity is an initial problem.

Overall, the best therapeutic approach to sarcopenic obesity, with the most effective and reliable data to date, is lifestyle modification, including regular combined aerobic and resistance training, with dietary modifications that should include calorie restriction to decrease fat mass and increased protein intake to increase muscle mass and functional capacity to improve quality of life and reduce mortality [22, 52].

Longer term studies assessing the impact of a multimodal approach on sarcopenic obesity and cardiovascular complications in patients will be of great interest.

Early detection of this condition remains important and tailored interventions should be considered to reduce its prevalence and associated adverse outcomes. New therapeutic strategies are required to improve the unfavourable prognosis.

Conflicts of interest

No potential conflict of interest relevant to this article reported.

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FACTORS AND LEVEL OF PHYSICAL PERFORMANCE OF SCHOOLCHILDREN AGED 13–14 YEARS

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ABSTRACT

There is an insufficiency of data on the characteristics of physical performance of schoolchildren in the critical period of ontogenesis associated with pubertal development.
The aim of the study. *To determine the factors and level of physical performance of schoolchildren aged 13–14 years, taking into account pubertal development.*
Methodology. *The study involved healthy male adolescents aged 13–14 years (n = 165). Five stages of puberty were determined. To diagnose the level of physical performance, a complex of functional and ergometric tests and a battery of motor tests were used. The structure of performance was determined based on the factor analysis.*
Results and discussion. *We determined the factors characterizing physical performance: aerobic capacity; absolute aerobic power; anaerobic alactic performance; anaerobic glycolytic performance; relative aerobic power. The identified factors are associated with zones of relative power. It has been established that during puberty, changes in indicators combined into different factors occur non-linearly and non-simultaneously. The results of the study show that subjects of the same age with stages II, III and IV of puberty differ in the level of key bioenergetic performance criteria. Transition to higher stages of puberty is accompanied with progressive dynamics of most indicators associated with factors of anaerobic performance, while indicators of aerobic power and capacity change in different directions, showing in some cases a tendency to temporarily decrease.*
Conclusion. *It is advisable to use the results of the study when organizing various types of monitoring the functional state and regulation of aerobic and anaerobic physical activity in adolescents aged 13–14 years at different stages of puberty. The obtained materials can serve as a scientific basis for improving the physical education system in order to increase the functional capabilities of children's bodies during the critical period of ontogenesis associated with pubertal development.*

Key words: *factor analysis, physical performance and muscle energy, stages of puberty, adolescent boys*

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ФАКТОРЫ И УРОВЕНЬ ФИЗИЧЕСКОЙ РАБОТОСПОСОБНОСТИ ШКОЛЬНИКОВ 13–14 ЛЕТ

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РЕЗЮМЕ

Существует недостаток данных об особенностях физической работоспособности школьников в критический период онтогенеза, связанный с процессом полового созревания.

Цель исследования. *Выявить факторы и уровень физической работоспособности школьников 13–14 лет с учётом полового созревания.*

Методика. *В исследовании приняли участие здоровые подростки мужского пола 13–14 лет (n = 165). Определяли пять стадий полового созревания. Для диагностики уровня физической работоспособности использовали комплекс функциональных и эргометрических проб и батарею моторных тестов. Структуру работоспособности определяли на основе факторного анализа.*

Результаты и обсуждение. *Идентифицированы факторы, характеризующие физическую работоспособность: аэробная ёмкость; абсолютная аэробная мощность; анаэробная алактатная работоспособность; анаэробная гликолитическая работоспособность; относительная аэробная мощность. Выделенные факторы ассоциируются с зонами относительной мощности. Установлено, что в процессе полового созревания изменения показателей, объединённых в разные факторы, происходят нелинейно и неодновременно. Результаты исследования показывают, что испытуемые одного возраста со II, III и IV стадиями полового созревания отличаются по уровню ключевых биоэнергетических критериев работоспособности. С переходом на более высокие стадии полового созревания наблюдается прогрессивная динамика большинства показателей, связанных с факторами анаэробной работоспособности, тогда как показатели аэробной мощности и ёмкости изменяются разнонаправленно, проявляя в отдельных случаях тенденцию к временному снижению.*

Заключение. *Результаты исследования целесообразно использовать при организации различных видов контроля функционального состояния и нормирования физических нагрузок аэробной и анаэробной направленности у подростков 13–14 лет с разными стадиями полового созревания. Полученные материалы могут служить естественнонаучным основанием для совершенствования системы физического воспитания в целях повышения функциональных возможностей организма детей в критический период онтогенеза, связанный с процессом полового созревания.*

Ключевые слова: *факторный анализ, физическая работоспособность и мышечная энергетика, стадии полового созревания, мальчики-подростки*

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BACKGROUND

Physical performance is a reliable indicator of the functional state of a person, reflecting the level of his health, physiological and mental reserves of the body, efficiency, power and capacity of energy sources, the degree of adaptation to intensive muscular activity. Identification of patterns of formation of the muscular energy system and human performance during ontogenesis is one of the most important tasks of sports and age physiology, preventive medicine, health-improving physical culture, theory and methods of youth sports. This is largely determined by the fact that the transformation of individual elements of this system in the process of development occurs heterochronically and unevenly, determining the specificity of the body's adaptation to physical work of aerobic and anaerobic orientation [1-4]. Of particular importance is information about the state of muscular energy and human performance during the critical period of ontogenesis associated with the process of puberty. During puberty the activity of the hypothalamic-pituitary system, which mediates the restructuring of the functioning of the endocrine glands and key physiological systems, changes significantly [5–8].

Today, the question of the factors determining the physical performance of adolescents during puberty remains open. These factors are generally considered to be relatively independent aspects of performance, reflecting the activity of various functional systems integrated into the dominant functional system responsible for the implementation of muscular activity [9, 10]. To a large extent, this is due to the fact that during puberty, there are significant transformations in the mechanisms of energy supply for muscle activity [2, 4], which cannot but affect the change in the number and composition of factors characterizing physical performance, the boundaries of various power zones, the ratios of the development levels of aerobic and anaerobic capabilities and motor fitness. In this regard, there is a need to identify the factor structure and level of physical performance of adolescents, as well as to determine valid indicators for its assessment during puberty.

It is well known that during puberty, the functional state and reserve capacities of the body are determined not only by the passport age, but also by the biological age of children [5, 2, 4, 11]. At this stage of development, significant differences in the level of sexual maturity are observed among adolescents of the same passport age [12, 13], which must be taken into account when diagnosing the functional capabilities of the body, standardizing aerobic and anaerobic loads, and choosing adequate physical activity regimens.

THE AIM OF THE STUDY

To determine the factors and level of physical performance of schoolchildren aged 13–14 years, taking into account pubertal development.

METHODS

The study involved 13–14-year-old male adolescents who did not attend sports clubs (n = 165; average age 13.5 ± 0.03 years). The study was carried out in accordance with the principles of the World Medical Association Declaration of Helsinki and was approved by the Ethics Committee of the Institute of Child Development, Health and Adaptation (Protocol No. 1 dated February 02, 2023).

The study was conducted at an air temperature of 18–24°C, several hours after eating. The room where the testing was carried out provided conditions for providing emergency and first medical aid. A doctor with extensive experience in carrying out this type of functional research participated in the work. Exclusion criteria for testing physical performance were: acute illnesses 2 weeks before the start of the study; signs of acute respiratory infection at the time of examination. The study was conducted only on healthy adolescents with no medical contraindications for physical education and sports, on days of optimal performance between 9:00 and 12:00 a.m.

The factors of physical performance and informative indicators suitable for its diagnostics were determined. In adolescents with different stages of puberty, the values of informative indicators of physical condition characterizing each of the identified factors were compared.

The study used a heterogeneous battery of ergometric and functional tests that comprehensively assessed physical performance [10]. The maximum oxygen consumption (VO_2max) according to Dobeln, watt-pulse (WP), physical working capacity at a pulse of 170 beats/min (PWC_{170}), maximum strength (MS), pulse debt accumulation intensity (PDAI) and time for performing bicycle ergometric loads "to failure" of 3 and 5 W/kg were determined. Using the Muller equation, individual constants characterizing the capacity of the aerobic (b) source and the degree of heterogeneity of the working skeletal muscles (a), indicators of the work power with the retention time of 1, 40, 240 and 900 s (W_1, W_{40}, W_{240} and W_{900}) were found [2, 14].

The testing model assumed the use of bicycle ergometer loads. To calculate the parameters of the Mueller equation, two loads "to failure" of 3.0 and 5.0 W/kg were performed, VO_2max – one "standard" load of 2.5 W/kg body weight for 5 min, PWC_{170} - a load of increasing power with rest intervals. The load steps were 1.5, 2.5 and 3.5 W/kg body weight. The duration of work at each step was 5 min; the rest interval between steps was 3 min. The first two stages of work were implemented by all subjects. If the heart rate (HR) after the second stage of work did not reach 150 beats/min, the third stage was performed with a power of 2.5–3.5 W/kg [15]. Heart rate was recorded using a Polar heart rate monitor (Polar Electro, Finland). The interval between two tests for maintaining loads "to failure" was 7 days, in all other cases – 2 days. The pedaling speed was constant and amounted to 60 rpm. As a criterion for "failure

to perform the work" a decrease in the pedaling frequency by more than 10% was considered. During the testing of adolescents' performance, no significant clinical signs indicating the need to stop work were detected, supporting the idea that the assessment of physical performance of adolescents through strenuous physical exercise is a safe procedure if precautions are taken [2, 15].

The battery of control exercises included: 6-minute run; 4 × 9 m shuttle run; 20 m standing start; standing long jump, torso lifting from a supine position in 1 min; forward bend. The overall physical fitness score (OPFS) was calculated by summing the points obtained for completing each motor test [10].

According to the method of D.V. Kolesov and N.B. Selverova, 5 stages of puberty were determined. All stages of puberty were identified in the sample under examination (stage I – 4 schoolchildren; stage II – 63 schoolchildren; stage III – 58 schoolchildren; stage IV – 37 schoolchildren; stage V – 3 schoolchildren). However, the results of testing adolescents with stage I and stage V were not analyzed due to their small number. It was found that the groups of adolescents with different stages of puberty did not statistically significantly differ in passport age.

Statistical data processing was performed using the Statistica software package (StatSoft Inc., USA). Factor analysis as the principal component method was used to study the structure of physical performance. The possibility of performing factor analysis was assessed using the Kaiser – Meyer – Olkin (KMO) criterion. The sample was considered acceptable if the value of this criterion exceeded 0.5. The statistical significance of differences was determined by applying parametric and nonparametric criteria of statistical significance of estimates for unrelated sample populations. The probability level $p < 0.05$ was used to assess the statistical significance of differences. The described research algorithm was also used to identify the structure of physical performance in children aged 7–8 and 9–10 years [10].

RESULTS AND DISCUSSION

Based on multivariate statistical analysis, factors characterizing the muscular energy and physical performance of students during puberty were identified. The factors were interpreted based on the analysis of the physiological content of the indicators included in them, taking into account the values of the weighting coefficients.

After factorization of the intercorrelation matrix, significant factors were identified that reflect the fundamental characteristics of muscle energy and physical performance of adolescent boys during puberty: I – aerobic capacity (oxidative system); II – absolute aerobic power (oxidative system); III – anaerobic alactic performance (phosphagen system); IV – anaerobic glycolytic performance (lactic system); V – relative aerobic power (oxidative system) (table 1).

The basis of the internal structure of the aerobic capacity factor (38% of the total variance) is created by its close relationships with the coefficients "b" and "a" of the Muller equation, the time of physical performing "to failure" of a load of 3 W/kg (t3 W/kg), W_{900}, W_{240}, $PDAI_{3\ W/kg}$ and the results of a 6-minute run (table 1).

All these physiological variables characterize to one degree or another volume of aerobic work performed. The exceptions are the W_{240} and 6-minute run indicators, which reflect the mixed aerobic-anaerobic nature of energy supply, also included in the factors of anaerobic glycolytic and anaerobic alactate performance. The greatest weight loads for the aerobic capacity factor were characterized by the coefficient "b" of the Mueller equation ($r = 0.98$) and $t_{3\ W/kg}$ ($r = 0.97$).

The absolute (17% of variance) and relative (5% of variance) aerobic power factors included VO_2 max, WP, and PWC_{170}. It should be noted that in the first case, these physiological indicators were characterized by high negative factor coefficients, and in the second case – by a high and medium degree of positive correlation. In both factors, VO_2max ($r = -0.94$ and $r = 0.86$) and WP ($r = -0.94$ and $r = 0.85$) had the maximum weight loads.

The performance factor associated with the phosphagen energy supply system (13% of variance) correlated with the PDAI after sprint running, motor fitness assessment (MFA), W_1, MS, results of shuttle running, long jump, 20 m standing start, 6-minute run. Most of the above mentioned indicators were characterized by a medium degree of correlation with the factor under review. The most significant statistical relationship with it was demonstrated by the PDAI after sprint running ($r = -0.93$) and MFA ($r = 0.75$).

The performance factor associated with the lactic acid energy supply system (8% of variance) combined $t_{5\ W/kg}$, W_{40}, W_{240}, $PDAI_{5\ W/kg}$, and the results of the torso lifting test. The maximum values of the factor coefficients were $t_{5\ W/kg}$ ($r = 0.91$) and W_{40} ($r = 0.91$).

Taking into account the results of multivariate statistical analysis, the influence of puberty on the performance indicators included in each factor was assessed. Differences due to the degree of puberty were identified (table 2).

Depending on the stage of puberty, statistically significant differences ($p < 0.05$–0.001) were found between most of the variables included in the aerobic capacity factor. Differences were observed for individual indicators between stages II and III on the one hand, and stage IV on the other (table 2). In this case, boys with stage III had higher values compared to stage II and especially stage IV. It is important to note that adolescents with stage III were characterized by a high generalized assessment of aerobic capacity (fig. 1).

Physiological variables associated with aerobic power factors also depended on the stage of puberty. The highest ($p < 0.05$–0.001) values of absolute aerobic performance indicators were observed at the stage IV,

TABLE 1

FACTOR STRUCTURE OF PHYSICAL PERFORMANCE OF MALE ADOLESCENTS AGED 13–14 YEARS

Indicators	Factors				
	I	II	III	IV	V
Coefficient «b», r.u.	0.981	-	-	-	-
$t_{3\ W/kg}$, s	0.966	-	-	-	-
W_{900}, W/kg	0.901	-	-	-	-
Coefficient «a», r.u.	0.871	-	-	-	-
W_{240}, W/kg	0.766	-	-	-	-
$PDAI_{3\ W/kg}$, bpc	-0.686	-	-	-	-
6-minute run, m	0.508	-	-	-	-
VO_2max, l/min	-	-0.938	-	-	-
WP, kgf-m/b	-	-0.937	-	-	-
PWC_{170}, kgm/min	-	-0.925	-	-	-
PDAI (sprint running), bpc	-	-	-0.926	-	-
MFA, score	-	-	0.746	-	-
Shuttle running, s	-	-	-0.710	-	-
Long jump, cm	-	-	0.675	-	-
W_1, W/kg	-	-	0.632	-	-
MS, kg/kg	-	-	0.632	-	-
20 m standing start, s	-	-	-0.603	-	-
6-minute run, m	-	-	0.515	-	-
$t_{5W/kg}$, s	-	-	-	0.913	-
W_{40}, W/kg	-	-	-	0.909	-
Torso lifting from a supine position in 1 min, time	-	-	-	0.782	-
W_{240}, W/kg	-	-	-	0.539	-
$PDAI_{5W/kg}$, bpc	-	-	-	-0.508	-
VO_2max, l/min*kg	-	-	-	-	0.856
WP, kgf-m/b*kg	-	-	-	-	0.845
PWC_{170}, kgm/min*kg	-	-	-	-	0.746
Variance, %	38	17	13	8	5

Note. MFA – motor fitness assessment.

and the lowest – at the stage II (table 2). In contrast, relative aerobic performance indicators showed a tendency to decrease as the transition from the stage II to the stage IV occurred, which in some cases was statistically significant ($p < 0.05–0.001$). Similar dynamics were demonstrated by the integral estimates of absolute and relative aerobic power.

Lower relative values of VO_2max in 13–14 year old adolescents with stage IV probably reflect a significant increase in body weight and a temporary decrease in the capacity of the oxygen transport system, which during this period can only be partially compensated for by improved functioning of the autonomic nervous system.

TABLE 2

PHYSICAL PERFORMANCE PARAMETERS (M ± M) RELATED TO DIFFERENT FACTORS IN ADOLESCENTS AGED 13–14 YEARS AT DIFFERENT STAGES OF PUBERTY

Indicators	Stages of puberty		
	II	III	IV
Factor 1 (aerobic capacity)			
Coefficient «b», r.u.	$12.5 \pm 0.3^{**}$	12.7 ± 0.3	$11,5 \pm 0,3^{x}$
$t_{3\ W/kg}$, s	$838.5 \pm 46.8^{*}$	$988.3 \pm 54.0^{+}$	$628,5 \pm 80,3^{x}$
Coefficient «a», r.u.	$5.5 \pm 0.2^{**}$	5.5 ± 0.2	4.8 ± 0.2^{x}
W_{900}, W/kg	2.8 ± 0.1	2.8 ± 0.1	2.6 ± 0.1^{x}
W_{240}, W/kg	3.6 ± 0.1	$3.8 \pm 0.1^{++}$	3.5 ± 0.1^{xxx}
$PDAI_{3\ W/kg}$, bpc	$0.4 \pm 0.1^{**}$	0.5 ± 0.1	0.7 ± 0.1
6-minute run, m	$1261.9 \pm 17.8^{*}$	1275.3 ± 18.7	1319.0 ± 13.9
Factor 2 (absolute aerobic power)			
VO_{2max}, l/min	$2055.6 \pm 38.1^{***}$	$2184.5 \pm 43.1^{+}$	2539.0 ± 48.7^{xxx}
WP, kgf-m/b	$7.8 \pm 0.2^{***}$	$9.8 \pm 0.2^{+++}$	10.7 ± 0.3^{x}
PWC_{170}, kgm/min	$542.8 \pm 18.5^{***}$	$591.4 \pm 15.8^{+}$	755.1 ± 29.2^{xxx}
Factor 3 (anaerobic alactic capacity)			
PDAI (sprint running), bpc	$14.5 \pm 0.6^{*}$	14.3 ± 0.6	16.7 ± 0.7^{x}
Shuttle running 4 × 9 m, s	$10.5 \pm 0.1^{*}$	10.4 ± 0.1	10.3 ± 0.1
MFA, score	$18.8 \pm 0.4^{***}$	$19.3 \pm 0.4^{+++}$	$21,7 \pm 0,4$
Long jump, cm	$173.0 \pm 1.9^{***}$	176.7 ± 2.8	190.9 ± 1.6^{xxx}
W_1, W/kg	10.3 ± 0.4	10.5 ± 0.3	11.5 ± 0.4^{x}
MS, kg/kg	$1.60 \pm 0.03^{**}$	1.61 ± 0.03	1.74 ± 0.04^{x}
20 m standing start, s	$3.78 \pm 0.03^{***}$	3.74 ± 0.04	3.47 ± 0.02^{xxx}
6-minute run, m	$1261.9 \pm 17.8^{*}$	1275.3 ± 18.7	1319.0 ± 13.9
Factor 4 (anaerobic glycolytic capacity)			
$t_{5W/kg}$, s	42.4 ± 2.3	46.7 ± 2.8	46.3 ± 3.6
W_{40}, W/kg	5.0 ± 0.1	5.1 ± 0.1	5.1 ± 0.1
Torso lifting from a supine position in 1 min, time	$45.4 \pm 1.0^{**}$	44.6 ± 1.2	40.2 ± 1.5^{x}
W_{240}, W/kg	3.6 ± 0.1	$3.8 \pm 0.1^{++}$	3.5 ± 0.1^{xxx}
$PDAI_{5W/kg}$, bpc	$3.72 \pm 0.27^{*}$	3.75 ± 0.26	4.59 ± 0.27^{x}
Factor 5 (relative aerobic power)			
VO_{2max}, l/min*kg	$51.2 \pm 1.0^{***}$	$47.1 \pm 0.9^{++}$	44.4 ± 0.9
WP, kgf-m/b*kg	0.195 ± 0.004	$0.212 \pm 0.004^{++}$	0.187 ± 0.005^{xxx}
PWC_{170}, kgm/min*kg	13.5 ± 0.5	12.8 ± 0.3	13.2 ± 0.5

Note. *, **, *** – statistical significance of differences between stages II and IV of puberty; +, ++, +++ – between stages II and III of puberty; x, xx, xxx – between stages III and IV of puberty at $p < 0.05$, 0.01 и 0.001, respectively.

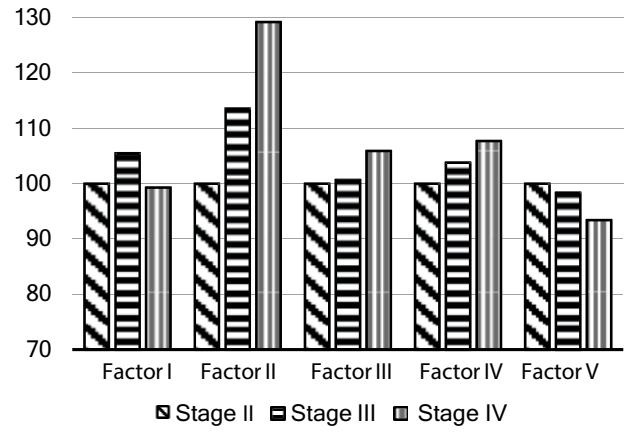

FIG. 1.
Integral physical performance parameters of adolescents with different stages of puberty: integral parameters for adolescents with II stages of puberty were taken as 100%»

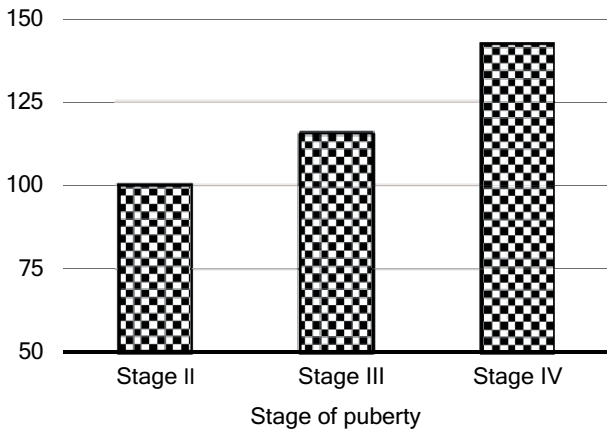

FIG. 2.
Body weight (%) in adolescents at different stages of puberty: the average values of body weight in adolescents with II stage of puberty were taken as 100%»

This conclusion is based in particular on the analysis of the data we obtained, showing that the average values of body weight in adolescents aged 13–14 years statistically significantly (p = 0.000) increase with the increase in the stage of puberty, with students with the stage II being characterized by below average and average, with stage III – above average, with stage IV – a high assessment of the indicator.

It is important to note that the average values in adolescents with stage II were close to the average values for 12 years of age, in adolescents with stage III – for 13 years of age, and in adolescents with stage IV – for 15 years of age [16]. The most pronounced differences were found when comparing stage III and stage IV (fig. 2).

Comparison of the indicators associated with the anaerobic alactic performance factor revealed a general trend of their improvement ($p < 0.05–0.001$) with an increase in the stage of puberty (table 2). Similar dynamics were also manifested in changes in the integral assessment of anaerobic alactic performance (fig. 1).

Analysis of the dynamics of the parameters included in the anaerobic glycolytic performance factor revealed a weak tendency for higher values of a number of physiological variables characterizing the capabilities of the lactic acid source in adolescents with stage IV compared to adolescents with stage II (table 2). However, statistically significant differences ($p < 0.05$) were found only in relation to the $PDAI_{5W/kg}$ anaerobic glycolytic performance factor. An exception is the oppositely directed dynamics of the results of the torso lifting test, which apparently reflects not the transformation of anaerobic energy during puberty, but the accelerated increase in torso mass and the increase in the muscle lever arm traction when performing this control exercise during the pubertal growth spurt.

In general, progressive changes in the anaerobic glycolytic capabilities of adolescents are well illustrated by the dynamics of integral indicators of physical performance during puberty (fig. 1). The obtained materials confirm the idea, established in age physiology, physiology of muscle activity and preventive medicine, that the development of anaerobic alactate and anaerobic glycolytic capabilities of the body is significantly accelerated in the final stages of puberty.

DISCUSSION

The study materials indicate that the greatest factor loads in the structure of physical performance of adolescents aged 13–14 years have the parameters of aerobic, anaerobic glycolytic, anaerobic alactate capabilities of the body and indicators of related motor abilities. The identified factors are associated with V.S. Farfel's relative power zones: the capacity and power of the oxidative system characterize the functional readiness to perform moderate and high-power muscle activity, anaerobic glycolytic capacity characterizes the effectiveness of implementing a submaximal power load, anaerobic alactate capacity characterizes the ability to perform work of maximum power. It is important to note that the factors considered are also interconnected with indicators of the development of students' conditioning motor abilities.

Three factors characterizing aerobic performance accounted for more than 60% of the total variance in the sample. These are aerobic capacity, absolute and relative aerobic power. Aerobic power, as is known, determines the intensity of work and reflects the highest rate of adenosine triphosphate (ATP) formation due to a given energy source, aerobic capacity limits the amount of work performed and characterizes the total amount of ATP that can be resynthesized due to available reserves of energy substrates [17]. The dynamics of the capacity indicators of aerobic and anaerobic processes, in contrast to the power indicators,

in the ontogenetic aspect has been practically not studied [2]. At the same time, the obtained results determine the need to assess the aerobic performance of adolescents not only on the basis of traditional power indicators, but also metabolic capacity. The coefficient "b" of the Muller equation and the time of maintaining a load of 3 W/kg can be used as informative indicators of aerobic capacity. These physiological variables, as our studies have shown, have high factorial validity. The coefficient "b" of the Muller equation and the time of maintaining a load of 3 W/kg can be used as informative indicators of aerobic capacity. These physiological variables, as our studies have shown, have high factorial validity.

The presence of two relatively independent factors associated with the power of the aerobic source of energy supply for muscular activity in adolescents is apparently due to the fact that absolute indicators of the body's aerobic performance increase significantly due to a sharp increase in total body weight during the pubertal growth spurt and poorly reflect real changes in the oxygen transport and utilization system (fig. 2). In contrast, relative indicators characterize the true change in aerobic capacity during puberty. Therefore, it is advisable to use VO_2max and WP values related to body mass as informative parameters of aerobic power. These results are in full agreement with the data of other studies, which have shown that during puberty there is an increase in the absolute values of VO_2max, associated mainly with an increase in skeletal muscle mass, while the relative values of this indicator change little [2, 6].

Anaerobic performance is represented by two factors. They accounted for more than 21% of the total sample variance. The anaerobic alactate performance factor included indicators reflecting the maximum power and efficiency of performing extremely intensive anaerobic loads, as well as the level of development of speed-strength, strength and speed motor abilities. The PDAI after sprint running and the general motor fitness assessment were highly informative in relation to this factor.

The anaerobic glycolytic performance factor included indicators related to the corresponding energy supply source and the level of strength endurance development. The most informative variables were the duration of maintaining a load of 5 W/kg and the power of work with the maximum implementation time of 40 s.

It was established that the values of physiological indicators associated with the identified factors of physical performance depended on the stage of puberty. It is known that changes in physical performance and muscle energy during puberty are controlled by sex hormones that affect the formation of energy supply mechanisms and the metabolic capabilities of skeletal muscles. During this period, testosterone supplements the anabolic effects of growth hormone in men [6]. It plays a key role in regulating physical performance and motor fitness at various stages of puberty: it affects body composition, bone tissue development, aerobic and anaerobic capacity, muscle strength, circulatory system function, muscle enzyme activity, use of energy substrates, and erythropoiesis [3, 6, 8, 18]. It is important to note that during puberty, circulating testosterone concentrations in men increase, with a dose-response relationship observed between circulating testosterone, muscle mass and strength, and circulating hemoglobin [8].

The results of the study, showing that with an increase in the stages of puberty, the indices of aerobic power and capacity change in different directions and are consistent with the data of other studies. It was established that in the prepubertal period and at the beginning of puberty, the physical performance of children increases mainly on the basis of the intensive development of the aerobic mechanism of energy supply. At the initial stages of puberty, a higher percentage of type I fibers is observed in skeletal muscles, which ensure the implementation of physical activity primarily through aerobic resynthesis of ATP [14, 19, 20]. The power of the aerobic system at this time increases significantly, in particular due to the enhanced development of the capillary network, an increase in the number of mitochondria in skeletal muscles in relation to the area of myofibrils, and the activity of oxidative enzymes [2, 21]. A further increase in performance during puberty occurs mainly due to the intensive development of anaerobic energy supply mechanisms, against the background of weakly expressed dynamics of relative aerobic power [2, 22, 23].

Our data on high aerobic capacity in male adolescents with stage III of puberty are confirmed in the scientific literature. It has been shown that at this stage, there is an increase in the functional capabilities of the oxygen transport and utilization system, associated with the processes of growth and development of the body. During this period, the heart and lungs grow intensively, the systolic volume, volumetric blood flow velocity, and vital capacity of the lungs increase. All this creates favorable conditions for improving the supply of oxygen to tissues and the development of energy supply mechanisms for muscle activity [24]. Stage III of puberty is characterized by the first phase of muscular pubertal differentiation, which contributes to the manifestation of obvious "traits of aerobic metabolism" in most muscle fibers: the size and number of mitochondria increase, and the activity of oxidative enzymes increases [2, 24]. The composition of skeletal muscles is transformed towards an increase in the proportion of type I fibers, a temporary increase in the power of the aerobic threshold and a corresponding expansion of the aerobic energy supply zone occur [2]. It is assumed that this is largely due to changes in endocrine functions, the capabilities of the oxygen transport system and the organization of tissue energy. For example, data on the relationship between the concentration of circulating testosterone and the timing and manifestations of puberty in male adolescents are well known. Characteristic clinical signs of masculinization, such

as muscle growth, increased body length, body hair, voice changes, and increased hemoglobin levels; appear only when the concentration of circulating testosterone in mid-puberty reaches the level of adult men [8]. As a result, there is a significant increase in the amount of hemoglobin, which provides the biological effect of increasing the blood oxygen capacity, enhancing oxygen transport to tissues and increasing aerobic energy expenditure [6, 8], while there is a linear relationship between changes in the hemoglobin level and aerobic performance. Perhaps, testosterone under these conditions promotes an increase in energy expenditure due to increased mitochondrial biogenesis in skeletal muscles [25], and also regulates the aerobic capacity of skeletal muscles by increasing the expression of myoglobin [26]. A significant increase in aerobic capacity at stage III of puberty may be due to changes in the level of cortisol [6] and thyroid activity, causing activation of oxidative metabolism of muscles [7, 14, 27].

The development of anaerobic alactic and anaerobic glycolytic mechanisms during the pubertal period occurs both synchronously and heterochronically, which determines the structure of energy processes and the specificity of the motor abilities formation associated with them. The most pronounced growth of anaerobic capabilities is noted in the final stages of puberty, when the definitive structure of energy supply for muscle activity is formed [11, 22, 23]. The development of anaerobic sources occurs to a large extent in conjunction with changes in basal concentrations of sex hormones and depends on the stage of puberty [6, 8, 11].

According to available data, during the transition from the initial to the final stage of puberty, under the influence of male sex hormones in skeletal muscles, an increase in the activity of key enzymes of anaerobic glycolysis and an increase in the thickness of IIB subtype (fast glycolytic) fibers is noted [2, 3, 8]. Based on the use of the Wingate test, a significant increase in anaerobic power in boys during puberty was revealed [18], while an average degree of correlation was found between peak and average anaerobic power on the one hand and testosterone levels on the other [28]. It has also been shown that the motor fitness of male adolescents improves during puberty [13]. Differences between the stages of puberty are manifested primarily in relation to strength and speed-strength abilities [4]. With an increase in the degree of sexual maturity, not only do motor abilities improve, but body length and weight also increase sharply [4]. Significant differences in anthropometric indicators make it possible to predict the stage of puberty with high accuracy based on changes in physical development [29]. In general, in adolescents with stage IV of puberty, the boundaries of the zones of maximum and submaximal relative power are noticeably expanded.

The differences we identified in the physical condition of adolescents of the same passport age largely reflect the classic variants of individual morphofunctional development – normal, slow (individual retardation) and accelerated (individual acceleration) [15, 30]. In the last two cases, development can be harmonious and inharmonious. With inharmonious individual acceleration, for example, a temporary decrease in the functional capabilities of the oxygen transport system and a decrease in the effectiveness of its reactions to standard and maximum physical loads can be observed [15].

As noted above, the rate of puberty has a noticeable effect on the structure of physical performance and muscle energy. There is evidence that individuals with a slow rate of puberty have a higher efficiency of the aerobic source of energy supply for muscle activity [6, 20, 30], while with an accelerated rate of maturation, there is an increased efficiency of the anaerobic glycolytic source of ATP resynthesis [6, 22]. In adolescents with a slow rate of puberty, a higher percentage of oxidative muscle fibers (type I) is observed in the skeletal muscles, characterized by the predominance of the aerobic source of energy supply for muscles [1, 19, 20], an increased density of mitochondria and a higher activity of oxidative enzymes [1, 11, 20]. A reflection of this can be seen in the trend described in our study of a temporary decrease in the relative value of VO_2max in 13–14-year-old adolescents with stage III and especially stage IV of puberty in relation to stage II. In general, it is assumed that in individuals with high rates of puberty, the activity of anaerobic glycolysis enzymes is higher than in individuals with low rates of maturation, while the activity of aerobic enzymes, on the contrary, is higher in adolescents with low rates of puberty compared to subjects with advanced maturation [11].

The obtained results show that students of the same calendar age with different levels of sexual maturity may have a pronounced specificity in the development of muscle energy and physical performance, which must be taken into account in the process of school physical education, health and sports training. Teenage boys aged 13-14 years, who are in the initial stages of puberty, are distinguished by high functional readiness to perform physical work of an aerobic nature and favorable conditions for the effective development of general endurance, while those in the final stages of puberty – to perform work of an anaerobic alactate and anaerobic glycolytic nature, as well as the development of strength, speed-strength and speed abilities. All this indicates that separate standards for assessing physical performance and motor fitness should be developed for adolescents of the same passport age with high and slow rates of biological development. Based on the data obtained, it is necessary to identify groups of adolescents with the initial and final stages of puberty in the process of physical training. This will allow for a differentiated "training" effect on the development of aerobic and anaerobic components of physical performance and related motor abilities, taking into account changes in the adaptive capabilities of the body during puberty. The results of the study should be taken

into account when selecting valid and reliable indicators of physical performance in different zones of relative power, creating systems for its comprehensive assessment within wide limits of available loads, standardizing training effects and developing effective programs for physical exercise during puberty.

CONCLUSION

The study identified five relatively independent factors characterizing the physical performance of 13-14 year old male adolescents within a wide range of available loads: aerobic capacity; absolute aerobic power; anaerobic alactic performance; anaerobic glycolytic performance; relative aerobic power. These factors are considered as key links in the dominant functional system that ensures adaptation to intense muscular activity and correlate well with V.S. Farfel's relative power zones.

Good indicators of diagnostics of factors of aerobic and anaerobic performance in adolescents at different stages of puberty were revealed. Analysis of physical performance in adolescents aged 13–14 years showed that during puberty, changes in indicators combined into different factors occur nonlinearly and non-simultaneously. The obtained results indicate that subjects of the age group studied, at stages II, III and IV of puberty, differ significantly in the level of key bioenergetic criteria for assessing physical performance. It has been established that with the increase in the stage of puberty, progressive changes occur in most indicators of anaerobic alactic and anaerobic glycolytic performance, while the indicators of aerobic power and capacity change in different directions, in some cases showing a tendency to a temporary decrease. The motor abilities associated with the factors studied change in a similar manner.

The obtained data on the factor structure and level of physical performance of 13-14 year old students should be taken into account when developing measures to standardize physical activity of aerobic, anaerobic glycolytic and anaerobic alactate nature in the process of systematic physical exercise classes, for operational, current and stage-by-stage monitoring of the functional state of adolescents at different stages of puberty. The results of the study can serve as a basis for the effective use of physical activity of various metabolic orientations in order to improve the functional capabilities of the students' body during the critical period of ontogenesis associated with the process of puberty.

In conclusion, it should be noted that in the pubertal period, the basis for complex control of load parameters in the process of physical improvement of adolescents with different stages of puberty should be based on taking into account the structure and level of physical performance, as well as the morphofunctional maturity of the leading physiological body systems that ensure the implementation of intense muscular activity.

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Conflicts of interest

No potential conflict of interest relevant to this article reported.

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OBSTETRICS AND GYNAECOLOGY

DIAGNOSTIC SIGNIFICANCE OF INTERLEUKIN LEVELS IN BLOOD SERUM IN PREMENOPAUSAL WOMEN WITH CHRONIC ENDOMETRITIS AND NORMAL WEIGHT OR OVERWEIGHT

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ABSTRACT

Background. *Chronic endometritis (CE) is an inflammatory hysteropathy causing miscarriage and infertility. High invasiveness of the main method of CE diagnosis and vague clinical picture necessitate the need for the development of less invasive approaches to establish the presence of this disease.*

The aim of the study. *To establish a significant association of the concentration of pro- and anti-inflammatory interleukins in the blood serum with the presence of chronic endometritis in premenopausal women without concomitant endocrine diseases.*

Materials and methods. *This re-analysis of the data is based on the results of a cross-sectional study conducted between May 2017 and December 2019 which included 198 premenopausal women. In all participants, body weight and height were measured with the calculation of the body mass index, the concentration of Creactive protein, pro- and anti-inflammatory cytokines in the blood serum was determined, and a pipelle biopsy was performed to determine the CD138 expression in the endometrial stroma. Non-parametric methods, as well as ROC analysis, were used for statistical analysis.*

Results. *Eighty six women were included in the re-analysis of the data, 37 of them had a confirmed diagnosis of chronic endometritis. Statistically significantly higher values of interleukin 1 (IL-1) concentration ($p = 0.0028$) and IL-1/tumor necrosis factor α ratio ($p < 0.001$) were determined in women with CE and normal body weight; threshold values of these parameters were ≥ 1.35 pg/ml (sensitivity 75 %, specificity 83 %; 95% confidence interval (95% CI): 0.88–2.15) and ≥ 1.03 (sensitivity 85 %, specificity 78 %; 95% CI: 0.81–1.27) respectively. Such a relationship was not revealed in women with overweight.*

Conclusions. *The obtained results can be the basis for conducting a larger-scale study with determining the concentration of cytokines not only in the blood serum, but also in the endometrium of women with CE, which will allow the development of a minimally invasive method for determining the risk of the presence of chronic endometritis in premenopausal women.*

Key words: *chronic endometritis, interleukin 1, tumor necrosis factor α, cytokines, chronic inflammation, overweight*

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ДИАГНОСТИЧЕСКАЯ ЗНАЧИМОСТЬ УРОВНЕЙ ИНТЕРЛЕЙКИНОВ В СЫВОРОТКЕ КРОВИ У ЖЕНЩИН РЕПРОДУКТИВНОГО ВОЗРАСТА С ХРОНИЧЕСКИМ ЭНДОМЕТРИТОМ И НОРМАЛЬНОЙ ИЛИ ИЗБЫТОЧНОЙ МАССОЙ ТЕЛА

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РЕЗЮМЕ

Обоснование. *Хронический эндометрит (ХЭ) является воспалительным заболеванием матки, приводящим к невынашиванию беременности и бесплодию. В связи с высокой инвазивностью основного метода диагностики ХЭ и смазанностью клинической картины является актуальной разработка менее инвазивных подходов установления наличия данного заболевания.*

Цель исследования. *Установление значимой ассоциации концентрации про- и противовоспалительных интерлейкинов в сыворотке крови с наличием хронического эндометрита у женщин репродуктивного возраста без сопутствующих эндокринных заболеваний.*

Материалы и методы. *Настоящий ре-анализ данных проведён на основе результатов кросс-секционного исследования, проведённого в период с мая 2017 по декабрь 2019 г. с участием 198 женщин репродуктивного возраста. У всех участниц измеряли массу тела и рост с расчётом индекса массы тела, определяли концентрацию С-реактивного белка, про- и противовоспалительных цитокинов в сыворотке крови и проводили пайпель-биопсию с определением экспрессии CD138 в строме эндометрия. Для статистического анализа использовали непараметрические методы анализа, а также ROC-анализ.*

Результаты. *В ре-анализ данных включили 86 женщин, из которых у 37 подтвердили диагноз ХЭ. У женщин с ХЭ на фоне нормальной массы тела установлены статистически значимо более высокие значения концентрации интерлейкина (ИЛ) 1 ($p = 0,0028$) и отношения ИЛ-1/фактор некроза опухоли α ($p < 0,001$) с пороговыми значениями данных параметров ≥ 1,35 пг/мл (чувствительность 75 %, специфичность 83 %; 95%-й доверительный интервал (95% ДИ): 0,88; 2,15) и ≥ 1,03 (чувствительность 85 %, специфичность 78 %; 95% ДИ: 0,81; 1,27) соответственно. У женщин с избыточной массой тела такой зависимости не выявлено.*

Выводы. *Полученные результаты могут быть основой для проведения более масштабного исследования с установлением концентрации цитокинов не только в сыворотке крови, но и в эндометрии женщин с ХЭ, что позволит разработать малоинвазивный метод определения риска наличия данного заболевания у женщин репродуктивного возраста.*

Ключевые слова: *хронический эндометрит, интерлейкин 1, фактор некроза опухоли α, цитокины, хроническое воспаление, избыточная масса тела*

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BACKGROUND

One of the main consequences of chronic endometritis (CE) is a decrease in endometrial receptivity against the background of inflammation, which can subsequently cause the development of miscarriage and infertility in women of reproductive age. This occurs as a result of increased expression of proinflammatory cytokines in the uterine cavity, which suppress endometrial growth and angiogenesis factors and lead to dysregulation of the decidualization processes of uterine mucosa cells. These processes cause thinning of the endometrium and reduce its ability to implant [1–3].

CE is a disease that causes difficulties for doctors, since along with decreased receptivity and an increased risk of infertility, this condition is very difficult to diagnose due to non-specific symptoms, and in some women it may not manifest itself clinically. Based on this, it is difficult to establish the exact prevalence of this disease in the general population. According to available data, CE occurs on average in 10-11% of women of reproductive age [4].

To date, the most effective and reproducible method for diagnosing CE is a pipelle biopsy to determine the content of plasma cells expressing CD138 in the endometrial stroma [5, 6]. Despite the high sensitivity and specificity of this research method, there are some difficulties in its use. In particular, the procedure for collecting endometrial samples for analysis is a minor gynecological operation and requires indications for appointment [6, 7]. In this regard, the risk of missing the presence of the disease in the patient is quite high.

For many years, researchers have been trying to develop less invasive and more accessible methods for diagnosing CE, including finding a relationship between some blood serum parameters and the presence of this disease [8, 9]. Of particular interest are serum levels of pro- and anti-inflammatory cytokines, since their imbalance is recorded in the uterine mucosa of women with CE [10]. Some researchers have found an increase in the concentration of proinflammatory cytokines in menstrual or venous blood in patients with CE [11]. However, these studies did not take into account additional parameters that may affect the concentration of cytokines in the blood, such as systemic inflammation due to the presence of concomitant diseases, including hormonal disorders and obesity [12, 13].

Thus, **the aim of this study** was to establish a significant association between the concentration of pro- and anti-inflammatory interleukins in the blood serum and the presence of chronic endometritis in women of reproductive age without concomitant endocrine diseases.

MATERIALS AND METHODS

Research design

A cross-sectional study, described in detail in our previously published works [8, 14], was conducted from May 2017 to December 2019. A total of 198 women of reproductive age (33.71 ± 5.93 years) were examined. All participants were recruited during annual preventive examinations at the Scientific Centre for Family Health and Human Reproduction Problems. All women who took part in the study signed informed consent for the examination. In working with patients, the ethical principles set out in the World Medical Association Declaration of Helsinki (1964, 2013 edition), the Federal Law of the Russian Federation of 11-21-2011 No. 323-FZ «On the Fundamentals of Health Protection of Citizens in the Russian Federation» and the «Rules of Clinical Practice in the Russian Federation», approved by Order of the Ministry of Health of Russia dated 06-19-2003 No. 266 were observed. This study was approved by the local Ethics Committee of the Scientific Centre for Family Health and Human Reproduction Problems (protocol No. 2.1 dated February 24, 2016).

The inclusion criteria for this reanalysis were: the presence or absence of CE; the presence of data on body mass index (BMI) and serum interleukin concentration. The exclusion criterion was the presence of a concomitant diagnosis of polycystic ovary syndrome in patients. At the time of the study, all patients had no signs of acute local or systemic inflammation.

Instrumental methods

Instrumental and clinical research methods have been described in detail previously [8, 14]. Based on the examinations conducted, BMI was calculated for all patients, and the diagnosis of CE was established based on the results of morphological and immunohistochemical studies of endometrial samples.

Laboratory methods

Blood serum for research was obtained by centrifuging tubes at 3000 rpm for 10 min. The serum was stored in disposable Eppendorf tubes at a temperature of –80°C. Quantitative determination of the concentration of interleukin (IL) 1, IL-6, IL-8, tumor necrosis factor (TNF) α, interferon (INF), and C-reactive protein (CRP) was performed using test systems of Vector-Best LLC (Russia) on an ELx808 enzyme immunoassay analyzer (BioTek, USA) according to the manufacturer's instructions.

Statistical methods

The statistical analysis included descriptive statistics, testing of statistical hypotheses, ROC analysis and determination of the odds ratio (OR). The Kolmogorov – Smirnov test was used to determine the proximity to the normal distribution law of continuous variables. The description of continuous variables is presented as the median and the lower and upper quartiles. To test the statistical hypothesis of equivalence in the location of two general populations for independent random samples, the Mann – Whitney U-test was used, and for several general populations, the Kruskal – Wallis test was used. To establish a correlation between the values of the BMI and CRP parameters,

the Spearman's rank correlation test was used. To establish the threshold values of the parameters and their 95% confidence intervals (95% CI), the characteristic curve analysis (ROC analysis) was performed. Sensitivity and specificity were calculated; the area under the ROC curve (AUC) was estimated. The OR was also calculated for each threshold value.

RESULTS

Of the 198 participants, data from 86 women were used for the present reanalysis according to the inclusion and exclusion criteria (fig. 1).

The women were then divided into two groups depending on the presence or absence of CE, and the values of serum pro- and anti-inflammatory cytokine concentrations, as well as their relationships between the groups, were compared (table 1).

Based on the comparison results, we found statistically significantly higher values of IL-1 concentration ($p = 0.0027$) and IL-1/TNF-α ratio ($p < 0.0001$) in women with CE compared to the values of these parameters in women in the control group.

In order to assess at what value of IL-1 concentration or IL-1/TNF-α ratio the presence of CE is observed in women, a ROC analysis was conducted, and based on the results we determined the threshold values for the analyzed parameters (fig. 2).

Thus, in women with CE, the IL-1 concentration values were ≥ 1.3 pg/ml (sensitivity 78 %, specificity 59 %; 95% CI: 0.76; 2.15 pg/ml), and the IL-1/TNF-α ratio was ≥ 0.89 (sensitivity 81 %, specificity 63 %; 95% CI: 0.69; 1.37). We also calculated the OR for the established threshold values, which for IL-1 was 3.732 (95% CI: 1.503; 9.267), for the IL-1/TNF-α ratio – 6.243 (95% CI: 2.356; 16.546).

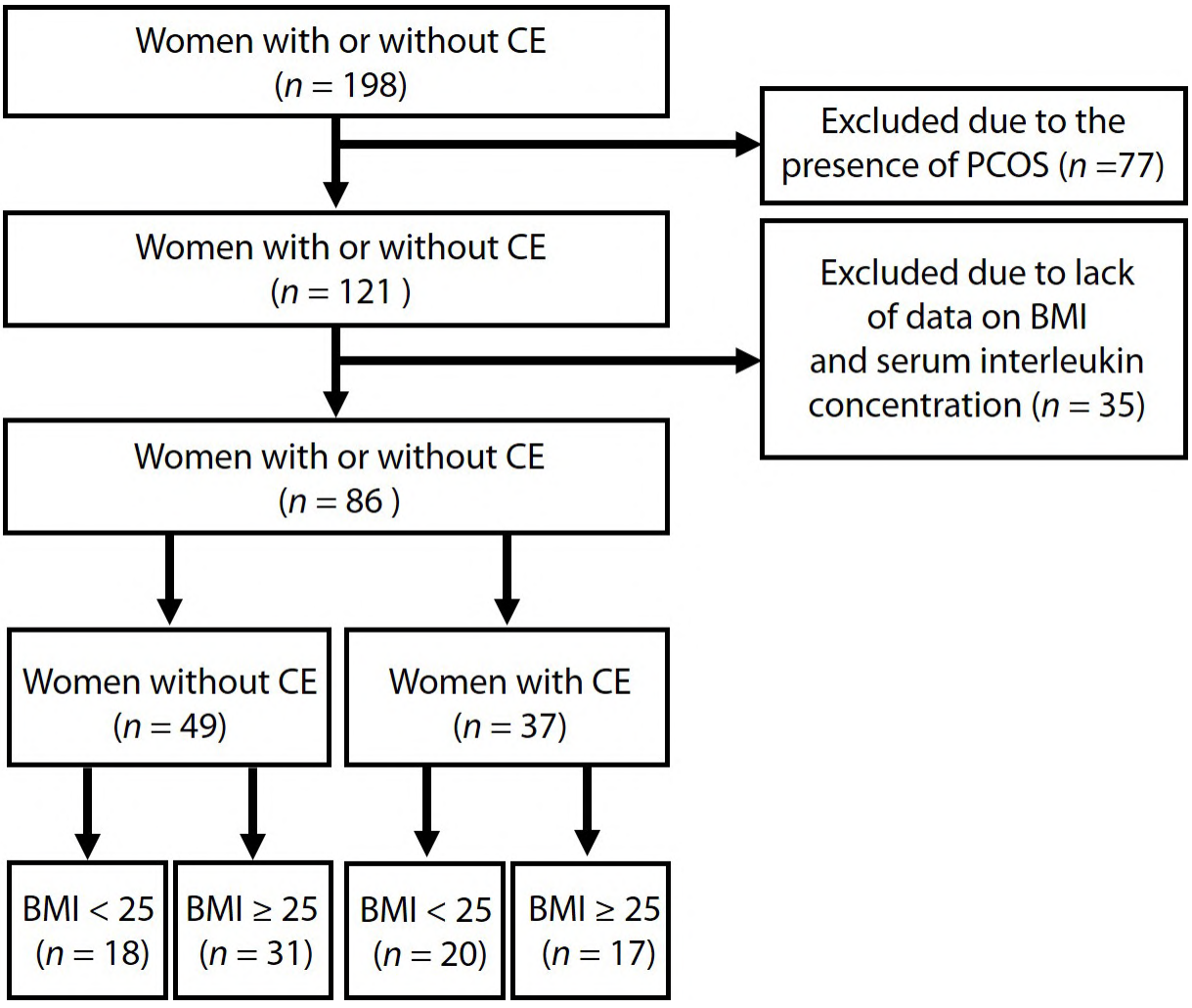

FIG. 1.
Study design: PCOS – polycystic ovarian syndrome

TABLE 1

MAIN CHARACTERISTICS AND CONCENTRATION OF INTERLEUKINS IN PREMENOPAUSAL WOMEN WITH OR WITHOUT CHRONIC ENDOMETRITIS

Parameters	Control (*n* = 49)	CE (*n* = 37)	*p*
Age, years	38 (34; 41)	39 (36; 43)	0.330
BMI, kg/m^2	25.92 (22.51; 30.14)	23.84 (21.05; 28.38)	0.105
	Proinflammatory cytokines		
IL-1, pg/ml	**0.95 (0,70; 1,85)**	**1.7 (1.10; 2.25)**	**0.0027**
IL-6, pg/ml	1.4 (0.75; 2.25)	0.9 (0.50; 2.10)	0.1640
IL-8, pg/ml	14 (6.75; 33.00)	13 (7.30; 28.30)	0.7762
TNF-α, pg/ml	1.7 (1.30; 2.20)	1.3 (0.85; 1.85)	0.0976
INF, pg/ml	0.6 (0.30; 1.05)	0.9 (0.35; 1.40)	0.1271
	Anti-inflammatory cytokines		
IL-10, pg/ml	1.5 (0.27; 2.25)	1.6 (0.70; 3.40)	0.3135
	Relationships between pro- and anti-inflammatory cytokines		
INF/IL-10	0.44 (0.145; 0.790)	0.43 (0.205; 0.89)	0.6712
IL-6/IL-10	1 (0.52; 1.55)	0.46 (0.255; 1.435)	0.0862
IL-1/IL-10	0.68 (0.275; 1.465)	0.88 (0.54; 1.89)	0.1496
IL-8/IL-10	9.6 (5.75; 35.31)	7.5 (2.58; 22.00)	0.1608
IL-6/TNF-α	0.73 (0.510; 1.820)	0.64 (0.395; 1.450)	0.5163
IL-10/TNF-α	0.94 (0.310; 1,680)	1 (0.470; 2.630)	0.1788
IL-1/TNF-α	**0.77 (0.43; 1.1)**	**1.19 (0.890; 2.295)**	**< 0.0001**

Note. Data are presented as medians and lower and upper quartiles; p is the level of statistical significance when comparing parameter values between groups using the Mann – Whitney test.

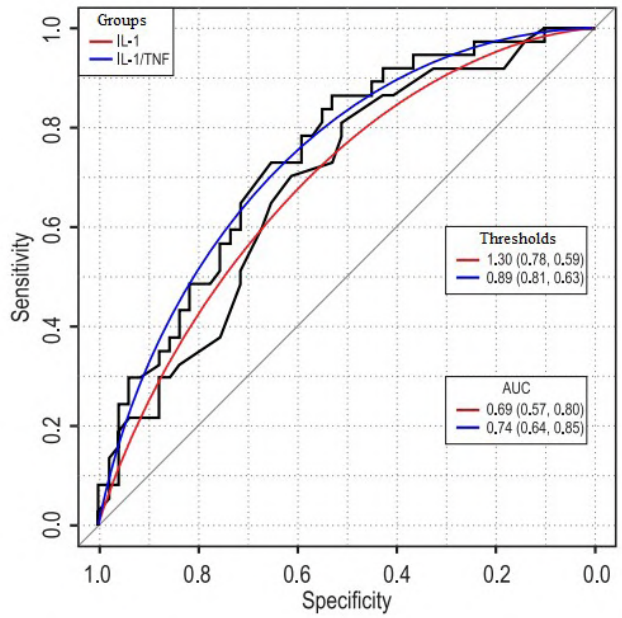

FIG. 2.
ROC curve of IL-1 concentration or IL-1/TNF-α ratio in premenopausal women with or without chronic endometritis against the background of normal weight or overweight: IL1 – ROC curve of IL-1 concentration in patients with or without chronic endometritis; IL1/TNF – ROC curve of IL-1/TNF-α ratio in patients with or without chronic endometritis; the cut-off point for IL-1 concentration is 1.3 (95% CI: 0.76; 2.15); the cut-off point for IL-1/TNF-α ratio is 0.89 (95% CI: 0.69; 1.37)

The low specificity of the obtained cut-off points could be due to the fact that the groups included both women with normal body weight and overweight and obese women, who could have chronic inflammation against the background of elevated BMI values. It should be noted that we did not find statistically significant differences in BMI values in the studied groups: 25.92 (22.51; 30.14) kg/m^2 in healthy women versus 23.84 (21.05; 28.38) kg/m^2 in patients with CE (p = 0.105). Therefore, we further compared the values of interleukin concentrations and their ratios between women with or without CE depending on BMI. We also assessed the concentration of CRP in the blood as a marker of chronic systemic inflammation in overweight and obesity in women of the studied groups (table 2) [15].

TABLE 2

MAIN CHARACTERISTICS AND CONCENTRATION OF INTERLEUKINS IN PREMENOPAUSAL WOMEN WITH OR WITHOUT CHRONIC ENDOMETRITIS DEPENDING ON BODY MASS INDEX

Parameters	Control (n = 49)		CE (n = 37)		
	BMI < 25 kg/m^2 (n = 18) 1	BMI ≥ 25 kg/m^2 (n = 31) 2	BMI < 25 kg/m^2 (n = 20) 3	BMI ≥ 25 kg/m^2 (n = 17) 4	p^a
Age	38 (33.5; 41)	38 (34; 41)	39 (36; 43)	39 (36; 42.75)	p_{all} = 0.4031
BMI, kg/m^2	**21.48 (20.13; 23.29)**	**28.42 (26.27; 31.78)**	**21.18 (20.06; 23.28)**	**28.79 (27.12; 29.77)**	**p_{all} < 0.001**
					p_{1-3} = 0.704^b
p^b	**< 0.001**		**< 0.001**		**p_{1-4} < 0.001^b**
					p_{2-4} = 0.1069^b
	Proinflammatory cytokines				
IL-1, pg/ml	**0.8 (0.5; 1.25)**	**1.2 (0.72; 1.9)**	**1.65 (1.013; 2.975)**	**1.8 (1.1; 2.15)**	**p_{all} = 0.0103**
					p_{1-3} = 0.0059^b
p^b	0.1187		0.982		**p_{1-4} = 0.0024^b**
					p_{2-4} = 0.1069^b
IL-6, pg/ml	0.955 (0.575; 1.825)	1.5 (1.04; 2.4)	0.9 (0.425; 2.5)	0.9 (0.55; 2.1)	0.2159
IL-8, pg/ml	18.5 (6.775; 45.4)	12 (5.4; 33)	12 (7.05; 30)	14 (7.65; 28.3)	0.7762
TNF-α, pg/ml	1.8 (1.325; 2.4)	1.7 (1.3; 2)	1.3 (0.8; 1.775)	1.4 (1; 2)	0.342
INF, pg/ml	0.75 (0.25; 1.35)	0.6 (0.3; 0.9)	0.85 (0.325; 1.575)	0.9 (0.4; 1.3)	0.4036
CRP, IU/l	**1.3 (0.55; 2.20)**	**2.3 (1.15; 6.35)**	**0.7 (0.40; 1.90)**	**2.8 (1.00; 3.05)**	**p_{all} = 0.0018**
					p_{1-3} = 0.2803^b
p^b	**0.0130**		**0.0142**		**p_{1-4} = 0.1036^b**
					p_{2-4} = 0.5538^b
	Anti-inflammatory cytokines				
IL-10, pg/ml	1.55 (0.55; 2.3)	1.5 (0.09; 2.3)	2 (0.75; 3.4)	1.3 (0.6; 3.1)	0.6008
	Relationships between pro- and anti-inflammatory cytokines				
INF/IL-10	0.465 (0.215; 0.805)	0.375 (0.095; 0.553)	0.385 (0.178; 0.618)	0.430 (0.270; 1.620)	0.3679
IL-6/IL-10	0.870 (0.658; 1.485)	0.895 (0.588; 1.615)	0.595 (0.248; 1.160)	0.440 (0.260; 2.000)	0.2020
IL-1/IL-10	0.705 (0.338; 1.833)	0.655 (0.230; 1.225)	0.610 (0.420; 2.833)	1.130 (0.690; 1.600)	0.4176
IL-8/IL-10	18.66 (7.95; 36.82)	8.03 (5.28; 28.06)	7.37 (2.64; 24.84)	7.50 (2.39; 20.00)	0.2687
IL-6/TNF-α	0.725 (0.545; 1.678)	0.880 (0.580; 2.190)	0.655 (0.425; 1.495)	0.640 (0.385; 1.305)	0.6363
IL-10/TNF-α	1.000 (0.410; 1.343)	0.940 (0.050; 1.880)	1.775 (0.405; 3,093)	0.890 (0.540; 2.425)	0.4826
IL-1/TNF-α	**0.675 (0.315; 0.9475)**	**0.79 (0.44; 1.19)**	**1.260 (1.015; 2.683)**	**1.140 (0.815; 2.015)**	**p_{all} = 0.0022**
					p_{1-3} = 0.0011^b
p	**0.7619**		**0.2661**		**p_{1-4} = 0.0157^b**
					p_{2-4} = 0.2661^b

Note. Data are presented as medians and lower and upper quartiles; p is the level of statistical significance when comparing parameter values between groups using the Mann – Whitney test.

We found statistically significantly higher IL-1 concentration and IL-1/TNF-α ratio in women with CE regardless of BMI compared to the results in women without CE with normal body weight. However, we did not find any significant differences in the values of these parameters in women without CE and with CE against the background of overweight and obesity. This can be explained by the presumable presence of chronic systemic inflammation in women with overweight and obesity [15], which is confirmed by a statistically significantly elevated CRP level in such women regardless of the presence of CE. In addition, in women with overweight and obesity, we found a statistically significant positive correlation of CRP with BMI ($r = 0.495$; $p < 0.001$), which was absent in women with normal body weight ($r = 0.050$; $p = 0.763$).

We next performed ROC analysis to establish risk thresholds for the presence of CE for IL-1 or IL-1/TNF-α ratio in women with normal body weight (fig. 3).

We found that in women with CE and normal body weight, the IL-1 concentration was ≥ 1.35 pg/ml (sensitivity 75 %, specificity 83 %; 95% CI: 0.88; 2.15), and the IL-1/TNF-α ratio was ≥ 1.03 (sensitivity 85 %, specificity 78 %; 95% CI: 0.81; 1.27). The OR for the established threshold values was: for IL-1 – 8.167 (95% CI: 1.885; 35.381), for the IL-1/TNF-α ratio – 15 (95% CI: 3.027; 74.32).

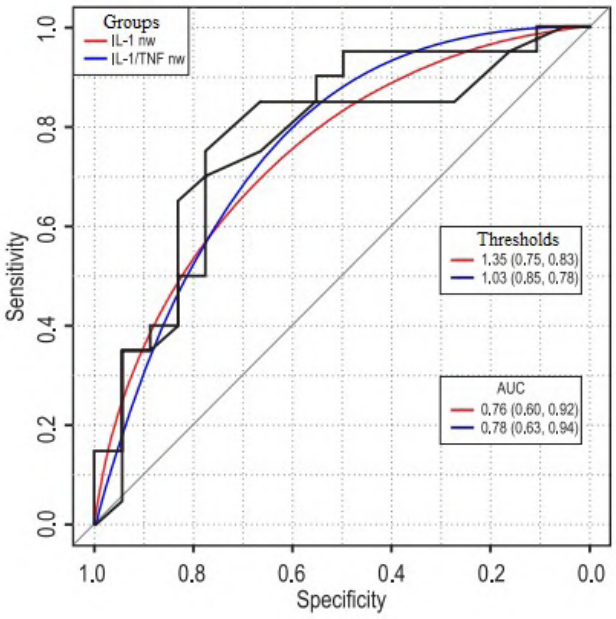

FIG. 3.
ROC curve of IL-1 concentration or IL-1/TNF-α ratio in premenopausal women with or without chronic endometritis against the background of normal weight: IL1 nw – ROC curve of IL-1 concentration in patients with normal weight; IL1/TNF nw – ROC curve of IL-1/TNF-α ratio in patients with normal weight; the cut-off point for IL-1 concentration is 1.35 (95% CI: 0.88; 2.15); the cut-off point for IL-1/TNF-α ratio is 1.03 (95% CI: 0.81; 1.27))

DISCUSSION

In this study, we examined the levels of pro- and anti-inflammatory cytokines in the blood serum of women with and without CE, including their dependence on BMI. Based on the results of the study, we found that women with CE, regardless of BMI, have statistically significantly higher values of serum IL-1 concentrations and IL-1/TNF-α ratios. We also determined the threshold values for these parameters in relation to the presence of CE using ROC analysis. However, the sensitivity of certain threshold values of IL-1 and IL-1/TNF-α concentrations turned out to be relatively low, and therefore we decided to analyze the levels of pro- and anti-inflammatory cytokines in women with and without CE, depending on BMI, since the presence of excess body weight and obesity can provoke chronic systemic inflammation and affect the level of serum cytokines [12], which can clinically manifest itself as an increase in the level of CRP [15]. According to the analysis, we also found a statistically significant association of higher IL-1 and IL-1/TNF-α values with the presence of CE only in women with normal body weight, but not in overweight and obese women. At the same time, in overweight women, regardless of the presence or absence of CE, we found a significantly higher level of CRP than in women with normal body weight, which indirectly confirms the contribution of increased BMI to the development of systemic inflammation. Subsequent ROC analysis with the establishment of threshold values showed more pronounced sensitivity and specificity for certain points compared to the values established for women without taking into account BMI. Thus, we found that for women of reproductive age with normal body weight, CE is accompanied by an increase in the concentration of IL-1 ≥ 1.35 pg/ml and the value of the IL-1/TNF-α ratio ≥ 1.03.

Proinflammatory cytokines in the uterine mucosa are produced by neutrophils, macrophages, and epithelial cells in response to regulatory factors under physiological conditions and bacterial and viral antigens in case of a pathological process. Thus, neutrophils in the uterine mucosa, under the influence of bacterial lipopolysaccharides (LPS), can produce IFN-γ, as well as IL-12 and TNF-α. IFN-γ, in turn, activates macrophages [16], which are located in the subepithelial stroma of the endometrium and are the first to recognize foreign antigens [17]. LPS can also directly stimulate macrophages to produce proinflammatory IL-1β, which causes the secretion of human β-defensin-2 by endometrial epithelial cells to resist bacterial invasion [18]. The development of inflammation in the endometrium can also be promoted by T-helpers 1 (Th1), which produce TNF-α [19]. In addition, proteins that provide direct stimulation of the production of proinflammatory cytokines (TNF-α and IL-1β) are expressed on epithelial cells, monocytes and dendritic cells of the endometrium [20]. Thus, with the development of CE under the influence of bacterial LPS and other proinflammatory factors

in the uterine cavity, the expression of cytokines responsible for the inflammatory response significantly increases.

Proinflammatory cytokines (IL-1, IL-6, IL-8, IL15, TNF-α) along with many hormones (estrogen, progesterone) and factors of decidualization and degradation of the endometrium extracellular matrix (integrin β3, IGFBP1 and metalloproteinases) are factors regulating endometrial receptivity, which determines the success of embryo implantation. In patients with CE, changes in the expression and functioning of these factors are noted, which can cause the development of infertility and habitual miscarriage against the background of this disease [3].

Thus, W.J. Wang et al. (2019) showed that the endometrium of women of reproductive age with CE has increased expression of proinflammatory IL-17 and decreased expression of anti-inflammatory IL-10 [2]. In our previous studies, we found increased secretion of IL-1β, IL-4, IL-6, IL-10, as well as IFN-γ and TNF-α in the endometrium of women with CE compared to the results in healthy women [21, 22]. C. Tortorella et al. (2014) also found an increased concentration of proinflammatory IL-1β, IL-6, and TNF-α in the menstrual blood of women with chronic endometritis. At the same time, with regard to CE screening, they revealed higher sensitivity for such indicators as the IL-6/TNF-α and IL-6/IL-1β ratios [23].

Currently, the most effective and reproducible method for diagnosing CE is the detection of CD138+ cells in the endometrial stroma during a Pipelle biopsy [5]. Despite the high sensitivity and specificity of this diagnostic method, it has a number of limitations. In particular, a Pipelle biopsy is a minor gynecological operation, and its use requires indications. At the same time, it is known that CE can often be asymptomatic. Such difficulties necessitate the search for new, less invasive methods for diagnosing CE [4, 6, 7].

Thus, some researchers have attempted to identify an association between the level of cytokines in the blood serum and the presence of CE. According to the results of the study by L.V. Tkachenko et al. (2020), an increase in the concentration of proinflammatory cytokines was found in uterine cavity aspirates, as well as in the blood serum of women with CE: IL-1β, IL-2, IL-6, TNF-α and IFN-γ [10]. I.P. Koltsov et al. (2011) recorded increased secretion of IL-8 by blood monocytes in women with chronic endometritis [24]. Yu.A. Sorokin et al. (2022) in a study of the effectiveness of CE treatment by introducing cavitated 0.9% sodium chloride solution into the uterine cavity revealed statistically significantly higher serum values of IL-1β (7.65 [6.3–8.98] vs. 1.22 [0.99–1.45]; $p < 0.05$) and TNF-α (2.75 [1.42–4.08] vs. 1.48 [1.29–1.67]; $p < 0.05$) in patients of reproductive age with CE compared to the values of similar parameters in women without CE [25]. These results are partially consistent with the data of our study that in women with CE, the concentration of IL-1 in the blood serum is ≥ 1.3–1.35 mg/ml.

Serum cytokine levels can change under the influence of not only local but also systemic inflammation. One of the common causes of chronic systemic inflammation is obesity. Thus, obese people may have elevated levels of proinflammatory cytokines in the blood serum [12], as well as CRP [15]. Based on this fact, we analyzed the levels of proinflammatory cytokines in women of reproductive age with or without CE depending on the body mass index. As a result, we found that an increase in the serum level of the proinflammatory cytokine IL-1 and the IL-1/TNF-α ratio is statistically significant only in the group of women with CE against the background of normal body weight. At the same time, a significant increase in the CRP level was observed only in overweight and obese women, regardless of the presence of CE. This may indirectly confirm the contribution of excess body weight to the development of chronic systemic inflammation, expressed in a relative increase in the CRP level.

The advantage of this study is that the level of interleukins in women with CE was assessed, among other things, taking into account BMI and CRP levels, since overweight and obesity can provoke chronic systemic inflammation [12, 15]. Also, one of the exclusion criteria was the presence of polycystic ovary syndrome in patients, since this disease is also associated with systemic inflammation and can affect serum cytokine levels [13]. The disadvantages of this study include the small patient sample size, which does not allow extrapolating the research results to the general population. Also, the lack of data on the levels of proinflammatory cytokines directly in the endometrial tissues with the determination of their correlation with the concentration of cytokines in the blood serum does not allow us to fully assert that the established patterns in the form of increased levels of IL-1 and the IL-1/TNF-α ratio are directly related to the presence of CE in patients. In addition, we did not assess the presence of other chronic diseases in patients that may be accompanied by the development of chronic systemic or local inflammation, which could have influenced the analysis results of the level of interleukins and CRP in the examined patients.

CONCLUSION

The conducted reanalysis of the cross-sectional study data of women of reproductive age with or without CE revealed a significantly higher serum concentration of IL-1 and higher values of the IL-1/TNF-α ratio in patients with CE against the background of normal body weight compared to the results in healthy patients with normal body weight with the establishment of threshold values of these parameters. At the same time, we did not find significant changes in the level of serum cytokines in patients with or without CE against the background of overweight and obesity, which can

be explained by the presence of systemic inflammation in them, which also affects the level of pro- and anti-inflammatory cytokines. The absence of a significant change in IL concentrations in patients of this group may be associated with dysregulation of the immune response against the background of overweight and obesity associated with an increased level of CRP.

Thus, it is necessary to conduct additional studies with a larger sample size, as well as assessing the level of cytokines not only in the blood serum, but also in the endometrium of women with or without CE to confirm the obtained results and identify a direct relationship between the studied parameters in the blood serum and endometrium. This will allow developing new a new method of minimally invasive diagnostics or determining the risk of CE in women of reproductive age.

Conflicts of interest

No potential conflict of interest relevant to this article reported.

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ULTRASONOGRAPHIC THRESHOLD OF OVARIAN STRUCTURE IN PREMENOPAUSAL WOMEN OF DIFFERENT ETHNICITY

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ABSTRACT

The polycystic ovarian morphology (PCOM) is a generally accepted ultrasound marker for ovulatory dysfunction, is one of the criteria for polycystic ovary syndrome (PCOS) and is established based on the assessment of ovarian volume (OV) and the follicle number per ovary (FNPO), taking into account the upper normal values determined in healthy premenopausal women. However, there is a necessity for regular revision of the PCOM characteristics depending on ethnic and age characteristics.

The aim. *To develop differentiated standards for assessing the ultrasonographic ovary structure in premenopausal women of various ethnicity.*

Materials and methods. *From March 2016 to December 2019, a multicenter cross-sectional prospective study was conducted in Eastern Siberia (Irkutsk region) and in the neighboring Republic of Buryatia. The study included 1134 participants: 715 women of Caucasian origin, 312 Asian women, 107 women of mixed ethnic subpopulation.*

Results. *It has been established that for Caucasians, it is advisable to diagnose PCOM when the ovarian volume is 9 cm^3 and/or FNPO ≥ 12; for women of the Asian population – when the ovarian volume is 7 cm^3 and/or FNPO ≥ 11; for women of mixed ethnicity – when the ovarian volume is 8 cm^3 and/or FNPO ≥ 9. An important advantage of our study is that all participants were recruited from a non-selective multi-ethnic population of women with comparable socio-demographic characteristics living in the same geographical conditions.*

Conclusion. *Differentiated approach for identifying the polycystic ovarian morphology in premenopausal women of different ethnic groups requires using ethnically differentiated normative readings.*

Key words: *PCOS, polycystic morphology, follicle number per ovary, ovarian volume, pelvic ultrasound, premenopausal women, ethnicity*

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УЛЬТРАСОНОГРАФИЧЕСКИЕ НОРМАТИВЫ СТРУКТУРЫ ЯИЧНИКОВ У ЖЕНЩИН РЕПРОДУКТИВНОГО ВОЗРАСТА РАЗЛИЧНОЙ ЭТНИЧЕСКОЙ ПРИНАДЛЕЖНОСТИ

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РЕЗЮМЕ

Поликистозная структура яичников (ПКЯ) является общепризнанным ультразвуковым маркером овуляторной дисфункции, служит одним из критериев синдрома поликистозных яичников (СПКЯ) и устанавливается на основании оценки объёма яичников (ОЯ) и количества фолликулов на яичник (КФЯ) с учётом верхних нормальных значений, определяемых в здоровых популяциях женщин репродуктивного возраста. Однако отмечается необходимость регулярного пересмотра характеристик ПКЯ в зависимости от этнических и возрастных особенностей.

Цель. *Разработать дифференцированные нормативы для оценки ультрасонографической структуры яичников у женщин репродуктивного возраста различной этнической принадлежности.*

Материалы и методы. *В период с марта 2016 по декабрь 2019 г. проведено многоцентровое поперечное (кросс-секционное) проспективное исследование на территории Восточной Сибири (Иркутская область) и в сопредельной Республике Бурятия. В исследование вошли 1134 участницы: 715 женщин европеоидной принадлежности, 312 – азиатской, 107 – смешанной этнической субпопуляции.*

Результаты. *Установлено, что для европеоидов ПКЯ целесообразно диагностировать при объёме яичников 9 см^3 и/или КФЯ ≥ 12; для женщин азиатской популяции – при объёме яичников 7 см^3 и/или КФЯ ≥ 11; для женщин смешанной этнической принадлежности – при объёме 8 см^3 и/или КФЯ ≥ 9. Важным преимуществом нашего исследования является то, что все участницы были рекрутированы в неселективной мультиэтнической популяции женщин с сопоставимыми социально-демографическими характеристиками, проживающих в одинаковых географических условиях.*

Выводы. *Для дифференцированного подхода к выявлению поликистозной структуры яичников у женщин репродуктивного возраста различных этнических групп необходимо принимать этнически дифференцированные нормативные значения.*

Ключевые слова: *СПКЯ, поликистозная структура, количество фолликулов на яичник, объём яичника, УЗИ органов малого таза, женщины репродуктивного возраста, этника*

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INTRODUCTION

Polycystic ovary structure (PCOM) is a generally accepted ultrasound marker of ovulatory dysfunction and, since 2003, following the adoption of the Rotterdam Consensus [1], has served as one of the criteria for polycystic ovary syndrome (PCOS) [2–4]. Currently, PCOM is defined based on the assessment of ovarian volume (OV) and follicle number per ovary (FNPO) taking into account the upper normal values determined in healthy populations of women of reproductive age [2, 4]. The latest guidelines on the diagnosis and management of PCOS, published in 2023 [4], propose to consider the provisions adopted in Rotterdam as basic for the diagnosis of PCOS [4, 5]. However, it is noted that there is a need for regular revision of the characteristics of PCOM, taking into account ethnic and age characteristics. The following updated criteria for defining PCOM in women of reproductive age are relevant: the most effective ultrasound marker for identifying PCOM in adults with transvaginal access should be considered FNPO, while FNPO ≥ 20 in at least one ovary should be considered the threshold value for polycystic ovary structure. As an equally effective marker of PCOM, along with OV ≥ 10 ml, it is again proposed to consider follicle number per cross-section (FNPS) ≥ 10 in at least one ovary. When using old ultrasound technologies and/or transabdominal ultrasound (US) and/or when the image quality is insufficient for an accurate assessment of the follicle number in the entire ovary, the following criteria are necessary for establishing polycystic ovary structure: OV ≥ 10 ml or FNPS ≥ 10 in both ovaries. When describing the ultrasound, it is proposed to use clear standardized protocols for assessing polycystic ovary structure, including at least the following: date of the last menstruation (or phase of the cycle); characteristics of the sensor used; high-quality count of the total follicle number measuring 2–9 mm per ovary. It is mandatory to measure the ovary in three dimensions and calculate the volume of each ovary; description of other ovarian features and/or pathologies, including ovarian cysts, corpora lutea, dominant follicles (≥ 10 mm) (which should not be taken into account when calculating ovarian volume). It is recommended to rely on the contralateral ovary FNPO for the diagnosis of PCOM in the presence of a dominant follicle in the ovary being evaluated. Uterine features and/or pathologies, including endometrial thickness and structure, should not be ignored.

Ethnic differences in the number of follicles and/or ovarian volume are actively studied. Thus, in Chinese women, the sufficient criteria for defining PCOM [6] are smaller ovarian volumes and the follicle number compared to women of the European population: ≥ 6.3 cm^3 and ≥ 10 follicles, respectively. Lower values compared to the Western population were also demonstrated by Turkish women. The threshold criteria for PCOM for them are OV = 6.43 cm^3 and FNPO ≥ 8 [7]. In the population of Korean patients, the follicle number is considered a more significant criterion for polycystic disease than ovarian volume due to the smaller ovarian volume characteristic of Asian women [8].

The volume of the ovaries and the number of follicles change during a woman's reproductive period, reaching a maximum value in adolescence, with a gradual decrease in adulthood and a rapid decrease at menopause. For example, in women over 35 years of age, the prevalence of PCOM is 7.8 %, compared to 21 % in younger women [9]. Moreover, the decrease in the number of follicles occurs faster than the decrease in ovarian volume [10]. Age-related processes in women involve a decrease in the number of growing antral follicles [10].

The relevance of detecting PCOM is determined by the fact that even in women with normal menstrual function and without clinical signs of hyperandrogenism (HA), PCOM is associated with higher levels of androgens and insulin and a lower pregnancy rate [11]. However, hirsutism, ovulatory dysfunction and menstrual cycle disorders were equally present in patients with normal ovarian volume and enlarged ovaries.

The diagnostic significance of PCOM depends on age and ethnic characteristics, which requires large-scale epidemiological studies to determine the population characteristics of PCOM. Standardization of diagnostic criteria for PCOM is the key to effective diagnostics of PCOS and, accordingly, prevention of complications and concomitant diseases associated with PCOS.

THE AIM OF THE STUDY

To develop differentiated standards for assessing the ultrasonographic ovary structure in premenopausal women of various ethnicity.

HYPOTHESIS

Ultrasonographic characteristics of the ovaries vary among women depending on ethnicity and age.

MATERIALS AND METHODS

Study design and population. Subjects were recruited during a cross-sectional prospective study (ClinicalTrials.gov: NCT05194384) conducted in two large regions of Eastern Siberia (Irkutsk Region and the Republic of Buryatia) from March 2016 to December 2019 [12, 13]. The study included women of reproductive age subject to annual medical examination at their place of work. The study was conducted in accordance with the World Medical Association Declaration of Helsinki (1964) and approved by the local Ethics Committee of the Scientific Centre for Family Health and Human Reproduction Problems (Irkutsk) (protocol No. 2.1 dated February

24, 2016). Informed consent was obtained from all subjects. The study was conducted as part of the research work "Prediction of metabolic and psychoemotional disorders in women of different age groups with hyperandrogen disorders for the development of personalized approaches to prevention and treatment" (registration number 123051600030-1). The study was carried out using the equipment of the Centre for the Development of Progressive Personalized Medical Technologies of the Scientific Centre for Family Health and Human Reproduction Problems.

Inclusion criteria for the sample: age from 18 to 44 years inclusive; signing of informed consent; participant's willingness to fully comply with all study procedures; availability throughout the study period. **Exclusion criteria:** age under 18 and over 45 years; unwillingness to participate or difficulty understanding informed consent or the aims and requirements of the study; presence of factors that interfere with the participant's full compliance with the study conditions. A total of 1,490 women of reproductive age were invited to participate in the study, 92 of whom were not included in the study due to lack of informed consent. In total, 1,398 women of reproductive age were included in the study.

Hyperandrogenism was defined as hirsutism (Hs) greater than 4 (≥ 5 points) according to the cut-off values for the modified Ferriman – Gallwey visual scale (mFG) previously established by us for the entire population using 2k-cluster analysis, and/or hyperandrogenemia – with a blood serum total testosterone (tT) concentration ≥ 73.90 ng/dl and/or a free androgen index (FAI) value ≥ 6.90 for Caucasians and a blood serum tT concentration ≥ 41.03 ng/dl and/or FAI ≥ 2.92 for Asians and/or a dehydroepiandrosterone sulfate (DHEA-S) level of 355 mg/dl for the entire population (according to the cut-off values reflecting the 98th percentile concentrations of tT, DHEA-S, and FAI in the blood serum in the reference cohort). Oligomenorrhea was defined as a menstrual cycle duration < 21 or > 35 days or a menstrual frequency of less than 9 cycles per year (according to the Rotterdam Consensus recommendations) [1], and in the case of an intact menstrual cycle – a decrease in the blood serum progesterone level on days 20–24 of the cycle below 3–4 ng/ml. Primary amenorrhea was defined as the absence of menstruation during life or the failure to reach menarche by the age of 15 years or 3 years after thelarche [14]. Secondary amenorrhea was defined as the absence of previously regular menstruation for 3 months, and in the case of previously irregular menstruation – the absence of menses for 6 months.

The clinical methods of the study included a questionnaire survey, general medical and gynecological examinations. During the objective examination, the hirsute number was assessed using mFG [15] in accordance with the standardized scoring technology. During the examination by the gynecologist, all women underwent the following: assessment of the condition of the mammary glands; gynecological bimanual examination of the pelvic organs; Pap smear study.

To determine the hormone levels in each patient, blood was collected from the cubital vein on an empty stomach from 8 to 9 a.m., after a 15-minute rest (according to the generally accepted method) and taking into account the phases of the menstrual cycle, using disposable vacuum systems BD Vacutainer (Becton, Dickinson and Company, USA).

Determination of concentrations of thyroid stimulating hormone (TSH), free thyroxine (Free T4), prolactin (PRL), luteinizing hormone (LH), follicle-stimulating hormone (FSH), 17-OH-progesterone, sex hormone-binding globulin (SHBG) and progesterone was carried out using the competitive solid-phase enzyme immunoassay method using the Alkor-Bio test systems (Russia). The study of total tT was carried out using a liquid chromatography–mass spectrometry (LC-MS) LCMS-8060 (Shimadzu, Japan). DHEA-S in blood serum was determined using a chemiluminescent enzyme immunoassay analyzer Immulite 1000 (Siemens, USA).

Instrumental examination methods included pelvic ultrasound performed by three experienced specialists trained to perform ultrasound examinations uniformly with coefficients of variation of examination results less than 6 %, using only the Mindray M7 device (Mindray Bio-Medical Electronics Co., China) with a transvaginal probe (5.0–8.0 MHz) for sexually active subjects. Ovarian volume was calculated using the formula for an oblate ellipsoid: length × width × height × π/6 [4, 5, 16]. The number of follicles was determined by scanning each ovary from the inner to the outer edge in longitudinal section (follicle number per ovary). Follicles were measured in two planes of the ovary, and the follicle diameter was determined as the average value of two diameters (longitudinal and transverse).

Sample size calculations were carried out using the formula:

$$n = [(z_{1-\alpha})^2(P(1 - P) / D^2)],$$

where n is the sample size for the study; $z_{1-\alpha} = 1.96$ (at $\alpha = 0.05$); P is the estimated prevalence of PCOS according to previously published data; D is the absolute error.

As a result, the sample size that would allow us to identify a significant diagnostic potential of the ovarian volume and follicle count values per ovary using ROC curves was at least 198 women for the entire sample.

The study data were entered and managed (report generation, data export for statistical analysis) using the REDCap information system, which is deployed on the server of Scientific Centre for Family Health and Human Reproduction Problems.

The statistical analysis methods included descriptive statistics, testing statistical hypotheses, analysis of relationships between variables, and construction of statistical models. Interval estimation of proportions and frequencies is performed by calculating 95%

confidence intervals (95% CI). To test statistical hypotheses, we used parametric Student's t-test, nonparametric Mann – Whitney test, parametric one-way analysis of variance (ANOVA, ANalysis Of VAriance) or nonparametric rank analysis of variance according to Kruskal – Wallis and median test, z-test, χ^2 criterion. The significance level is defined as 0.05. ROC analysis was used to determine the upper limit of the norm (ULN) for ultrasonographic parameters.

RESULTS

Among the overall population of women of reproductive age included in the study and who underwent a complete examination, 1134 participants had satisfactory visualization of the ovaries based on the results of ultrasonography, and we divided them in the following groups: women with a regular cycle and no signs of hyperandrogenism – group 0 (control group) ($n = 642$); a group of women with PCOS according to NIH (National Institutes of Health) criteria – participants were defined by the combination of the presence of oligomenorrhea/oligoanovulation (OA) [4, 5] and hyperandrogenism (hirsutism and/or hyperandrogenemia) [4, 5, 17] – group 11 ($n = 82$); a group with the presence of any one criterion of PCOS according to NIH (hyperandrogenemia or hirsutism or oligoanovulation) – group 11 ($n = 410$) (fig. 1).

The number of women in subgroups depending on ethnicity is presented in the diagram (fig. 1).

The main sociodemographic, anthropometric characteristics, menstrual and reproductive history of women of reproductive age by groups are presented in Tables 1 and 2.

Women with PCOS according to NIH criteria and study participants with any one symptom of PCOS were younger compared to the representatives of the control group ($p < 0.001$ and $p = 0.013$, respectively). The ethnic composition of the analyzed groups was comparable.

Women with PCOS had higher weight and waist circumference (WC) compared to women in the control group and participants with any one symptom of PCOS ($p < 0.001$ and $p = 0.018$; $p = 0.003$, and $p = 0.020$, respectively). We noted a trend towards higher blood pressure values in the group of women with two symptoms of PCOS compared to the control group ($p = 0.007$). As expected, participants with PCOS had a higher incidence of ovulatory dysfunction, hyperandrogenemia, and hirsutism compared to the group with one criterion ($p < 0.001$ for all frequencies). The mFG index was statistically significantly different in both groups compared to the control group ($p < 0.001$ for all groups), as well as between women with one and two PCOS criteria, with predictably higher values in group 11 ($p < 0.001$) (table 2).

The average age at menarche did not differ between the study groups. However, women with two symptoms of PCOS had a longer menstrual cycle length compared with both the control group ($p < 0.001$) and the group with one criterion ($p < 0.001$); however, women in group 12 also had a statistically significantly longer cycle length than the control group ($p < 0.001$). Accordingly, the frequency of cycles per year was significantly lower in the PCOS group compared with both the group with one criterion ($p < 0.001$) and the control group ($p < 0.001$), and in the group with one criterion compared with the control group ($p < 0.001$). When assessing the reproductive history (table 2), we noted a lower number of pregnancies and births in women in the groups with one and two PCOS criteria compared to the control group ($p < 0.001$ for both groups), with the lowest rate in the PCOS group. The lower frequency of pregnancy can probably also explain the lack of statistically significant differences in the frequency of ectopic and non-viable pregnancies, as well as therapeutic abortions at the request of a woman in the representatives of the groups with one and two symptoms of PCOS compared to the control group. At the same time, we noted a higher number of antenatal fetal deaths in the group of women with one criterion of PCOS compared to the group with two criteria and the control group, and a higher number of spontaneous abortions in women with two criteria of PCOS compared to the control group and the group with one criterion ($p < 0.001$). Certainly, these data confirm the negative impact of both hyperandrogenism and oligoanovulation on the reproductive function of women and justify the need for early detection, management and treatment of such patients.

There were no significant differences in the mean levels of prolactin, TSH, FSH, and 17-OH between the groups, although the participants with PCOS had slightly higher 17-OH values, which were within the normal range, compared with women with one criterion for PCOS and the control group. The representatives of the group with two criteria of PCOS demonstrated higher levels of testosterone, DHEA-S, LH/FSH ratio, FAI, and AMH and, accordingly, the lowest SHBG value compared with the control group and women with one criterion ($p < 0.001$ for all values). However, women with one criterion of PCOS similarly differed statistically significantly in these hormones from the control group ($p < 0.001$ for all values) (table 3).

As for FNPO and OV, the following main results were obtained: among the studied groups, antral follicles number and OV for both the right and left ovary were increased in the groups with hyperandrogenism. Among the groups with the presence of PCOS criteria, the highest values of antral follicles number and OV were found in the group with two criteria (table 3).

Determination of cut-off points for ovarian volume and follicle number in overall population and by ethnicity

In the next step, we evaluated the ultrasound characteristics of the ovaries of the women included in the study.

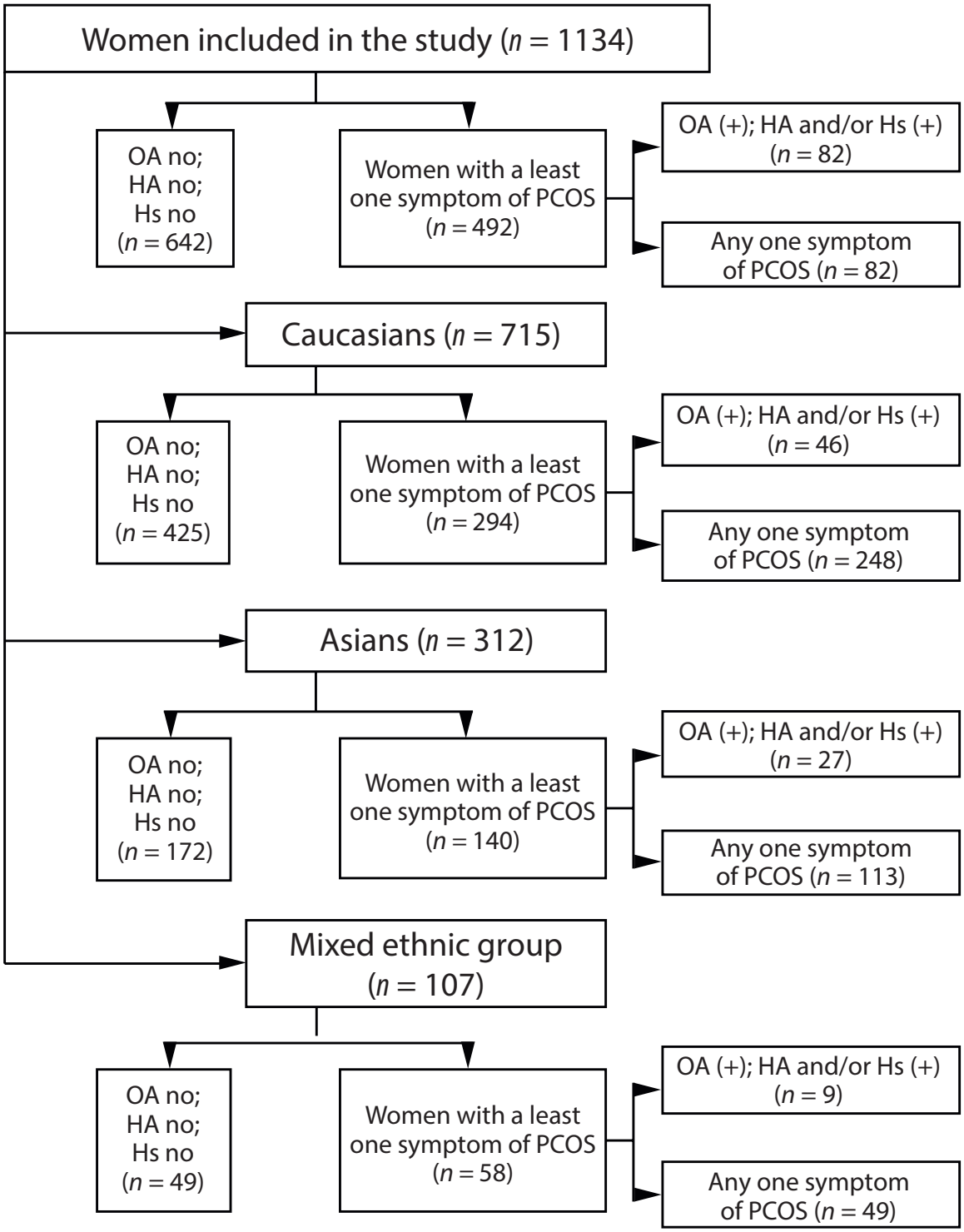

FIG. 1.
Diagram of the distribution of women into subgroups with no PCOS criteria, with one and two criteria overall, and according to ethnicity

TABLE 1

SOCIO-DEMOGRAPHIC CHARACTERISTICS OF WOMEN IN THE STUDY GROUPS

Variables	Group without OA, HA, Hs (*n* = 642)	NIH group (*n* = 82)	Group with any one symptom (*n* = 410)	*p*
	0	11	12	
Age (years), M (SD), Me (LQ; UQ)	35.12 ± 6.17 36.0 (31.0; 40.0)	31.65 ± 6.55 32.0 (26.25; 36.0)	33.67 ± 6.55 34.0 (29.0; 39.0)	$p_u = 0.000_{0–11}$ $p_u = 0.000_{0–12}$ $p_u = 0.013_{11–12}$
	Ethnic composition			
Caucasians, n/N (%)	421/642 (65.58 %)	46/82 (56.10 %)	248/410 (60.49 %)	$p_{\chi2} > 0.05$
Asians, n/N (%)	172/642 (26.79 %)	27/82 (32.93 %)	113/410 (27.56 %)	$p_{\chi2} > 0.05$
Mixed ethnicity, n/N (%)	49/642 (7.63 %)	9/82 (10.98 %)	49/410 (11.95 %)	$p_{\chi2} > 0.05$

Note. $p_{\chi2}$ – χ^2 test; p_u – Mann – Whitney test.

TABLE 2

ANTHROPOMETRIC CHARACTERISTICS, MENSTRUAL AND REPRODUCTIVE HISTORY OF PREMENOPAUSAL WOMEN

Variables	Group 0 (*n* = 642)	Group 11 (*n* = 82)	Group 12 (*n* = 410)	*p*
BMI (kg/m^2), M (SD), Me (LQ; UQ)	25.42 ± 5.11 24.7 (21.5; 28.18)	27.78 ± 6.09 26.9 (22.82; 31.85)	26.17 ± 6.02 24.9 (21.8; 29.4)	$p_u = 0.000_{0–11}$ $p_u = 0.175_{0–12}$ $p_u = 0.018_{11–12}$
Waist circumference (cm), M (SD), Me (LQ; UQ)	77.76 ± 12.02 76.0 (68.0; 85.0)	82.91 ± 14.41 82.0 (72.0; 93.0)	79.05 ± 13.83 76.0 (69.0; 86.0)	$p_u = 0.003_{0–11}$ $p_u = 0.346_{0–12}$ $p_u = 0.020_{11–12}$
SBP (mm Hg), M (SD), Me (LQ; UQ)	78.43 ± 9.85 78.0 (71.0; 84.0)	80.7 ± 10.75 80.0 (74.0; 84.75)	78.65 ± 9.91 79.0 (71.25; 84.0)	$p_u = 0.109^{-11}$ $p_u = 0.424_{0–12}$ $p_u = 0.280_{11–12}$
DBP (mm Hg), M (SD), Me (LQ; UQ)	121.69 ± 13.75 121.0 (113.0; 128.0)	126.2 ± 14.78 124.0 (115.25; 135.0)	122.7 ± 13.66 122.0 (113.0; 130.0)	$p_u = 0.007_{0–11}$ $p_u = 0.170_{0–12}$ $p_u = 0.069_{11–12}$
Oliganovulation, n/N (%)	0/642 (0.00 %)	82/82 (100 %)	208/410 (50.73 %)	$p_{\chi2} = 0.000_{11–12}$
Hyperandrogenemia, n/N (%)	0/642 (0.00 %)	58/82 (70.73 %)	162/410 (39.51 %)	$p_{\chi2} = 0.000_{11–12}$
Hirsutism, n/N (%)	0/642 (0.00 %)	38/82 (46.34 %)	52/410 (12.68 %)	$p_{\chi2} = 0.000_{11–12}$
Hirsutism (scores), M (SD), Me (LQ; UQ)	0.56 ± 1.01 0.0 (0.0; 1.0)	3.99 ± 3.62 4.0 (0.25; 6.0)	1.61 ± 2.51 0.0 (0.0; 2.0)	$p_u = 0.000_{0–11}$ $p_u = 0.000_{0–12}$ $p_u = 0.000_{11–12}$
Age at menarche (years), M (SD), Me (LQ; UQ)	13.3 ± 1.35 13.0 (12.0; 14.0)	13.29 ± 1.72 14.0 (12.0; 14.0)	13.2 ± 1.45 13.0 (12.0; 14.0)	$p_u = 0.843_{0–11}$ $p_u = 0.384_{0–12}$ $p_u = 0.556_{11–12}$
Duration of the menstrual cycle, M (SD), Me (LQ; UQ)	27.67 ± 2.2 28.0 (27.0; 29.0)	35.22 ± 12.49 32.0 (28.0; 38.0)	29.27 ± 5.3 28.0 (27.0; 30.0)	$p_u = 0.000_{0–11}$ $p_u = 0.000_{0–12}$ $p_u = 0.000_{11–12}$
Minimum duration of the menstrual cycle (days), M (SD), Me (LQ; UQ)	26.17 ± 2.39 26.0 (25.0; 28.0)	27.8 ± 6.5 28.0 (25.0; 30.0)	25.8 ± 4.49 27.0 (24.0; 28.0)	$p_u = 0.001_{0–11}$ $p_u = 0.945_{0–12}$ $p_u = 0.004_{11–12}$
Maximum duration of the menstrual cycle (days), M (SD), Me (LQ; UQ)	29.39 ± 2.74 30.0 (28.0; 30.0)	65.95 ± 37.43 51.0 (40.0; 90.0)	40.73 ± 28.68 31.0 (28.0; 40.0)	$p_u = 0.000_{0–11}$ $p_u = 0.000_{0–12}$ $p_u = 0.000_{11–12}$

TABLE 2 (*continued*)

Number of menstrual cycles per year, M (SD), Me (LQ; UQ)	12.23 ± 1.23 12.0 (12.0; 12.0)	9.2 ± 2.56 9.0 (8.0; 11.75)	11.41 ± 1.86 12.0 (11.0; 12.0)	$\mathbf{p_u = 0.000_{0–11}}$ $\mathbf{p_u = 0.000_{0–12}}$ $\mathbf{p_u = 0.000_{11–12}}$
Pregnancies				
Number of pregnancies, M (SD), Me (LQ; UQ)	2.7 ± 2.54 2.0 (1.0; 4.0)	1.66 ± 2.48 1.0 (0.0; 2.0)	2.32 ± 2.18 2.0 (1,0; 3,0)	$\mathbf{p_u = 0.000_{0–11}}$ $\mathbf{p_u = 0.019_{0–12}}$ $\mathbf{p_u = 0.000_{11–12}}$
Number of births, M (SD), Me (LQ; UQ)	1.37 ± 1.01 1.0 (1.0; 2.0)	0.74 ± 0.89 0.0 (0.0; 1.0)	1.27 ± 1.11 1.0 (0.0; 2.0)	$\mathbf{p_u = 0.000_{0–11}}$ $\mathbf{p_u = 0.035_{0–12}}$ $\mathbf{p_u = 0.000_{11–12}}$
Number of stillbirths, M (SD), Me (LQ; UQ)	0.02 ± 0.17 0.0 (0.0; 0.0)	0.0 ± 0.0 0.0 (0.0; 0.0)	0.0 ± 0.07 0.0 (0.0; 0.0)	$p_u = 0.193_{0–11}$ $\mathbf{p_u = 0.039_{0–12}}$ $p_u = 0.529_{11–12}$
Number of self-induced miscarriages, M (SD), Me (LQ; UQ)	0.2 ± 0.56 0.0 (0.0; 0.0)	0.07 ± 0.31 0.0 (0.0; 0.0)	0.16 ± 0.54 0.0 (0.0; 0.0)	$\mathbf{p_u = 0.000_{0–11}}$ $p_u = 0.092_{0–12}$ $p_u = 0.135_{11–12}$
Number of ectopic pregnancies, M (SD), Me (LQ; UQ)	0.04 ± 0.24 0.0 (0.0; 0.0)	0.06 ± 0.24 0.0 (0.0; 0.0)	0.04 ± 0.23 0.0 (0.0; 0.0)	$p_u = 0.275_{0–11}$ $p_u = 0.720_{0–12}$ $p_u = 0.205_{11–12}$
Number of non-viable pregnancies, M (SD), Me (LQ; UQ)	0.03 ± 0.22 0.0 (0.0; 0.0)	0.04 ± 0.19 0.0 (0.0; 0.0)	0.02 ± 0.15 0.0 (0.0; 0.0)	$p_u = 0.546_{0–11}$ $p_u = 0.939_{0–12}$ $p_u = 0.531_{11–12}$
Number of medical abortions, M (SD), Me (LQ; UQ)	1.06 ± 1.85; 0.0 (0.0; 2.0)	0.76 ± 1.87 0.0 (0.0; 0.75)	0.88 ± 1.41 0.0 (0.0; 1.0)	$\mathbf{p_u = 0.003_{0–11}}$ $p_u = 0.295_{0–12}$ $\mathbf{p_u = 0.016_{11–12}}$
Infertility, n/N (%)	120/633 (18.96 %)	30/82 (37.04 %)	104/404 (25.74 %)	$\mathbf{p_{\chi 2} = 0.000_{0–11}}$ $\mathbf{p_{\chi 2} = 0.018_{0–12}}$ $p_{\chi 2} = 0.071_{11–12}$

Note. $p_{\chi 2}$ – χ^2 test; p_u – Mann – Whitney test.

TABLE 3

HORMONAL CHARACTERISTICS OF PREMENOPAUSAL WOMEN AND DATA OF PELVIC ULTRASOUND

Variables	Group without OA, HA, Hs (*n* = 642) 0	NIH group (*n* = 82) 11	Group with any one symptom (*n* = 410) 12	*p*
TSH (mIU/ml), M (SD); Me (LQ; UQ)	372.01 ± 256.99 314.5 (221.0; 445.5)	403.91 ± 315.13 319.0 (239.5; 467.0)	393.92 ± 251.01 329.0 (235.0; 483.0)	$p_u = 0.807_{0–11}$ $p_u = 0.065_{0–12}$ $p_u = 0.869_{11–12}$
PRL (mU/l), M (SD); Me (LQ; UQ)	1.73 ± 1.2 1.5 (1.1; 2.1)	1.75 ± 1.05 1.6 (1.0; 2.28)	1.94 ± 1.95 1.5 (1.1; 2.2)	$p_u = 0.756_{0–11}$ $p_u = 0.340_{0–12}$ $p_u = 0.826_{11–12}$
LH (mIU/ml), M (SD); Me (LQ; UQ)	8.03 ± 10.89 5.3 (3.2; 7.9)	12.94 ± 13.06 8.5 (5.28; 16.95)	10.06 ± 12.31 6.0 (3.7; 10.85)	$\mathbf{p_u = 0.000_{0–11}}$ $\mathbf{p_u = 0.000_{0–12}}$ $\mathbf{p_u = 0.000_{11–12}}$
FSH (mIU/l), M (SD); Me (LQ; UQ)	7.02 ± 8.25 5.4 (3.8; 7.3)	6.91 ± 5.07 6.15 (4.32; 7.7)	9.34 ± 5.9 5.5 (3.9; 7.52)	$p_u = 0.100_{0–11}$ $p_u = 0.260_{0–12}$ $p_u = 0.289_{11–12}$
LH/FSH ratio, M (SD); Me (LQ; UQ)	1.24 ± 1.12 0.93 (0.66; 1.5)	1.91 ± 1.31 1.61 (1.02; 2.38)	1.4 ± 1.07 1.04 (0.67; 1.83)	$\mathbf{p_u = 0.000_{0–11}}$ $\mathbf{p_u = 0.022_{0–12}}$ $\mathbf{p_u = 0.000_{11–12}}$

TABLE 3 (*continued*)

Testosterone (g/dl), M (SD); Me (LQ; UQ)	247.03 ± 132.3 246.38 (153.5; 326.87)	571.09 ± 659.06 432.47 (306.46; 609.6)	403.15 ± 384.43 298.2 (209.63; 434.57)	$p_u = 0.000_{0–11}$ $p_u = 0.000_{0–12}$ $p_u = 0.000_{11–12}$
SHBG, M (SD); Me (LQ; UQ)	82.86 ± 54.5 67.45 (43.7; 105.65)	60.08 ± 43.87 44.3 (29.72; 75.4)	76.32 ± 52.78 64.8 (38.1; 98.9)	$p_u = 0.000_{0–11}$ $p_u = 0.027_{0–12}$ $p_u = 0.003_{11–12}$
FAI, M (SD); Me (LQ; UQ)	1.41 ± 1.09 1.16 (0.63; 1.92)	4.52 ± 4.92 3.36 (1.82; 5.36)	3.1 ± 6.78 1.65 (0.94; 3.5)	$p_u = 0.000_{0–11}$ $p_u = 0.000_{0–12}$ $p_u = 0.000_{11–12}$
DHEA-S (mg/dl), M (SD); Me (LQ; UQ)	158.59 ± 65.38 150.0 (110.5; 200.0)	244.67 ± 109.9 233.0 (168.75; 316.25)	190.59 ± 98.33 170.0 (118.0; 240.0)	$p_u = 0.000_{0–11}$ $p_u = 0.000_{0–12}$ $p_u = 0.000_{11–12}$
17-OH-progesterone, M (SD); Me (LQ; UQ)	5.5 ± 3.35 5.2 (2.48; 7.7)	6.01 ± 3.18 5.6 (4.3; 6.9)	5.83 ± 4.78 4.9 (2.9; 6.9)	$p_u = 0.222_{0–11}$ $p_u = 0.912_{0–12}$ $p_u = 0.107_{11–12}$
AMH, M (SD); Me (LQ; UQ)	2.78 ± 2.45 2.1 (1.0; 4.0)	6.58 ± 5.51 5.3 (2.65; 8.12)	4.66 ± 4.74 3.2 (1.7; 6.1)	$p_u = 0.000_{0–11}$ $p_u = 0.000_{0–12}$ $p_u = 0.001_{11–12}$
Ultrasound characteristics of the ovaries *All ovaries*				
OV	6.36 ± 2.57 5.93 (4.71; 7.46)	9.66 ± 5.43 8.82 (5.96; 11.40)	7.35 ± 3.73 6.53 (4.83; 9.09)	$p_u = 0.000_{0–11}$ $p_u = 0.000_{0–12}$ $p_u = 0.001_{11–12}$
FNPO	6.74 ± 2.83 6.00 (5.00; 8.00)	10.92 ± 4.97 12.00 (7.00; 13.5)	7.97 ± 3.70 7.00 (5.00; 10.00)	$p_u = 0.000_{0–11}$ $p_u = 0.000_{0–12}$ $p_u = 0.001_{11–12}$
Left ovaries				
OV	8.12 ± 9.0 6.34 (4.92; 8.57)	10.25 ± 8.67 8.68 (6.02; 11.69)	8.33 ± 6.75 6.9 (5.0; 9.7)	$p_u = 0.000_{0–11}$ $p_u = 0.000_{0–12}$ $p_u = 0.001_{11–12}$
FNPO	6.4 ± 2.65 6.0 (5.0; 8.0)	10.44 ± 5.31 10.0 (7.0; 13.0)	7.26 ± 3.38 7.0 (5.0; 9.25)	$p_u = 0.000_{0–11}$ $p_u = 0.000_{0–12}$ $p_u = 0.000_{11–12}$
Right ovaries				
OV	9.06 ± 23.65 6.72 (5.23; 8.84)	11.23 ± 7.15 9.74 (6.67; 12.55)	8.98 ± 8.19 7.46 (5.34; 10.54)	$p_u = 0,000_{0–11}$ $p_u = 0,005_{0–12}$ $p_u = 0,000_{11–12}$
FNPO	6.68 ± 2.79 6.0 (5.0; 8.0)	10.77 ± 4.8 12.0 (7.0; 14.0)	7.66 ± 3.84 7.0 (5.0; 10.0)	$p_u = 0.000_{0–11}$ $p_u = 0.000_{0–12}$ $p_u = 0.000_{11–12}$

Note. p_u – Mann – Whitney test.

The exclusion criterion for an ovary was the presence of a mass, cyst, or follicle of 10 mm or more in diameter detected during the ultrasound scan performed in this study. A total of 1665 ovaries that met the inclusion criteria were ultimately included in the analysis. In the control group, 927 ovaries were evaluated (group 0 in the diagram); in the group with at least one PCOS criterion (group 12 in the diagram), 597 ovaries were evaluated; in women with two PCOS criteria (group 11), 132 ovaries were evaluated (fig. 2). In women of Caucasian ethnicity, 1024 ovaries met the inclusion criteria, in Asian women, 458 ovaries, and in the mixed ethnicity group, 174 ovaries met the inclusion criteria. Next, the cut-off points (upper normal values) for ovarian volume and follicle number were determined to classify women without symptoms of PCOS with the group of participants demonstrating single criteria of PCOS and with women with PCOS

both in general and taking into account their ethnicity. To clarify the upper normal values for ovarian volume and of antral follicles number per ovary in women with any one criterion of PCOS, the following subgroups were identified when stratifying by ethnic groups. The overall population is group 0/12, which included 1052 participants, the average age was 34.4 ± 6.35 years, the number of ovaries in this group was 1524. Group 0c/12c – Caucasians (n = 669, number of ovaries – 953), the average age was 34.4 ± 6.4 years. Group 0a/12a – Asians (n = 285, number of ovaries – 412), average age 33.8 ± 6.7 years. Group 0m/12m – participants of mixed ethnicity (n = 98, number of ovaries – 159), average age 32.1 ± 7.3 years (fig. 2).

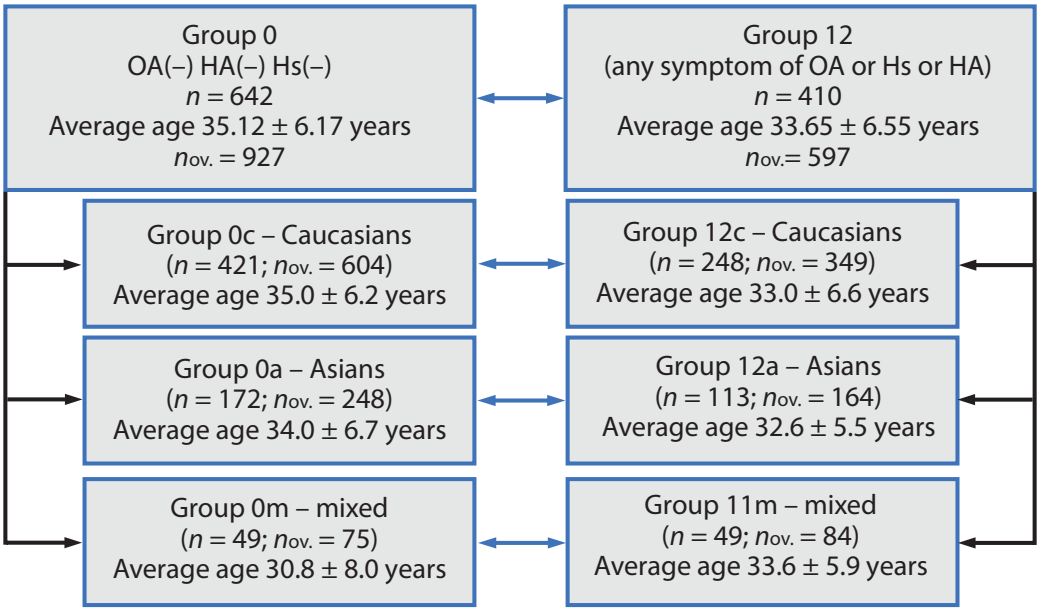

FIG. 2.
Inclusion diagram of study participants to determine cut-off points for ovarian volume and follicle number when classifying patients into groups 0–12 overall and by ethnicity

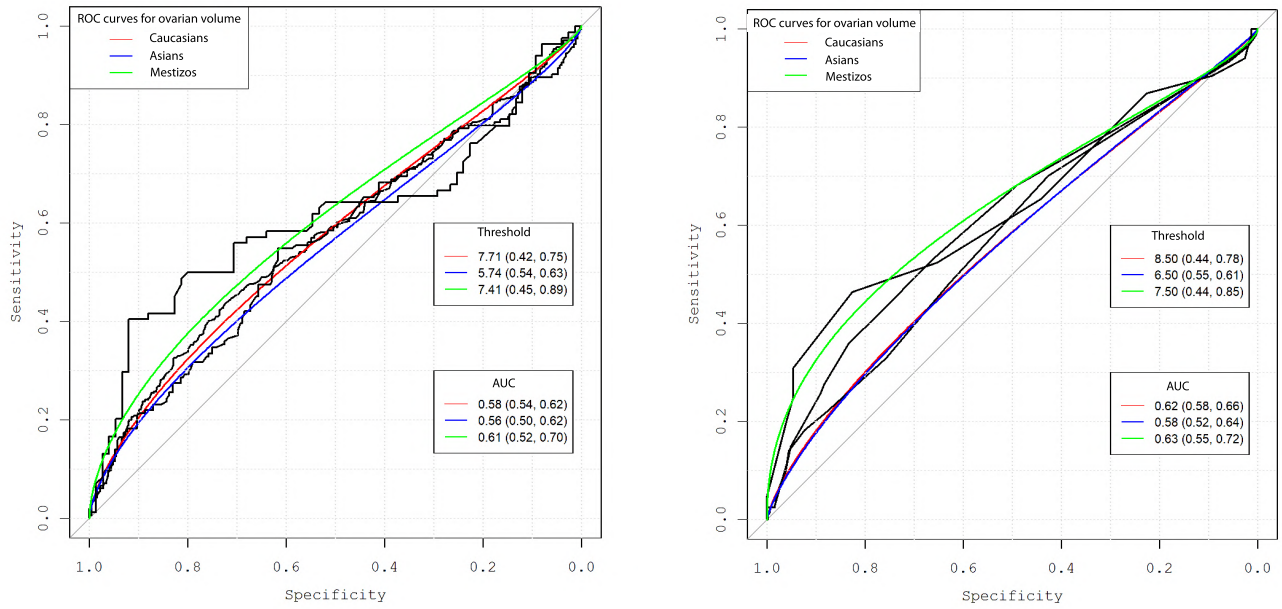

FIG. 3.
ROC-curves for ovarian volume and antral follicles number per ovary for premenopausal women (group 0/group 12 (any one sign of PCOS according to NIH criteria (hyperandrogenism/oligoanovulation)) for overall population, Caucasian women, Asian women, and women of mixed ethnicity

TABLE 4

CUT-OFF POINTS FOR OVARIAN VOLUME AND FOLLICLE NUMBER WHEN CLASSIFYING THE PATIENTS INTO GROUPS OVERALL AND BY ETHNICITY (GROUP 0/GROUP 12 (ANY ONE SIGN OF PCOS ACCORDING TO NIH CRITERIA (HYPERANDROGENISM/ OLIGOANOVULATION))

Parameters	Cut-off point	95% CI for cut-off points	AUC	95% CI for AUC	Sensitivity	Specificity	PSPR	PSNR
Ovarian volume								
All ethnic groups	7.55	(6.83; 8.75)	0.57	(0.54; 0.60)	0.39	0.77	0.11	0.54
Caucasians	7.71	(6.95; 9.79)	0.58	(0.54; 0.62)	0.42	0.75	0.14	0.51
Asians	5.74	(5.19; 9.36)	0.56	(0.50; 0.62)	0.54	0.63	0.24	0.35
Mixed ethnicity	7.41	(6.04; 7.47)	0.61	(0.52; 0.70)	0.45	0.89	0.11	0.67
Antral follicles number per ovary								
All ethnic groups	8.50	(6.50; 9.50)	0.60	(0.57; 0.63)	0.39	0.80	0.09	0.61
Caucasians	8.50	(6.50; 9.50)	0.62	(0.58; 0.66)	0.44	0.78	0.12	0.56
Asians	6.50	(5.50; 10.50)	0.58	(0.52; 0.64)	0.55	0.61	0.23	0.36
Mixed ethnicity	7.50	(7.50; 8.50)	0.63	(0.55; 0.72)	0.44	0.85	0.14	0.57

Note. PSPR – prognostic significance of a positive result; PSNR – prognostic significance of a negative result.

ROC-analysis was performed on the data set obtained in the groups of women presented in figure 3. The threshold values and diagnostic efficiency of the OV and antral follicles number per ovary are presented in table 4.

Determination of cut-off points for ovarian volume and follicle number when classifying the patients into groups 0–12 of women of Caucasian, Asian, and mixed ethnicity aged less than 35 years and aged 35 years and older inclusive

To clarify the cut-off values for OV and antral follicles number per ovary in women with any one criterion of PCOS, the following subgroups were identified when stratifying the patients by ethnic groups and age. The overall population is the 0/12 group, which included 1052 participants, the average age was 34.4 ± 6.35 years, the number of ovaries was 1524; then, depending on age, the women were divided into participants aged 18–34 years and 35–44 years. As a result, the distribution by groups is as follows: group 0a1/12a1 – 496 participants, the average age was 28.91 ± 3.84 years, 726 ovaries were subject to assessment; Group $0c_{a1}/12c_{a1}$ – Caucasians aged 18–34 years (n = 324, number of ovaries – 466), average age 28.94 ± 3.85 years; Group $0a_{1a}/12a_{1a}$ – Asians aged (n = 122, number of ovaries – 177), average age 28.84 ± 3.95 years; Group $0m_{1a}/12m_{1a}$ – participants of mixed ethnicity (n = 50, number of ovaries – 83), average age 28.88 ± 4.06 years (fig. 4).

Women aged 35–44 years were distributed as follows: group $0_{a2}/12_{a2}$ – 556 women, average age 39.59 ± 3.03 years, 798 ovaries; group $0c_{a2}/12c_{a2}$ – Caucasians aged 18–34 years (n = 345, number of ovaries – 487), average age 39.56 ± 3.08 years; group $0a_{a2}/12a_{a2}$ – Asians aged 35–44 years (n = 163, number of ovaries – 235), average age 39.69 ± 2.92 years; group $0c_{a2}/12c_{a2}$ – women of mixed ethnicity (n = 48, number of ovaries – 76), average age 39.42 ± 3.07 years (fig. 4).

Next, ROC analysis was performed on the data set obtained in the groups presented in figure 3. The threshold values and diagnostic efficiency of the OV and antral follicles number per ovary are presented in table 4.

The threshold values for OV in women with one criterion of PCOS were: for all ethnic groups – 7.55 cm^3; for Caucasians – 7.74 cm^3; for Asians – 5.74 cm^3; for representatives of mixed ethnicity – 7.41 cm^3. The area under the curve (AUC) for ovarian volume was 0.57, 0.58, 0.56 and 0.61, respectively. The threshold values for antral follicles number per ovary in women with one criterion of PCOS were: for all ethnic groups – 8.50; for Caucasians – 8.50; for Asians – 6.50; for representatives of mixed ethnicity – 7.50. The AUC of antral follicles number per ovary was 0.60, 0.62, 0.58 and 0.63, respectively (table 3).

The cut-off values for OV in women with any one PCOS criterion, taking into account age for all ethnic groups, for participants aged 18–34 years were 7.48 cm^3 (AUC = 0.63), which was statistically significantly higher than the cut-off levels for women aged 35–44 years (4.63 cm^3; AUC = 0.53; $p < 0.05$) (table 5). For Caucasians in the age group of 18–34 years, this value was 8.02 cm^3 (AUC = 0.62), which was statistically significantly higher than the cut-off levels for women aged 35–44 years (5.22 cm^3; AUC = 0.535; $p < 0.05$) (table 5).

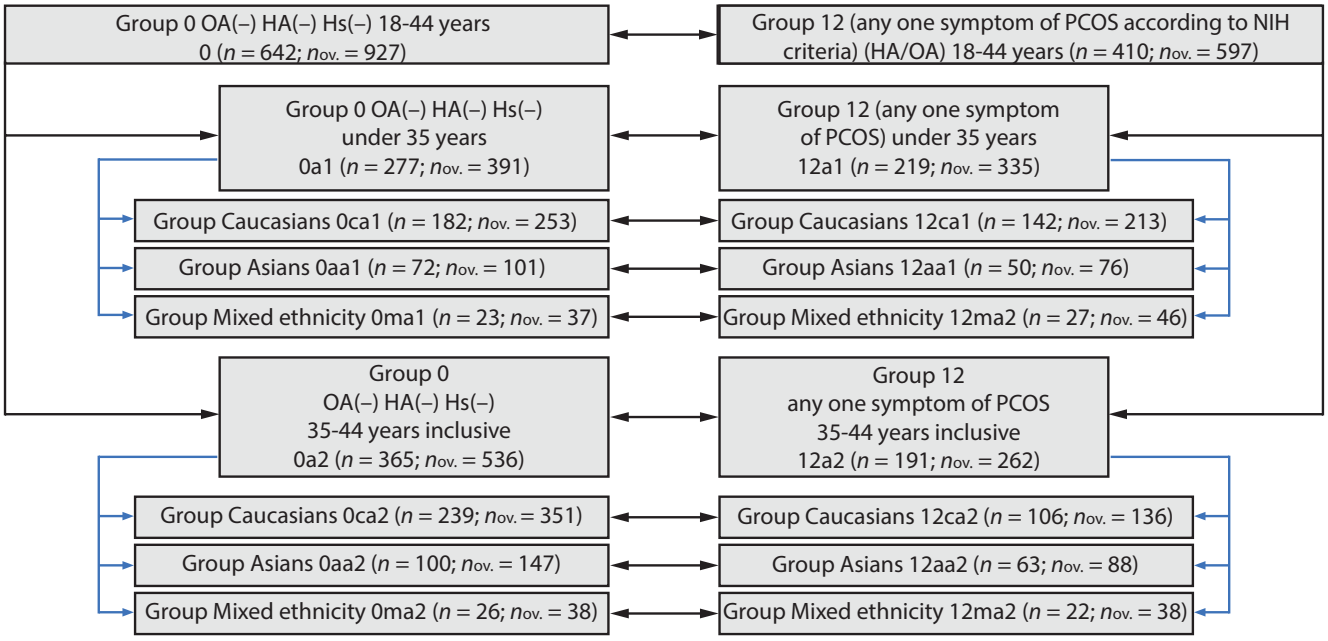

FIG. 4.
Inclusion diagram of study participants to determine cut-off points for ovarian volume and follicle number when classifying patients into groups 0–12 overall, by age and by ethnicity

TABLE 5

CUT-OFF POINTS FOR OVARIAN VOLUME AND FOLLICLE NUMBER WHEN CLASSIFYING PATIENTS UNDER 35 YEARS OF AGE AND 35–44 YEARS OF AGE INTO GROUPS 0–12

Parameters	Cut-off point	95% CI for cut-off points	AUC	95% CI for AUC	Sensitivity	Specificity	PSPR	PSNR
				Ovarian volume				
All ethnic groups under 35 years of age, 0/12a1 (*n* = 726)	7.48	(6.19; 9.13)	0.63	(0.59; 0.67)	0.53	0.69	0.22	0.40
All ethnic groups 35-44 years of age, 0/12a2 (*n* = 798)	4.63	(3.02; 5.42)	0.53	(0.49; 0.58)	0.40	0.72	0.27	0.23
Caucasians under 35 years of age, 0/12a1 (*n* = 466)	8.05	(6.33; 9.76)	0.62	(0.57; 0.67)	0.52	0.71	0.21	0.42
Caucasians 35-44 years of age, 0/12a2 (*n* = 487)	5.22	(3.65; 6.27)	0.55	(0.49; 0.61)	0.49	0.65	0.29	0.26
Asians under 35 years of age, 0/12a1 (*n* = 177)	5.30	(5.04; 9.53)	0.64	(0.55; 0.72)	0.78	0.51	0.49	0.19
Asians 35-44 years of age, 0/12a2 (*n* = 235)	4.73	(3.09; 6.18)	0.62	(0.54; 0.70)	0.26	0.87	0.21	0.18
Mixed ethnicity under 35 years of age, 0/12a1 (*n* = 83)	7.30	(5.56; 8.63)	0.66	(0.54; 0.78)	0.57	0.84	0.21	0.52
Mixed ethnicity 35-44 years of age, 0/12a2 (*n* = 76)	6.83	(5.79; 8.63)	0.62	(0.50; 0.73)	0.37	0.95	0.09	0.71

TABLE 5 (*continued*)

Antral follicles number per ovary								
All ethnic groups under 35 years of age, 0/12a1 (n = 726)	9.50	(7.50; 10.50)	0.63	(0.58; 0.67)	0.48	0.75	0.16	0.51
All ethnic groups 35-44 years of age, 0/12a2 (n = 798)	6.50	(6.50; 10.50)	0.52	(0.48; 0.57)	0.38	0.72	0.12	0.49
Caucasians under 35 years of age, 0/12a1 (n = 466)	9.50	(8.50; 11.50)	0.63	(0.58; 0.68)	0.53	0.72	0.20	0.45
Caucasians 35-44 years of age, 0/12a2 (n = 487)	6.50	(6.50; 10.50)	0.52	(0.45; 0.58)	0.43	0.68	0.16	0.44
Asians under 35 years of age, 0/12a1 (n = 177)	9.50	(5.50; 10.50)	0.60	(0.52; 0.69)	0.37	0.86	0.09	0.66
Asians 35-44 years of age, 0/12a2 (n = 235)	5.50	(1.0; 0.45)	0.59	(0.51; 0.67)	0.56	0.59	0.27	0.31
Mixed ethnicity under 35 years of age, 0/12a1 (n = 83)	7.50	(7.50; 8.50)	0.68	(0.57; 0.80)	0.57	0.86	0.25	0.49
Mixed ethnicity 35-44 years of age, 0/12a2 (n = 76)	7.50	(7.50; 8.50)	0.64	(0.53; 0.75)	0.34	0.87	0.07	0.69

Determination of cut-off points for ovarian volume and follicle count when classifying the patients into groups 0–11 overall and by ethnicity

To clarify the upper normal values for OV and antral follicles number per ovary in women with two signs of PCOS according to the NIH criteria, the following subgroups were identified when stratifying by ethnic groups. The overall population is group 0/11, which included 724 participants, the average age was 33.1 ± 7.2 years, the number of ovaries was 1059. Group 0c/11c – Caucasians (n = 467, the number of ovaries was 675), the average age was 34.8 ± 6.9 years. Group 0a/11a – Asians (n = 199, the number of ovaries was 294), the average age was 32.1 ± 6.9 years. Group 0m/11m – women of mixed ethnicity (n = 58, the number of ovaries was 90), the average age was 32.9 ± 8.3 years (fig. 5).

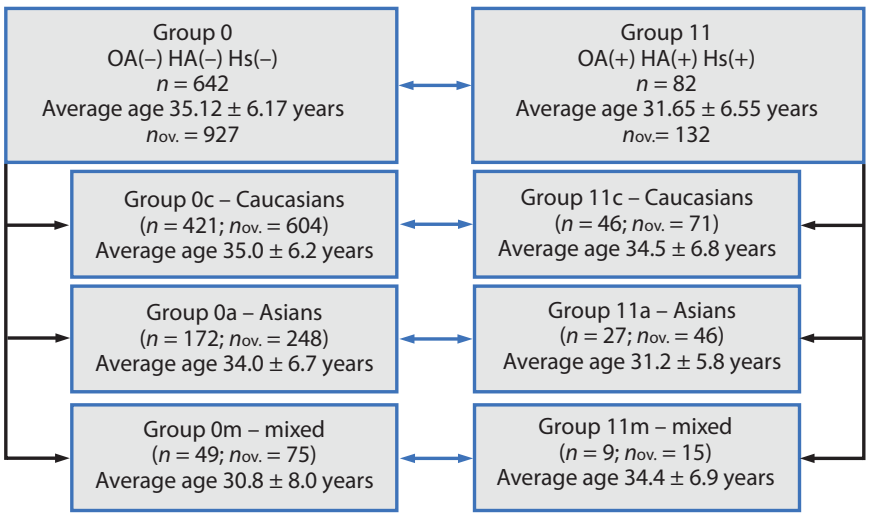

FIG. 5.
Inclusion diagram of study participants to determine cut-off points for ovarian volume and follicle number when classifying patients into groups 0–11 overall and by ethnicity

ROC-analysis was performed on the data set obtained in the groups presented in figure 6. The threshold values and diagnostic efficiency of OV and antral follicles number per ovary are presented in table 6.

The threshold values for OV in women with two PCOS criteria were: for all ethnic groups – 8.11 cm^3; for Caucasians – 8.4 cm^3; for Asians – 6.56 cm^3; for representatives of mixed ethnicity – 7.11 cm^3. The AUC

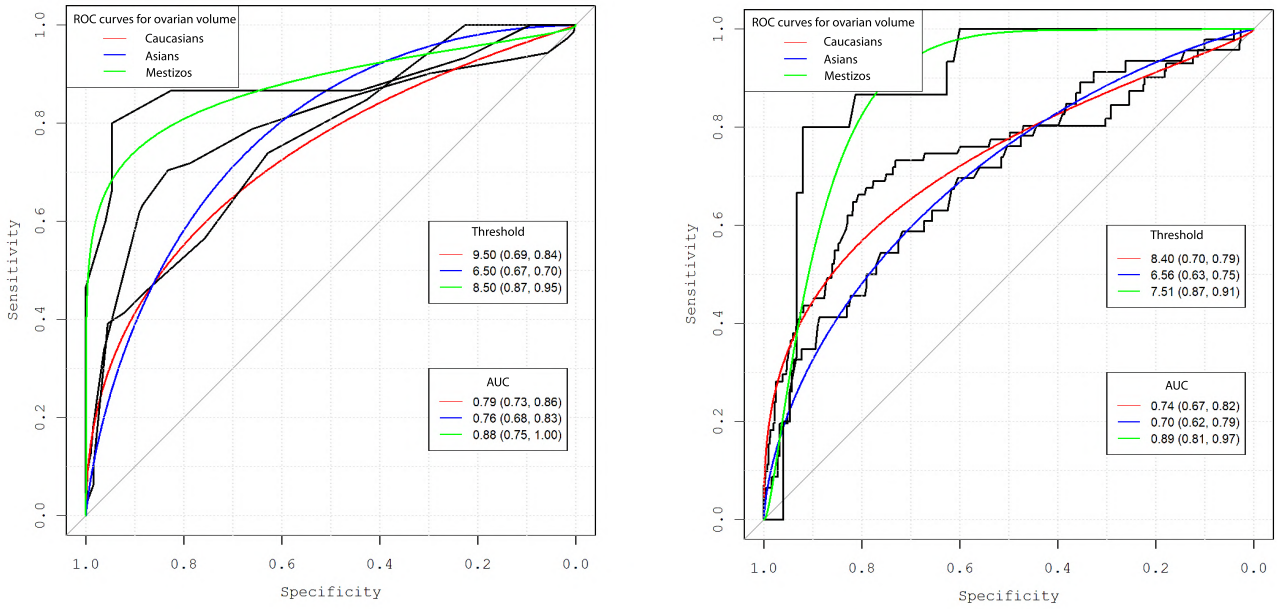

FIG. 6.
ROC-curves for ovarian volume and antral follicles number per ovary for premenopausal women (group 0/group 11 (PCOS according to NIH criteria (hyperandrogenism/oligoanovulation)) for overall population, Caucasian women, Asian women, and women of mixed ethnicity

TABLE 6

CUT-OFF POINTS FOR OVARIAN VOLUME AND FOLLICLE NUMBER WHEN CLASSIFYING PATIENTS INTO GROUPS OVERALL AND BY ETHNICITY (GROUP 0/GROUP 11 (PCOS ACCORDING TO NIH CRITERIA (HYPERANDROGENISM/OLIGOANOVULATION))

Parameters	Cut-off point	95% CI for cut-off points	AUC	95% CI for AUC	Sensitivity	Specificity	PSPR	PSNR
				Ovarian volume				
All ethnic groups	8.11	(7.62; 8.66)	0.73	(0.67; 0.78)	0.62	0.80	0.15	0.61
Caucasians	8.40	(7.61; 8.81)	0.74	(0.67; 0.82)	0.70	0.79	0.18	0.59
Asians	6.56	(4.71; 9.37)	0.70	(0.62; 0.79)	0.63	0.75	0.20	0.51
Mixed ethnicity	7.51	(5.73; 8.57)	0.89	(0.81; 0.97)	0.87	0.91	0.17	0.73
				Antral follicles number per ovary				
All ethnic groups	9.50	(7.50; 10.50)	0.78	(0.73; 0.82)	0.61	0.86	0.12	0.70
Caucasians	9.50	(8.50; 11.50)	0.79	(0.73; 0.86)	0.69	0.84	0.15	0.65
Asians	6.50	(6.50; 11.00)	0.76	(0.68; 0.83)	0.67	0.70	0.29	0.40
Mixed ethnicity	8.50	(7.50; 9.00)	0.88	(0.75; 1.00)	0.87	0.95	0.15	0.78

Note. PSPR – prognostic significance of a positive result; PSNR – prognostic significance of a negative result.

for ovarian volume was 0.73, 0.74, 0.71 and 0.89, respectively. The threshold values for antral follicles number per ovary in women with one PCOS criterion were: for all ethnic groups – 9.50; for Caucasians – 9.50; for Asians – 6.50; for representatives of mixed ethnicity – 8.50. The AUC of antral follicles number per ovary was 0.78, 0.79, 0.76 and 0.88, respectively (table 6). Considering the high values of sensitivity and specificity for the threshold levels of OV and antral follicles number per ovary both in the overall population and depending on ethnicity, we can conclude that the diagnostic ability of the studied variables is satisfactory.

Determination of cut-off points for ovarian volume and follicle count when classifying the female patients aged less than 35 years and aged 35 years and older inclusive (group 0-11)

At the next stage, to determine the upper normal values for OV and antral follicles number per ovary in women with two signs of PCOS according to the NIH criteria, the following subgroups were identified when stratified the patients by ethnic groups and age. The overall population is group 0/11, which included 724 participants, the average age was 34.72 ± 6.30 years, $n_{ov.}$ = 1059. The women were then divided depending on age into participants aged 18–34 years (B1) and 35–44 years (B2). Taking into account the age, the following groups were ultimately formed. Group $0_{a1}/11_{a1}$ included 329 participants, average age 28.96 ± 3.93 years, $n_{ov.}$ = 480. Group $0c_{a1}/11c_{a1}$ included Caucasians aged 18–34 years (n = 212; n_{ov} = 302), average age 28.83 ± 3.94 years. Group $0a_{a1}/11a_{a1}$ included Asians aged (n = 87; $n_{ov.}$ = 128), average age 29.55 ± 3.68 years. Group $0m_{a1}/11m_{a1}$ included individuals of mixed ethnicity (n = 30; $n_{ov.}$ = 50), average age 28.17 ± 4.49 years (fig. 7).

Women aged 35–44 years were distributed as follows: group $0_{a2}/11_{a2}$ – 395 participants, average age 39.52 ± 4.04 years, n_{ov} = 579; group $0c_{a2}/11c_{a2}$ – Caucasians aged 18–34 years (n = 255; $n_{ov.}$ = 373), average age 39.49 ± 3.08 years; group $0a_{a2}/11a_{a2}$ – Asians aged (n = 112; n_{ov} = 166), average age 39.44 ± 3.01 years; group $0m_{a2}/11m_{a2}$ – individuals of mixed ethnicity (n = 28; $n_{ov.}$ = 40), average age 40.14 ± 2.85 years (Fig. 7).

The threshold values for OV in women with two criteria of PCOS, taking into account age for all ethnic groups were: for participants aged 18–34 years – 8.39 cm^3 (AUC = 0.77); for women aged 35–44 years – 8.43 cm^3 (AUC = 0.58); for Caucasians in the age group 18–34 years – 8.57 cm^3 (AUC = 0.80); in the group of women aged 35–44 years – 8.51 cm^3 (AUC = 0.80) (table 7).

For Asian women aged 18–34 years, the threshold for ovarian volume was determined as 6.52 cm^3 (AUC = 0.75), for the age group 35–44 years – 6.75 cm^3 (AUC = 0.72). For 18–34-year-old participants in the mixed group, the threshold was 7.50 cm^3 (AUC = 0.86), for the age group 35–44 years – 7.50 cm^3 (AUC = 0.87) (table 7). We noted the absence of statistically significant differences in ovarian volume in women aged 18–34 and 35–44 years in all ethnic groups.

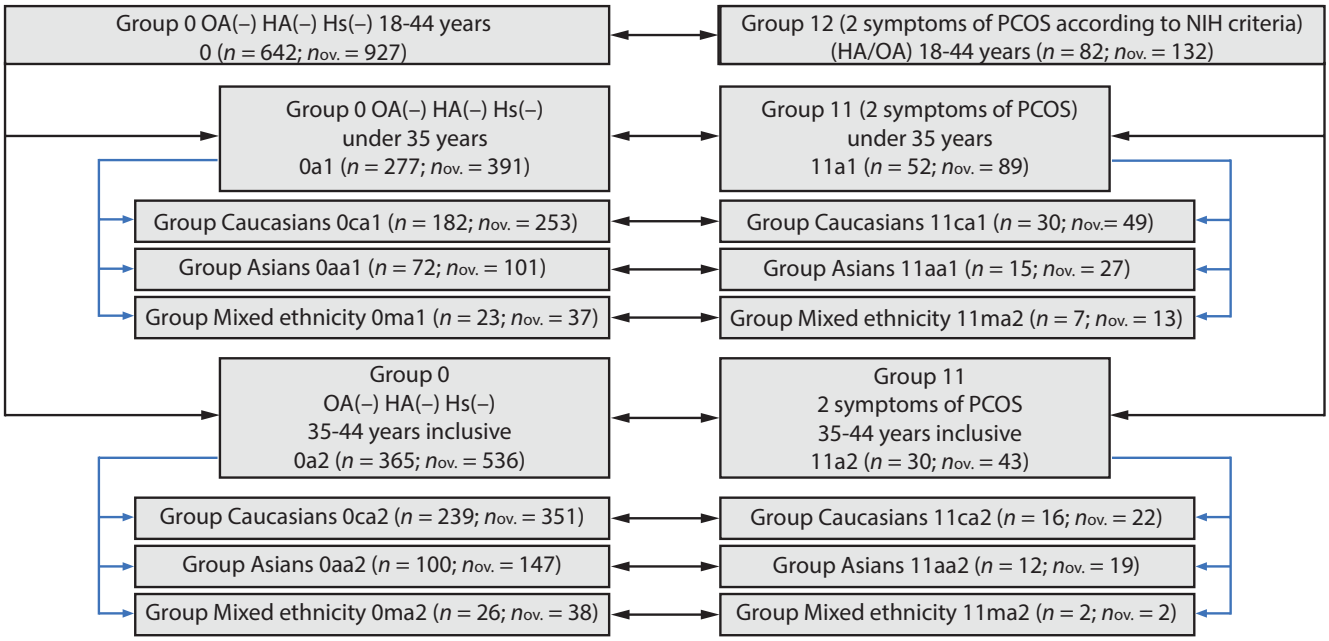

FIG. 7.
Inclusion diagram of study participants to determine cut-off points for ovarian volume and follicle number when categorized into groups 0–11 overall, by age and by ethnicity

TABLE 7

CUT-OFF POINTS FOR OVARIAN VOLUME AND FOLLICLE NUMBER WHEN CLASSIFYING PATIENTS UNDER 35 YEARS OF AGE AND 35–44 YEARS OF AGE INTO GROUPS 0–11

Parameters	Cut-off point	95% CI for cut-off points	AUC	95% CI for AUC	Sensitivity	Specificity	PSPR	PSNR
Ovarian volume								
All ethnic groups under 35 years of age, 0/11a1 (*n* = 815)	8.39	(7.58; 9.04)	0.77	(0.71; 0.82)	0.73	0.76	0.24	0.51
All ethnic groups 35-44 years of age, 0/11a2 (*n* = 579)	8.43	(4.58; 10.62)	0.58	(0.49; 0.68)	0.40	0.87	0.05	0.75
Caucasians under 35 years of age, 0/11a1c (*n* = 302)	8.57	(7.58; 9.06)	0.80	(0.72; 0.87)	0.84	0.74	0.29	0.48
Caucasians 35-44 years of age, 0/11a2c (*n* = 373)	8.51	(7.58; 9.17)	0.80	(0.73; 0.87)	0.41	0.87	0.06	0.75
Asians under 35 years of age, 0/12a1(*n* = 128)	6.52	(5.23; 9.53)	0.75	(0.64; 0.85)	0.78	0.67	0.35	0.37
Asians 35-44 years of age, 0/11a2a (*n* = 166)	6.85	(5.34; 9.50)	0.72	(0.62; 0.82)	0.84	0.42	0.54	0.15
Mixed ethnicity under 35 years of age, 0/11a1m (*n* = 50)	7.51	(5.73; 8.57)	0.86	(0.75; 0.97)	0.92	0.84	0.30	0.57
Mixed ethnicity 35-44 years of age, 0/11a2m (*n* = 40)	7.50	(5.73; 8.57)	0.87	(0.77; 0.97)	1.00	0.71	0.35	0.46
Antral follicles number per ovary								
All ethnic groups under 35 years of age, 0/11a1 (*n* = 815)	10.50	(8.50;11.50)	0.82	(0.77; 0.87)	0.73	0.85	0.19	0.63
All ethnic groups 35-44 years of age, 0/11a2 (*n* = 579)	6.50	(6.50; 9.50)	0.65	(0.55; 0.74)	0.58	0.72	0.73	0.49
Caucasians under 35 years of age, 0/11a1c (*n* = 302)	11.50	(9.50; 11.50)	0.84	(0.77; 0.90)	0.82	0.82	0.23	0.59
Caucasians 35-44 years of age, 0/11a2c (*n* = 373)	11.50	(9.50; 11.50)	0.84	(0.78; 0.90)	0.59	0.79	0.13	0.62
Asians under 35 years of age, 0/12a1 (*n* = 128)	10.50	(7.50; 11.00)	0.76	(0.65; 0.87)	0.63	0.91	0.11	0.75

TABLE 7 (*continued*)

Asians 35-44 years of age, 0/11a2a (*n* = 166)	6.50	(6.50; 11.00)	0.76	(0.68; 0.83)	0.67	0.70	0.29	0.40
Mixed ethnicity under 35 years of age, 0/11a1m (*n* = 50)	8.50	(7.50; 10.00)	0.96	(0.92; 1.00)	1.00	0.92	0.30	0.64
Mixed ethnicity 35-44 years of age, 0/11a2m (*n* = 40)	8.50	(7.50; 11.00)	0.96	(0.92; 1.00)	1.00	0.45	0.4	0.27

DISCUSSION

In our study, cut-off points for OV and antral follicles number per ovary were determined using pairwise comparisons of three groups: 1) women with regular cycles and no signs of hyperandrogenism versus 2) study participants with oligomenorrhea/olianovulation [4, 5] and hyperandrogenism (hirsutism and/or hyperandrogenemia) [4, 5, 17], the combination of which allows the diagnosis of PCOS without assessment of ovarian ultrasonographic characteristics, or 3) women with either oligoanovulation or any one sign of hyperandrogenism.

According to our data, for differentiation of the 1st and 2nd comparison groups in Caucasians, the best compromise between sensitivity and specificity was achieved with a diagnostic threshold for OV ≥ 9 cm^3, which did not depend on age (8.57 cm^3 and 8.51 cm^3 in the younger and older age groups, respectively) and was slightly lower compared to the results of previous studies presented by E. Carmina et al. [18], D. Dewailly et al. [19] and M.E. Lujan et al. [20], where the ULN was 10 cm^3.

For Asian women, the upper normal value for ovary that we determined also did not differ statistically significantly depending on age. Thus, at the age of 18–34 years, the ULN for ovary was determined as 6.52 cm^3, and for the age group of 35–44 years – 6.75 cm^3, which is consistent with the data obtained previously in Korean patients with PCOS [8], who had the right ovary volume of 6.4 cm^3, the left – 6.7 ml [8], as well as in Chinese women [21]: the ULN values for ovary were 6.3 cm^3 [22] and 7 cm^3 [23]. A lower OV in women of the Asian population with PCOS compared to Caucasians has been mentioned previously [24–26].

When comparing patients with two criteria for PCOS and a control group of Caucasian ethnicity, high values of sensitivity and specificity were determined at a threshold level of FNPO ≥ 12, while D. Dewailly et al. [19] and M.E. Lujan et al. [20] on a similar sample proposed an ULN for FNPO at a level of ≥ 19 and ≥ 26, respectively. The authors substantiated their results by the high sensitivity of modern ultrasound devices and assumed that the use of a lower threshold for assessing FNPO would lead to overdiagnosis of PCOM in young women with a high ovarian reserve.

Our study demonstrated the high performance of FNPO as a marker of PCOM in women of the Asian subpopulation. The best compromise between sensitivity and specificity was achieved with a threshold for FNPO ≥ 11, which is consistent with the results of estimating the ULN for FNPO in Asian women obtained by other researchers: ≥ 10 [22], > 11.25 for young women under 35 years old and > 10.75 for the older age group [27].

For women of mixed ethnicity, according to the results of our study, the ULN for OV was ≥ 8 cm^3, and for FNPO ≥ 9. The data obtained are unique, since similar studies have not been previously conducted in populations of women of mixed (Caucasian-Asian) ethnicity.

The obtained values can be explained by the contingent included in the study (a small number of young women aged 18–25 years) with an average age of participants in the 18–35 group of 28.83 ± 3.94 years. It is known that the period of the most rapid loss of follicles in both women with PCOS and those without PCOS occurs between the ages of 18 and 30 years [28].

Strengths of the study. Overall, given the high sensitivity and specificity values for the ovarian volume and antral follicles number cut-off levels which we determined both in the overall population and depending on ethnicity, we can conclude that the diagnostic ability of the studied variables is satisfactory. An important advantage of our study is that all participants were recruited from a non-selective multiethnic population of women with comparable socio-demographic characteristics and living in the same geographic conditions [12, 29]. We consider the population of Eastern Siberia as an ideal model for studying the characteristics of PCOM in Caucasians and Asians using ethnically dependent normative ranges of androgens [30].

Another advantage is that testosterone measurement for the diagnosis of hyperandrogenemia in our study was performed using the HPLC-MS/MS method [31], while our previously developed population-specific standards for assessing hirsutism were used.

Limitations of the study. In our study, the examination of patients was carried out using mid-range ultrasound equipment. At the same time, mid-range ultrasound devices are the most frequently used equipment in practical healthcare and our data are as close as possible to the realities of routine medical practice.

CONCLUSION

For a differentiated approach to identifying PCOM in women of reproductive age of different ethnic groups, it is necessary to take into account the normative values we have developed: thus, in Caucasians, it is advisable to diagnose PCOM with an ovarian volume of 9 cm^3 and/or FNPO ≥ 12; in women of the Asian population, an ovarian volume of 7 cm^3 and/or FNPO ≥ 11 is diagnostically significant, and for women of mixed ethnicity – 8 cm^3 and/or ≥ 9, respectively. When assessing age aspects, we noted the absence of statistically significant differences in the upper normal values of OV and FNPO in women aged 18–34 and 35–44 years in all ethnic groups.

Conflicts of interest

No potential conflict of interest relevant to this article reported.

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RISK FACTORS FOR DIMINISHED OVARIAN RESERVE IN WOMEN: CURRENT STATE OF THE PROBLEM

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ABSTRACT

Ovarian reserve is the basis of female fertility. The main markers of ovarian reserve are the level of anti-Mullerian hormone and the number of antral follicles. In addition to the natural age-related loss of follicles, many women experience a premature diminished ovarian reserve associated with a number of factors. This can be caused by both various diseases and environmental factors, lifestyle, and social aspects.

The aim of this review *was to examine the influence of external factors on the ovarian reserve and women fertility. A systematic analysis of data from modern scientific literature, domestic and foreign sources was carried out. The search involved such resources as PubMed, MEDLINE, Science Direct, eLibrary, Scopus, Cyberleninka. A detailed analysis of the influence of environmental pollution, lifestyle (sleep, nutrition, physical activity), previous surgeries, bad habits, obesity, psychological and social factors on the ovarian reserve and reproductive function of women was carried out. Significantly diminished ovarian reserve was noted with low sleep quality, excessive physical activity, and an unbalanced diet poor with animal proteins. Regular consumption of alcohol, smoking and exposure to certain chemical environmental pollutants cause premature follicle apoptosis and the onset of menopause. Circadian dysrhythmia, chronic stress and obesity can lead to the ovarian menstrual cycle disorders and the development of infertility in women. Previous parovarium surgeries are a significant risk factor for diminished ovarian reserve. Further population-based studies are needed to determine the precise mechanisms of influence of various factors on female fertility.*

Key words: *ovarian reserve, follicles, menopause, fertility, infertility, lifestyle, smoking, environmental factors*

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ФАКТОРЫ РИСКА СНИЖЕНИЯ ОВАРИАЛЬНОГО РЕЗЕРВА ЖЕНЩИН: АКТУАЛЬНОЕ СОСТОЯНИЕ ПРОБЛЕМЫ

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РЕЗЮМЕ

Овариальный резерв – основа женской фертильности. Основными маркерами овариального резерва являются уровень антимюллерова гормона и количество антральных фолликулов. Помимо естественной возрастной потери фолликулов, многие женщины сталкиваются с преждевременным снижением овариального резерва, связанным с множеством факторов. Причинами данного явления могут стать как различные заболевания, так и факторы внешней среды, образ жизни, социальные аспекты.

***Целью настоящего обзора** стало рассмотрение влияния внешних факторов на овариальный резерв и фертильность женщин. Проведён систематический анализ данных современной научной литературы, отечественных и зарубежных источников. В поиске были задействованы такие информационные ресурсы, как PubMed, MEDLINE, Science Direct, eLibrary, Scopus, Cyberleninka. Проведён подробный анализ влияния загрязнения окружающей среды, образа жизни (сон, питание, физические нагрузки), перенесённых операций, вредных привычек, ожирения, психологических и социальных факторов на овариальный резерв и репродуктивную функцию женщин. Отмечено существенное снижение овариального резерва при низком качестве сна, чрезмерных физических нагрузках, несбалансированной диете, обеднённой животными белками. Регулярное употребление алкоголя, курение табака и воздействие некоторых химических загрязнителей окружающей среды приводят к преждевременному апоптозу фолликулов и наступлению менопаузы. Циркадианная дизритмия, хронический стресс и ожирение могут приводить к нарушениям овариально-менструального цикла и развитию бесплодия у женщин. Перенесённые операции на придатках являются существенным фактором риска снижения овариального резерва. Для выяснения точных механизмов воздействия различных факторов на фертильность женщин необходимы дальнейшие популяционные исследования.*

Ключевые слова: *овариальный резерв, фолликулы, менопауза, фертильность, бесплодие, образ жизни, курение, экологические факторы*

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INTRODUCTION

According to Russian and international associations of human reproductive science and embryology, the incidence of infertility among married couples has increased in recent decades, while the average age of its onset has decreased. Today, infertility in couples is a common problem among young women and men. Environmental pollution, various somatic diseases, lifestyle, psychological stress, and social aspects are factors that negatively affect reproductive function. While men can completely renew their sperm pool in a relatively short period of time, for women the impact on eggs can be irreversible in some cases and significantly affect their ability to conceive [1].

The reproductive lifespan of women begins at puberty and ends at menopause, after the depletion of the ovarian follicle reserve [1]. Ovarian reserve is the basis of female fertility. In clinical practice, it is usually assessed using parameters such as the woman's age, antral follicle number, Anti-Müllerian hormone (AMH) level, and basal levels of sex hormones, including follicle-stimulating hormone (FSH), luteinizing hormone, and estradiol [2].

Numerous studies have identified a number of determining factors that can have a negative impact on ovarian reserve and fertility in women, but most factors should be considered in combination due to their interaction with each other and the complexity of interpretation [2]. This review examines in detail such risk factors for decreased ovarian reserve as environmental, social, psychological factors, lifestyle (sleep, nutrition, unhealthy habits, physical activity), obesity, and previous surgeries on the pelvic organs. Multicenter and population-based scientific studies are needed to study the impact of each factor in more detail.

ENVIRONMENTAL FACTORS

A growing number of epidemiological studies and experimental animal models suggests that exposure to a number of ubiquitously distributed reproductively toxic environmental chemicals (RTECs) may contribute to earlier menopause and premature ovarian failure. These include lead, mercury, toluene, perchlorates, bisphenol A and some phthalates, pesticides, perfluorochemicals, polychlorinated biphenyls, and polybrominated diphenol ethers [3, 4].

The negative impact of toxic substances on the ovaries can occur in three ways: disruption of the endocrine system; induction of oxidative stress; epigenetic changes [4, 5]. Depending on the moment of exposure to ovarian ontogenesis, the effect of RTECs on ovarian function can be temporary or permanent. This effect causes the greatest harm prenatally, affecting the ontogenesis of the ovaries in the female fetus at the time of follicle development, and affects the reproductive function of girls whose mothers were exposed to RTECs during pregnancy [6].

Environmental toxicants can trigger the formation of reactive oxygen species such as free radicals, oxygen ions, and hydrogen peroxide in the body cells. Oxidative stress is induced by cellular oversaturation with reactive oxygen species (ROS), when cellular machinery and endogenous antioxidants that regulate ROS levels are overloaded by exogenous reactive oxygen species. There is strong evidence that ROS are involved in the initiation of antral follicle apoptosis [7]. The role of oxidative stress in the pathogenesis of premature ovarian failure has been confirmed [7, 8].

Epigenetic modifications can occur as a result of environmental pollutants affecting DNA methylation, which can alter ovarian function. For example, bisphenol A, which is used in the manufacture of plastics, can destroy methyl groups and inhibit enzymes responsible for DNA methylation [5].

A number of scientific papers have shown the impact of chemical pollutants of the environment on any stage of follicle formation, as well as on the development of the ovaries in general. Evidence has been found that exposure to RTECs in the prenatal, neonatal, prepubertal periods and even in adulthood leads to disruption of ovarian function and a reduction in the duration of reproductive function in female rodents [3].

Due to their similarity to natural ligands, many environmental compounds have the ability to bind to sex hormone receptors (albeit with lower affinity) and can thus influence either the initial stages of ovarian reserve formation during fetal development or the maintenance of ovarian reserve in adults [9]. Exposure to chemicals can negatively affect ovarian reserve through aryl hydrocarbon or estrogen receptors. Thus, after binding to such an exogenous ligand, the aryl hydrocarbon receptor induces the synthesis of proapoptotic factors that promote follicular atresia.

Major international organizations, including the Endocrine Society [10], the World Health Organization and the International Pollutants Elimination Network [11], and the International Federation of Gynecology and Obstetrics [12], have noted an increase in chemical pollution in recent decades and emphasize the need for more detailed study of chemical impacts on the female reproductive system. For example, a study of the fertility of women living in the Aral Sea basin showed that they had a reduced number of antral follicles number compared to women of the same age living in a more ecologically favorable region [13]. The drainage of the Aral Sea has led to increased levels of various toxicants in the soil, water, and air, namely substances such as pesticides and heavy metals, which contribute to the development of various diseases among the local population. Cross-sectional studies show that exposure to certain environmental chemicals can compromise women's reproductive health and in some cases correlate with earlier onset of menopause [3]. However, in humans, adverse effects from chemical exposure are usually diagnosed after several years or decades, making it difficult

to determine the relationship between RTECs exposure and reproductive health [6].

Further epidemiological and experimental studies are needed to determine the direct and indirect effects of environmental chemical pollutants on ovarian function and to better understand their mechanisms of action.

LIFESTYLE

Sleep, nutrition and physical activity. Recently, more and more attention has been paid to the influence of a woman's lifestyle, diet, sleep and wakefulness patterns, and physical activity on the women reproductive function. Today, more and more women are into various diets, therapeutic fasting, extreme physical activity, and equally with men choose «difficult professions» with shift work schedules and high stress levels. Researchers have shown that intense physical exercise and poor sleep quality have a significant negative impact on the antral follicles number in women aged 31 to 36 years [14]. Excessive physical activity leads to menstrual irregularities, decreased ovulation frequency, disruption of endometrial development, and in some cases, amenorrhea and subfertility. The probable cause of these conditions is dysregulation of the hypothalamic-pituitary-ovarian axis due to a decrease in systemic stimulating signals for the release of gonadotropin-releasing hormone and impaired secretion of gonadotropins [15].

Adherence to a certain type of diet in some cases can also affect the ovarian reserve. Thus, it was shown that the transition to an exclusively plant-based diet without animal components, containing sugar and not supplemented with folic acid and vitamin B12, is associated with the risk of early menopause, while a "healthy" plant-based diet, additionally enriched with a vitamin complex, did not affect the timing of menopause [16]. In another study, the authors conclude that giving up vegetarianism contributes to longer-term preservation of fertility [17]. In a sample of more than 2,000 women, a relationship between regular consumption of dairy products and a reduced risk of early menopause was found [18]. Today, this is of particular importance, since adherence to veganism, which entails the rejection of dairy products, is widespread among modern women.

Long-term fasting can also have a negative impact on women's reproductive function. Thus, a diet violation leads to a decrease in the amplitude of the pulse secretion of thyroid-stimulating hormone: a decrease in its basal concentration in the blood serum, as well as a decrease in its peak level at night [19], which in turn leads to dysfunction of the thyroid gland and indirectly affects female fertility.

Misalignment of the biological clock with the sleep-wake cycle as a result of long and frequent flights, shift work, and stressful situations underlies the phenomenon of circadian dysrhythmia [20]. Circadian dysrhythmia is associated with a higher body mass index (BMI), obesity [21], and an increased risk of developing metabolic syndrome and type 2 diabetes [22]. The production of thyroid-stimulating hormone is also associated with circadian rhythms. Thus, in a study that included an examination of more than 5,000 patients, an increased risk of subclinical hyperthyroidism was found in patients who slept little (less than 7 hours per day), in contrast to people who slept more than 8 hours per day [23]. Hypothyroidism, in turn, is often the cause of endocrine infertility, as it is accompanied by increased prolactin levels, anovulatory menstrual cycles, luteal phase defects and changes in sex hormone levels [24, 25].

Circadian dysrhythmia can also have a direct effect on the secretion of reproductive hormones, leading to anovulation, and in some cases is combined with insulin resistance [26].

Smoking. According to numerous scientific studies, tobacco smoking has a number of consequences that are potentially harmful to a woman's reproductive function, as it can have an adverse effect on ovarian function, cause mutations in germ cells, and increase the risk of early miscarriages and adverse outcomes of assisted reproductive technology cycles [27].

Tobacco toxins have been shown to have a detrimental effect on the follicle pool and increase the rate of follicular atrophy and atresia, leading to a decrease in their number, changes in sex hormone levels, and ultimately to a decrease in fertility [2, 28]. Smoking has been found to be associated with a decrease in estradiol and estriol concentrations, an increase in testosterone levels, and a tendency toward an increase in serum FSH levels [29].

Other negative effects of tobacco toxins include increased rates of apoptosis as well as necrosis in various human tissues and increased apoptosis in primordial germ cells differentiated from human stem cells *in vitro* [30]. These effects may also manifest as increased rates of follicular apoptosis or ovarian dysfunction. Indeed, polycyclic aromatic hydrocarbons in cigarette smoke are toxic to follicles, which has been demonstrated in both animal models and numerous human studies [2, 28].

Smoking is associated with an increased rate of follicle loss in Caucasian women. In 14,620 middle-aged women in the Study of Women's Health across the Nation, smoking was associated with an earlier age at menopause (0.3–1.2 years earlier), but there was no dose-response effect with the number of cigarettes smoked per day [28]. Data were obtained indicating a higher incidence of early ovarian failure syndrome, reduced fertilization and implantation rates, pregnancy rates, and live birth rates in assisted reproductive technology programs in women who smoke [31, 32].

Among women of reproductive age, users of popular e-cigarettes have been found to have higher levels of several markers of toxin exposure (including nicotine metabolites, the tobacco carcinogenic biomarker

NNAL (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol), lead, and volatile organic compounds) [33]. This study showed that exclusive use of e-cigarettes may reduce exposure to some toxins compared with smoking combustible cigarettes, but e-cigarettes result in greater exposure to toxicants compared with quitting smoking completely. Even reduced levels of tobacco toxins in e-cigarette users may lead to potentially harmful reproductive health consequences [33, 34].

Alcohol is also one of the risk factors for decreased female fertility. The biological mechanisms of the negative impact of alcohol on reproductive function are still poorly understood. One hypothesis is that alcohol can reduce fertility by changing the concentration of endogenous hormones [35]. Another theory suggests a direct negative effect of alcohol on egg maturation, early blastocyst development, and implantation [36].

A group of researchers from Denmark showed on a cohort of more than 6 thousand women that regular consumption of 14 servings of alcohol per week is associated with an increase in estrogen concentration, which reduces the secretion of FSH, resulting in the suppression of folliculogenesis and ovulation, as well as the amount of bioavailable forms of estrogen. In this work, the «serving» of alcohol was calculated depending on its strength (beer – 330 ml, wine – 120 ml, hard liquor – 20 ml) [35].

Alcohol consumption is combined with the consumption of other toxins present in alcoholic beverages, such as ethyl carbamates, tetra-beta-carbolines or food additives, which have a toxic effect on oocytes, including through the development of oxidative stress, accelerating follicular apoptosis [36]. To date, researchers agree on the negative impact of alcohol consumption on female reproductive function.

Data from univariate and multivariate analyses [2] conducted in China on a cohort of 1,513 women showed a significant negative correlation between alcohol consumption and AMH levels in both the 20–30 and 31–36 age groups. In 2017, a group of scientists from China conducted a dose-response meta-analysis based on 19 studies with a total of more than 98 thousand women and established a linear dose-dependent relationship between a decrease in the ability to spontaneously conceive and an increase in the dose of alcoholic beverages consumed per 12.5 g of ethanol per day (relative risk – 0.98; 95% confidence interval: 0.97–0.99) [36]. The data from these studies demonstrate that abstinence from alcohol is important for maintaining reproductive function, which is especially important for young women under 36 years of age [2].

OBESITY

Obesity is a disease that is closely related to a person's lifestyle and is currently becoming increasingly global in scale. Today, over 2 billion people worldwide suffer from excess body weight and obesity [37]. Obesity has a negative impact on a woman's reproductive function, associated with changes in the regulation of the hypothalamic-pituitary-ovarian axis, a decrease in the quality of eggs and a disruption of the physiological processes of the endometrium, which has been proven in a cohort of over 47 thousand women from Denmark and is confirmed by data from Russian scientists [38, 39].

Obesity leads to ovarian-menstrual cycle disorders and an increase in the number of anovulatory cycles. It has been proven that decreased fertility in obese patients may be associated, in particular, with a decrease in ovarian reserve [40].

Depending on the degree of obesity, patients experience a progressive decrease in ovarian reserve parameters: AMH concentration levels, a decrease in ovarian volume and antral follicles number, in parallel with an increase in serum testosterone concentration [39, 40]. Other studies have shown that women of reproductive age with abdominal obesity against the background of insulin resistance have a decrease in ovarian reserve and accelerated aging processes of the reproductive system [41, 42]. Some researchers have observed an inverse correlation between AMH levels and BMI, including in polycystic ovary syndrome, which is also often associated with infertility, as shown in a sample of 489 women with infertility examined in the USA [43].

It is interesting that the body mass index in late adolescence is an important prognostic factor for fertility in the reproductive period: for example, women in the Nurses' Health Study, conducted in Boston, USA on a cohort of 1950 people [44], who were underweight at 18 years of age (BMI < 18.5 kg/m^2), needed on average 25 % more time to get pregnant than women with normal weight in adolescence. This suggests that the prepubertal period is a critical time for programming the implementation of reproductive function [15]. Large randomized multicenter clinical trials are needed to study in more detail the role of body mass index in reducing ovarian reserve, including women of different ages and races and comprehensively assessing the influence of concomitant factors.

LATE PREGNANCY PLANNING

One of the main factors influencing the ovarian reserve is age. Today, there is a worldwide trend towards an increase in the age of women giving birth for the first time. In Russia, from 1990 to the present, the average age of a mother at the time of the birth of her first child has increased from 22 to 26 years [45]; and in the USA, over the past 30 years, the proportion of women giving birth over 35 years has increased from 8 % to 18 % [46]. With age, women's fertility decreases, and the optimal age for the birth of a first child is from 20 to 30 years, while after 35 years, the ability to conceive spontaneously decreases, while the risk of giving birth

to a child with congenital malformations or chromosomal pathology increases [1].

On the other hand, social, cultural and economic factors, the use of contraception and the availability of assisted reproductive technologies shape the tendency in society to plan pregnancy at the age of 35 and older [47]. A study conducted in Spain on a sample of 326 women revealed that women who were in stable relationships became mothers at an older age (31.83 ± 0.29 years) than unmarried women. The average time required to achieve pregnancy increased with increasing maternal age and averaged 24 months for women around 35 years of age compared to 3 months or less for women around 29 years of age. Women 35 years of age and older were more likely to require medical assistance to achieve pregnancy [47]. At the same time, age-related decrease in ovarian reserve in combination with concomitant diseases (obesity, diabetes, etc.) often leads to the fact that by the time of pregnancy planning, a woman has less and less chance of spontaneous conception [1, 46].

PSYCHOLOGICAL FACTORS AND STRESS

Factors that have an adverse effect on the function of the endocrine system include chronic stress. Individual perception of increased physical and mental stress can affect the regulatory function through an increase in the level of corticosteroid hormones, and they in turn affect the functioning of the hypothalamic-pituitary-ovarian axis [48].

In 2015, an experimental study was published in animal models showing that stress-induced changes in neuroendocrine and immune responses lead to premature ovarian failure and early menopause [49]. The latest study by Chinese scientists has caused resonance: in animal models, it was shown that prolonged three-week stress consistently reduced plasma AMH and estradiol concentrations, induced loss of primordial and preantral follicles, increased granulosa cell apoptosis in growing follicles, and ultimately reduced litter size in rats. Based on these results, scientists suggest that in humans, chronic psychological stress also causes loss of ovarian reserve by accelerating activation of primordial follicles and destruction of growing follicles, leading to ovarian exhaustion and decreased fertility [50].

Some population studies have shown that the discovery of infertility in a family is a stress factor in itself and can lead to depression. According to the literature, infertile men and women are perceived by society as inferior, socially maladjusted, and negative social attitudes aggravate their plight. According to researchers from India, for both men and women, factors such as weak support from a spouse, financial constraints, and social maladjustment in the first years of marriage are stress factors in themselves [51]. Thus, patients find themselves in a "vicious circle" when infertility causes stress, which aggravates the course of the disease.

In the study of emotional state, a scale for assessing positive and negative affect is used [52]. Thus, a high level of positive affect is defined as a state of pleasant involvement, high energy and full concentration as opposed to despondency and lethargy. In a study of female fertility and stress on a sample of more than 1000 women from the USA, it was shown that women with a low level of positive affect may experience an accelerated decrease in antral follicle number, and, conversely, high positive affect may be a protective factor mitigating the negative impact of psychological stress on antral follicle number [53].

PELVIC ORGANS SURGERIES

Pelvic organ surgeries have a significant negative impact on the ovarian reserve, since surgical intervention involves the removal of part of the ovarian tissue and disruption of the blood supply to the reproductive organs. A univariate and multivariate analysis conducted in China on a cohort of 8,323 women confirmed that appendage surgeries have a significant negative impact on such ovarian reserve indicators as antral follicle number and AMH in women aged 20–30 and 31–36 years [42].

Appendage surgeries lead to disruption of the blood supply to the ovaries and maturation of antral follicles, and removal of ovarian tissue during enucleation of benign and malignant ovarian neoplasms leads to a decrease in the amount of ovarian cortex and, as a consequence, to a decrease in the ovarian reserve [54]. Many researchers associate the main traumatic effect on the ovary with the bipolar coagulation method, often used in surgical interventions, however, in the work of Korean researchers on a group of 125 patients [55], it was shown that the level of Anti-Müllerian hormone decreases after appendage surgeries, regardless of the method used in surgical hemostasis. It is also noted that repeated interventions on the ovaries are significantly more traumatic for the ovarian reserve than primary ones [56], and the postoperative decrease in AMH occurs much more intensively with initially reduced levels of this hormone. This should be taken into account when counseling and planning appendage surgery for women planning to have children.

Moreover, surgical interventions on the uterus do not have adverse consequences for the ovarian reserve; the antral follicles number or the level of Anti-Müllerian hormone do not change after myomectomy or other surgeries on the uterus [57].

Thus, when planning surgical intervention on the uterine appendages in women of reproductive age, it is necessary to conduct a thorough assessment of the ovarian reserve, as well as discuss possible methods of preserving fertility, for example, by performing superovulation stimulation and cryopreservation of oocytes before surgery, or alternative methods of surgical treatment, such as reduction

therapy for endometrioid ovarian cysts, instead of their resection, before entering into in vitro fertilization programs [58]. However, advocating for the preservation of the ovarian reserve of each woman of reproductive age, one should not lose sight of oncological vigilance, that is, in each specific case, use an individual approach.

CONCLUSION

The female reproductive system is a delicate mechanism that is influenced by many factors. Changes at any stage of gametogenesis can negatively affect the ability to conceive. Life in the modern world, environmental problems, social behavioral trends, psychological factors leave a certain imprint on the reproductive function in general and the ovarian reserve in particular. Although female fertility has a certain ability to adapt to a new lifestyle, environmental pollution, social behavior and other factors, age-related risk factors, surgical interventions on the appendages, intense physical activity, normalization of work and rest, as well as the use of toxins should be taken seriously. Scientific research shows that female fertility is more susceptible to the effects of external factors with age [2].

The ways to solve the problem of the risk of reducing the ovarian reserve include conducting conversations, education and competent prospective counseling of teenage girls and women of young reproductive age, which can prevent the negative impact of a number of factors and adjust the lifestyle and reproductive plans of women both in our country and around the world. If the influence of environmental factors is not always possible to identify and correct in time, then minimizing the impact of tobacco and alcohol toxins is possible for women striving to give birth to a healthy child.

The wide modern possibilities of "delayed childbearing" associated with cryopreservation of oocytes, embryos and ovarian tissue can partially solve the problem of age-related loss of ovarian reserve and late implementation of a woman's reproductive plans.

A balanced approach to the choice of method, volume and timing of surgical interventions on the uterine appendages, drawing up a correct plan for managing patients together with a gynecological surgeon and a reproductive specialist will help minimize the consequences of these surgeries in relation to the fertility of patients.

Giving up unhealthy habits, choosing a healthy lifestyle, stabilizing the sleep and wakefulness patterns, regular moderate physical activity and a balanced diet are the basis for the normal functioning of the hypothalamic-pituitary-ovarian system, and therefore ensuring the long-term preservation of normal reproductive function in women.

Conflicts of interest

No potential conflict of interest relevant to this article reported.

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BIOLOGY AND MEDICAL BIOLOGY

LIGAND-ASSOCIATED ACTIVATION OF VITAMIN D RECEPTORS AND POTENTIAL POINTS OF APPLICATION OF ITS EFFECTS IN THE MORPHOGENESIS OF IMMUNE INFLAMMATION: LITERATURE REVIEW

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ABSTRACT

According to recent data, vitamin D is classified as a substance with hormonal activity, which, in addition to classical, has "non-classical" effects caused by the complex relationship between vitamin D and effector cells of the immune system. This relationship is based on the expression of the vitamin D receptor (VDR) on immune cells, which is encoded by the corresponding VDR gene. Vitamin D receptor specifically binds the active form of vitamin D (1,25(OH)$_2$D$_3$). As a result, a D$_3$-VDR complex is formed, which mediates the effects of vitamin D through the formation of intracellular signaling pathways that transform the activity of certain target genes. However, it is not entirely clear how vitamin D realizes its effects at the cellular and receptor levels. According to the literature, studies of recent decades have revealed a significant role of vitamin D and immune checkpoint receptors (PD-1 (programmed cell death), PD-L (PD ligand), CTLA (cytotoxic T-lymphocyte associated protein)) in autoimmune diseases. This review outlines possible mechanisms for the interconnection of these pathways. A deeper understanding of the intercellular interactions mediated by ligand-associated activation of vitamin D receptors, D$_3$-VDR complex and immune checkpoint receptors (PD-1, PD-L, CTLA) in inflammation may become the basis for the development of new strategies for the diagnosis, prognosis and treatment of various diseases.

Key words: *vitamin D, VDR, immune granuloma, immune checkpoint proteins PD-1, PD-L, CTLA*

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ЛИГАНД-АССОЦИИРОВАННАЯ АКТИВАЦИЯ РЕЦЕПТОРОВ ВИТАМИНА D И ПОТЕНЦИАЛЬНЫЕ ТОЧКИ ПРИЛОЖЕНИЯ ЕЁ ЭФФЕКТОВ В МОРФОГЕНЕЗЕ ИММУННОГО ВОСПАЛЕНИЯ: ОБЗОР ЛИТЕРАТУРЫ

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РЕЗЮМЕ

Согласно последним данным, витамин D относят к веществам с гормональной активностью, который, помимо классических, имеет «неклассические» эффекты, обусловленные наличием сложной взаимосвязи между витамином D и эффекторными клетками иммунной системы. Данная взаимосвязь обусловлена экспрессией рецептора витамина D (VDR, vitamin D receptor) на иммунных клетках, который кодируется соответствующим геном VDR. Рецептор витамина D специфически связывает активную форму витамина D ($1,25(OH)_2D_3$). В результате образуется сложный комплекс D_3-VDR, который опосредует эффекты витамина D путём образования внутриклеточных сигнальных путей, трансформирующих активность определённых таргетных генов. При этом до конца не ясно, каким образом витамин D реализует свои эффекты на клеточном и рецепторном уровнях. По данным литературы, исследования последних десятилетий выявили значимую роль витамина D и рецепторов иммунных контрольных точек (PD-1 (programmed cell death), PD-L (PD ligand), CTLA (cytotoxic T lymphocyte associated protein)) в аутоиммунных заболеваниях. В этом обзоре излагаются возможные механизмы взаимосвязи данных путей. Более глубокое понимание межклеточных взаимосвязей опосредованных лиганд-ассоциированной активацией рецепторов витамина D, комплекса D_3-VDR и рецепторов иммунных контрольных точек (PD-1, PD-L, CTLA) в воспалении может стать основой для разработки новых стратегий диагностики, прогноза и лечения различных заболеваний.

Ключевые слова: *витамин D, VDR, иммунная гранулема, белки иммунных контрольных точек PD-1, PD-L, CTLA*

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INTRODUCTION

Until recently, the role of vitamin D was considered only from the standpoint of its effect on calcium and phosphorus metabolism in the body. However, over the past 15 years, new effects of vitamin D, such as neuroregenerative, neurosteroid and immunomodulatory effects have been identified [1]. Nevertheless, the role of vitamin D (VD), one of the most important vitamins in the body, remains insufficiently studied due to the pleiotropic nature of its effects. Modern data indicate a close relationship between vitamin D_3 (calcitriol) and immune cells [2], as well as autoimmune diseases [3, 4], which allows us to consider it as an important regulatory link in a complex system of intercellular interactions. On the other hand, studies in recent decades have also revealed a significant relationship between immune checkpoint proteins (ICPs) and immune cells [5, 6], which is manifested in fine regulation of the balance between tolerance and immunopathology. It is possible that these links in immune regulation are interconnected. However, due to limited information, which confirms the relevance of this problem, this literature review presents key information concerning modern concepts of the structural and functional organization of the VD gene and receptor (VDR, vitamin D receptor), «non-classical» effects of VD in the morphogenesis of immune inflammation, potential relationships between the D_3-VDR complex and immune checkpoint receptors and possible points of application of this relationship within the framework of granulomatous inflammation.

1. Modern concepts of the structural and functional organization of the gene and receptor of vitamin D

The literature contains a fairly broad range of data on the role of VD, the *VDR* gene, and the VDR receptor in the development of various pathological processes and diseases. To some extent, this is due to the fact that, according to modern scientific knowledge, VD has endocrine functional activity, similar to a hormone [7], which attaches to and interacts with its specific receptors (VDR) on various cells, thus exerting numerous effects on various body systems.

The *VDR* or *NR1I1* gene (nuclear receptor subfamily 1 group I member 1) is encoded by a relatively large gene (> 100 kb) and is localized on the submetacentric 12^{th} chromosome, its long arm (12q12-q14). The *NR1I1* gene has about 60 thousand nucleotide pairs and consists of 14 exons and intermediate introns. The *VDR* gene can be divided into two regions: coding and noncoding. The noncoding region of the *VDR* gene includes 6 of the 14 exons: 1A, 1B, 1C, 1D, 1E and 1F. The remaining 8 exons are part of the coding region of the *VDR* gene, which encodes information about the primary structure of the VDR gene protein, consisting of 4 functional domains [8].

The *NR1I1* gene exerts its effects through genomic (nuclear) and extragenomic mechanisms.

The genomic pathway leading to changes in gene transcription takes from several hours to several days [9]. The *VDR* gene encodes the nuclear VDR, which, together with the retinoic acid receptor (RAR), retinoid X receptor (RXR), and peroxisome proliferator-activated receptor (PPAR), is part of the second group of the nuclear receptor (NR) family [10]. The first group includes estrogen, androgen, progesterone, and mineralocorticoid receptors [11]. The second group of receptors can form heterodimers with each other (e.g., VDR-RXR), and also function and exert effects through interaction with certain ligands [10, 11].

In addition to the classical target cells (enterocytes, parathyroid cells, and nephrocytes), which are directly involved in maintaining calcium homeostasis, the *VDR* gene is also expressed in immune system cells (monocytes, macrophages, dendritic cells, lymphocytes) [12]. In addition, *VDR* gene expression was detected in brain neurons, cardiomyocytes, smooth muscle cells, vascular endothelium, breast cells, prostate cells, skin, and other organs [13].

The extragenomic pathway and its effects, according to the latest updated data, are mediated by the synthesis of secondary messengers (cyclic adenosine monophosphate, inositol triphosphate, etc.), which are associated with the steroid receptor MARRS (membraneassociated rapid response steroid), which contributes to a faster response – from several seconds to several minutes [14], – in contrast to the genomic pathway. Thus, the extragenomic effects of VD are realized much faster than the genomic ones.

Upon entering the cell, VD3 binds to VDR. VDR consists of 427 amino acids and has 4 main functional domains:

1. the highly variable N-terminal A/B domain is responsible for the transactivated functions of VDR induced by ligands, but its structural elements are poorly defined;
2. the DNA-binding domain (DBD) is the central DNA-binding domain;
3. the ligand-binding domain (LBD) is the ligand-binding domain at the C-terminus;
4. the flexible region connecting the DBD and LBD is called the hinge domain.

After binding to VD3, VDR forms an active D_3-VDR complex, which is transported across the nuclear membrane directly into the cell nucleus [15].

An even more complicated complex is then formed by binding of the D3-VDR complex to one of the three retinoid X receptors (RXRα, RXRβ, RXRγ). Subsequently, this D_3-VDR-RXR complex binds to VDREs (vitamin D response elements) on the surface of the DNA of target genes. VDREs are multiple regions of the genome whose activity is under the control of D_3. After binding of D_3-VDR-RXR to VDREs, activation or, conversely, suppression of the corresponding target genes occurs [16]. Thus, an important conclusion can be made that calcitriol mediates its effects through ligand-associated activation of vitamin D_3 receptors (D_3-VDR complex).

VDR is mainly concentrated in the nucleus, cytosol, and cytoplasmic membrane. VDR specifically binds the active form of VD ($1,25(OH)_2D_3$) and mediates its actions. Thus, the effects of VD are directly due to a complex interaction with its receptor VDR. This D_3-VDR complex cannot be considered separately, since it functions as a single mechanism. The absence, deficiency, or structural defect of any of the components of the complex (D_3 or VDR) disrupts the functioning of its components and the implementation of its effects – both genomic and extragenomic. A striking example of disruption of the D_3-VDR complex is VD deficiency in adults, usually associated with the development of osteoporosis and osteomalacia, as well as hereditary mutations of the VDR gene, which lead to the development of vitamin D-resistant rickets in children, characterized by muscle weakness, growth retardation, bone deformities, and secondary hyperparathyroidism.

It is currently known that ligand-associated activation of VD receptors has multiple effects, since VDR receptors are expressed in many tissues of the body [17]. The ubiquitous distribution of VDR reflects its pleiotropic biological activity [18]. In the nuclei of target cells, an active nuclear D_3-VDR complex functions, which controls the transcription of about 3% of the entire human genome. In addition, in the cytoplasmic membranes of cells, the D_3-VDR complex functions as a modulator of gene expression and a coordinator of a number of important biochemical processes [19].

Many genes with the expression regulated by ligand-associated activation of VDR have been described: for example, activation of the *DEFB4A* and *CAM*P genes encoding cathelicidin and defensin-β2 [20], as well as suppression of the IL-2 gene activity in activated T lymphocytes [21]. The listed genes are located "far" from chromosome 12 encoding VDR, but, nevertheless, are under the control of vitamin D. It is possible that the genes encoding ICT proteins are also under the influence of vitamin D. In addition, the D_3-VDR-RXR complex suppresses gene expression and synthesis of interferon γ (IFN-γ), which is a key cytokine of Th1 lymphocytes in humans, by competitively inhibiting the NF-kB factor (nuclear factor kappa-light-chain-enhancer of activated B cells) [22]. Thus, by increasing or decreasing the expression level of various genes, the D_3-VDR-RXR complex implements the "classical" and "non-classical" effects of VD.

At present, the biological effects of VD can be divided into "classical" (calcitropic, regulating phosphorus-calcium metabolism) and "non-classical" (regulation of metabolism and cell cycle, anti-inflammatory, antibacterial, antitumor, antihypertensive effects). Moreover, VD is directly involved in the regulation of the functioning of immune system elements.

2. "Non-classical" effects of vitamin D in the morphogenesis of immune inflammation

Morphogenesis of immune inflammation implies strengthening or weakening of the immune response during inflammation (including granulomatous inflammation) as a result of changes in the receptor and cytokine profile, cellular subpopulations, including under conditions of ligand-associated activation of vitamin D receptors.

Vitamin D deficiency, according to the latest data from world literature, has become a new pandemic of the 21st century, which is especially pronounced in northern latitudes, due to a deficiency of ultraviolet (UV) radiation in residents of megacities. In addition, vitamin D is pathogenetically associated with a progressive increase in the prevalence of various diseases, including autoimmune diseases such as type 1 diabetes mellitus, bronchial asthma, atopic dermatitis, alopecia areata, systemic lupus erythematosus (SLE), psoriasis, etc. [23]. This is far from a complete list of all diseases that are associated with VD deficiency. In particular, in northern latitudes, the prevalence of multiple sclerosis and rheumatoid arthritis is inversely proportional to the level of UV radiation, which may indirectly indicate the participation of VD in the manifestation and pathogenesis of these diseases [24]. Moreover, according to B. Terrier et al., vitamin D supplements statistically significantly increased the number of Treg lymphocytes and decreased the number of Th1 and Th17 cells [25]. However, the mechanism of the relationship between VD deficiency and autoimmune processes underlying the above-mentioned diseases is not fully understood.

Current data indicate that VD suppresses acquired immunity but stimulates innate immunity. The first evidence that VD is a significant stimulator of innate immunity may be data on the treatment of tuberculosis with fish oil [26], as well as the synthesis of antimicrobial peptides such as defensin-β2 and cathelicidin [27]. In addition, defensin-β2 transcription is directly activated by the D_3-VDR-RXR genomic complex [27, 28]. For example, cathelicidin gene expression is enhanced after pathogen recognition by TLRs (toll-like receptors) as a result of the interaction of mature monocytes with *Mycobacterium tuberculosis*, thus promoting increased synthesis and secretion of 1α-hydroxylase and VDR [29]. These studies provide more detailed explanations of the mechanisms which help VD potentiate the antimicrobial action of monocytes and macrophages, which are key effector cells in the fight against pathogens such as *Mycobacterium tuberculosis*.

The level of VDR expression changes dynamically during the formation and maturation of various effector cells of the immune system. On the one hand, naive T lymphocytes are characterized by a relatively low level of VDR expression, while mature forms of T lymphocytes are distinguished by a high level of VDR expression [30]. On the other hand, monocytes in the process of differentiation into macrophages and dendritic cells (DCs) show, on the contrary, a decrease in the level of VDR expression [31]. Thus, the level of VDR expression, and accordingly, the susceptibility of effector cells of the immune system to VD are manifested differently depending on the degree of cell maturity, which may play a key role in the complex system of regulation

of the immune response, as well as its specificity, reactivity and plasticity.

Peculiarities of the influence of the D_3-VDR complex on innate immune cells: macrophages and dendritic cells

Blood monocytes undergo differentiation into macrophages, which are the main cells of the human immune system, through which interaction and coordination of innate and acquired immunity occurs. Mature macrophages are capable of activating the immune response by chemotaxis, phagocytosis, and presentation of antigen to T-helpers (Th). In particular, macrophages, unlike monocytes, can undergo so-called polarization, i.e. differentiate into two phenotypes (M1 or M2) depending on inducing factors and cytokines. For example, macrophages with the M1 phenotype destroy bacteria, viruses and tumor cells, are formed under the direct influence of lipopolysaccharides, tumor necrosis factor α (TNF-α), IFN-γ, while macrophages with the M2 phenotype destroy extracellular pathogens and are formed upon stimulation of interleukin (IL) 4 and IL-13. Immunogenic macrophages with the M1 phenotype activate the Th1 immune response as a result of the synthesis of a certain spectrum of cytokines, while the tolerogenic phenotype of M2 macrophages shifts the balance of Th cells towards Th2 [32]. In addition, macrophages express VDR, which makes them susceptible to VD [33].

The D_3-VDR complex exerts mainly suppressive effects on monocytes by decreasing the expression of MHC II (major histocompatibility complex), TLR2 and TLR4 molecules, which leads to anergy of further responses. Moreover, ligand-associated activation of VDR reduces the expression level of CD40, CD80, CD86, which promote co-activation and stimulation of the immune response, and also suppresses the synthesis of IL-1α, IL-1β, IL-2, IL-6, IL-12, TNF-α and IFN-γ. Among the activating effects of the D_3-VDR complex, there is an increase in the synthesis of anti-inflammatory cytokines: IL-10, IL-4 and IL-5 [34].

Based on the foregoing, it follows that the D_3-VDR complex inhibits the immunogenic, proinflammatory responses of macrophages with the M1 phenotype, contributing to a decrease in their activity. On the other hand, the D_3-VDR complex polarizes macrophages towards the tolerogenic M2 phenotype.

However, other studies refute the theory that the D_3-VDR complex suppresses macrophages with the M1 phenotype. For example, according to data from N. Wafa et al., exposure to the D_3-VDR complex in the presence of *Pseudomonas aeruginosa* stimulated the synthesis of IL-1β, which increased the M1/M2 ratio [35].

Red bone marrow progenitor cells initially differentiate into immature DCs, which are transformed into mature DCs through migration and phagocytosis of various pathogens. During phagocytosis, antigenic determinants (epitopes) are formed from various microorganisms, which bind to MHC class II in the Bjorkman cleft and are expressed on the cell surface. At the same time, DCs express CD40, CD80, and CD86 (costimulatory proteins) and acquire the ability to migrate to regional lymph nodes, where they present a ready-made MHC-II complex bound to the epitope to Th0 cells [36]. When the D_3-VDR complex acts on immature DCs, effects similar to those of macrophages develop. On the one hand, the level of expression of costimulatory proteins and MHC class II is suppressed, which contributes to a decrease in the synthesis and secretion of IL-12, suppression of antigen presentation on the surface of DCs, and on the other hand, the synthesis of IL-10 is enhanced [37].

The activity of Th1 and Th17 lymphocytes, which play a key role in the pathogenesis of autoimmune diseases, sharply decreased as a result of a decrease in the synthesis of IL-12 and IL-23 by dendritic cells after ligand-associated activation of VDR [38]. Moreover, the D_3-VDR complex inhibits the differentiation of monocytes into DCs and their subsequent maturation [34]. This pattern may explain the reason for the increase in the number of tolerogenic DCs, since they consist to some extent of immature cells [39].

Features of the influence of the D_3-VDR complex on the components of acquired (adaptive) immunity (T- and B-lymphocytes)

The precursor of T lymphocytes, like all formed elements of the blood, is a pluripotent hematopoietic stem cell, and its marker is CD34. From the red bone marrow, early pre-T lymphocytes migrate to the thymus gland, where antigen-independent differentiation of T lymphocytes and the process of so-called "positive" and "negative" selection occur [40].

After selection and exit from the thymus, T lymphocytes, like macrophages, undergo polarization. According to the literature, Th0 lymphocytes can differentiate in one of four directions:

1. Th1 lymphocytes, which are capable of destroying foreign pathogens, virus-infected and oncotransformed cells, and can also cause autoimmune diseases and delayed type IV hypersensitivity reactions, synthesize IL-2, IL-12, IL-15, IFN-γ and TNF-α and thus activate cellular immunity;
2. Th2 lymphocytes, which synthesize anti-inflammatory cytokines such as IL-4, IL-5, IL-6, IL-10, IL-13, and participate in humoral immunity;
3. Th17 lymphocytes, which synthesize mainly IL-17. These cells protect against pathogens by synthesizing IL-8 and thus mobilizing neutrophils to the site of inflammation. In addition, Th17 lymphocytes damage their own cells and tissues in various autoimmune diseases [41]. On the one hand, as shown above, VDR-mediated activation inhibits Th17 lymphocytes and, accordingly, tissue damage in immune inflammation, which complicates treatment with immune checkpoint inhibitors; on the other hand, VD enhances PD-L1 expression on both epithelial and immune cells, which is reflected in their synergistic effect;
4. in Treg lymphocytes (T-suppressors), which have a specific $CD4^+CD25^+FOXP3^+$ phenotype. Treg cells synthesize IL-10, TGF-β and are functional antagonists

of Th1, Th2 and Th17 lymphocytes [42]. The main function of Treg cells is to prevent autoimmune reactions [43]. Moreover, Treg lymphocytes express CTLA-4 [44] and PD-1 [45].

In addition, another type of T-helper subpopulation is distinguished – the so-called Th3 lymphocytes with immunoregulatory and immunosuppressive functions, which are induced by the introduction of a foreign oral antigen. TGF-β is the main anti-inflammatory cytokine of these cells. Th3 cells have been described as $CD4^{+-}$ $FOXP3^{-}$-regulatory T cells, i.e., unlike the well-characterized Treg cells, Th3 lymphocytes do not express the FOXP3 transcription factor [46]. It is still unclear how the D_3-VDR complex acts on Th3 lymphocytes.

As mentioned above, the D_3-VDR complex inhibits IL-12 synthesis by macrophages and DCs. As a result, Th0 lymphocytes differentiate not into Th1 but into Th2 lymphocytes [47]. Treatment of T lymphocytes with VD promotes suppression of the synthesis and secretion of proinflammatory cytokines by Th1 lymphocytes (IL-2, IFN-γ, TNF-α) [48], and also initiates the secretion of anti-inflammatory cytokines by Th2 lymphocytes (IL-3, IL-4, IL-5, IL-10) [47]. Moreover, proliferation and homing of $CD4^{+}$ T cells to lymph nodes is significantly reduced as a result of suppression of E-selectin ligand synthesis in endothelial cells after ligand-associated activation of VDR [49].

It has been shown that higher levels of VD can induce a variety of anti-inflammatory functions, including an increase in Treg cell numbers. In addition, experimental studies have shown that other small molecules, including retinol, niacin, and short-chain fatty acids, can potentiate Treg cell functions. However, the relationship between VD therapy and changes in Treg cell numbers or function in patients or healthy volunteers has not been clearly defined [50].

B-lymphocytes are similar to T lymphocytes and are susceptible to the action of VD. On the one hand, VD directly inhibited the differentiation and proliferation of B lymphocytes in patients with SLE [51]. However, on the other hand, ligand-associated activation of VDR did not have a direct effect on B lymphocytes, suppressing their proliferation and differentiation, but only an indirect effect, by inhibiting active T lymphocytes. As a result, either the functional activity of B lymphocytes decreased, which was manifested by a decrease in the synthesis of IgM and IgE, or B cells died completely as a result of apoptosis [52].

In summary, it can be concluded that key cells of the immune system, such as monocytes, macrophages, dendritic cells, T- and B-lymphocytes, are susceptible to ligand-associated activation of VDR, which is reflected in the dynamics of cytokine synthesis and modification of their receptors as a result of the interaction of the D_3-VDR complex with the corresponding target genes. In addition, the active D_3-VDR complex helps to reduce the concentration of proinflammatory cytokines (IL-1, IL-6, IL-12, IL-17, etc.) as a result of inhibition of their biosynthesis and, conversely, to increase the concentration of anti-inflammatory cytokines (IL-3, IL-4, IL-5, IL-10 and IL-13). This cytokine rearrangement is a mirror image of the effect of the active D_3-VDR genomic complex on the polarization of naive Th0 lymphocytes into Th1 and Th17 cells and naive Th0 lymphocytes into Th2 and Treg lymphocytes. The above-mentioned effects of ligand-associated VDR activation may contribute to a decrease in the incidence of various immune and autoimmune reactions, primarily Th1-dependent, and may also contribute to the alleviation of the patient's clinical symptoms. In reality, the immune complex effect of ligand-associated VDR activation on the immune system is undoubtedly deeper and more multifaceted, and it remains to be studied.

3. Potential relationship of the D_3-VDR complex with immune checkpoint receptors

The immune checkpoint family is one of the key elements of the regulatory link of the immune response. According to the literature, the ICP family consists of several main proteins. First of all, these are the programmed cell death receptor PD-1 and the programmed cell death ligand PD-L, as well as the cytotoxic protein CTLA4. On the one hand, these ICP receptors are susceptible to the effects of various viruses, as well as neoplastic cells, which ultimately leads to the suppression of antiviral and antitumor immunity, respectively [53]. On the other hand, ICP inhibitors in the form of various drugs open up new prospects not only in the immunotherapy of tumor processes, transplant immunity, allergies, but also in the control of autoimmune processes.

T-lymphocyte is activated as a result of the simultaneous action of two key signals. First of all, this is the binding and interaction of proteins expressed on the surface of effector cells, namely the T cell receptor (TCR) of the lymphocyte with MCH antigen-presenting cells (APC), respectively, which is necessary for the specificity of the immune response. At the same time, the interaction of the CD28 protein expressed on the surface of the T-lymphocyte occurs in a similar way with CD80 (B7-1) or CD86 (B7-2) on the surface of APC, resulting in the formation of a secondary costimulatory signal that ensures the maintenance of the primary signal. However, the absence of the secondary signal contributes to the development of anergy or apoptosis of the T lymphocyte [54].

ICT proteins, along with other receptors and cytokines, provide a fine mechanism for regulating cytotoxic lymphocytes during their activation upon interaction of TCR with the peptide associated with MHC class I. At the same time, as a result of the interaction of PD-L on the surface of the target cell with PD-1 on the surface of T cells, two important events occur. First, the "switching off" or even death of the T-lymphocyte, and second, the survival and preservation of the target cell. This "rescue" mechanism also has two opposite outcomes. First of all, a positive outcome is associated with the suppression of the development of autoimmune aggression, while a negative outcome is used by tumor cells

to protect themselves from antitumor immune surveillance [55].

The PD-1 protein (CD279) is one of the best known ICT proteins, which is expressed on immunocompetent cells such as monocytes, macrophages, DCs, natural killers, T and B lymphocytes. The PD-1 protein, together with the complementary ligands PD-L1 (CD274 or B7-H1) and/or PD-L2 (CD273 or B7-DC), forms the B7:CD28 receptor family, which contains inhibitory tyrosine-containing amino acid sequences (ITIM, immunoreceptor tyrosine-based inhibition motif) in the intracellular domain [56]. In addition, activated TCR on the T-lymphocyte surface potentiates PD-1 expression, while induction by type I and II IFN molecules together with JAK2-associated proteins increases PD-L1 expression [57]. Thus, PD-1 protein and VDR are expressed on the same cells, which indirectly confirms their relationship.

As a result of binding and interaction of the PD-1 receptor with its ligand PD-L1 and/or PD-L2, an intracellular signal is generated that stimulates phosphorylation of two sequences – ITIM and ITSM (immune receptor tyrosine-based switch motif) – with subsequent activation of two phosphatases: SHP-1 and SHP-2 (Src homology region 2 domain-containing phosphatase) [58]. In turn, SHP-1 and SHP-2 suppress phosphorylation of the PI3K/Akt (phosphoinositide 3-kinase/protein kinase B) signaling pathway, association of ZAP-70 (Zeta-chain-associated protein kinase 70) and the CD3ζ complex, which leads to «switching off» of TCR on the surface of the T lymphocyte. On the one hand, T-cell inactivation is manifested by a decrease in their proliferation and functional activity, which is manifested in the form of a decrease in the synthesis of key cytokines, such as IL-2 and IFN-γ, on the other hand, their death occurs through apoptosis, which is a consequence of the inhibition of transcription factors NF-κB and AP-1 (activator protein 1). Similar effects in T-cells are caused by the D_3-VDR-RXR complex. Thus, it is possible that the potential relationship between vitamin D and ICT lies in the activation of the listed intracellular signaling pathways.

At the same time, cytotoxic T-lymphocyte-associated protein 4, also known as CTLA4 (CD152), is also expressed on the T-lymphocyte surface. CTLA-4 competes with the CD28 receptor for the B7 family ligand: B7-1 (CD80) and B7-2 (CD86). Upon binding to the B7 ligand, the activated CTLA-4 complex inhibits T-lymphocyte activation [59]. In addition, CTLA4 leads to the suppression of the downstream PI3K/Akt, cyclin D_3, CDK4/CDK6, and NF-kB pathways, thereby altering T-lymphocyte differentiation [60]. Ligand-associated activation of VDR has a similar inhibitory effect on NF-kB. At the same time, VD stimulates the PI3K/Akt pathway [61], unlike CTLA-4.

Based on modern scientific data, in addition to T lymphocytes, PD-1, PD-L1/2 and CTLA-4 are also expressed by tumor cells, thus suppressing neoplastic immune surveillance [62]. As an "antidote", corresponding monoclonal antibodies to ICT proteins were created, which neutralized the negative impact of malignant cells on the functions of T lymphocytes as a result of their "reanimation". These monoclonal antibodies are widely used in practical medicine.

Macrophages also express ICT proteins, namely PD-1. According to recent studies, PD-1 expression is more specific for the anti-inflammatory M2 phenotype of macrophages. Moreover, anti-PD-1 therapy can redirect macrophages from the M2 phenotype to the M1 phenotype [63]. Therefore, immune blockade of PD-1 will provoke an increase in phagocytosis activity and a decrease in tumor volume. At the same time, the active D_3-VDR complex transforms macrophages towards the tolerogenic M2 phenotype, which suggests a synergistic effect of VD with the PD-1 molecule.

In particular, the anti-inflammatory cytokines IL-10 and IL-4 stimulate PD-L1 expression on monocytes and PD-L2 expression on DCs [64]. Since the D_3-VDR complex increases the synthesis of IL-10 and IL-4, it is likely that it will also increase the expression of PD-L1 and PD-L2, respectively, but most likely indirectly (through intracellular signaling pathways) rather than directly. On the other hand, in severe COVID-19, vitamin D administration, on the contrary, inhibited PD-L1 expression [65]. Finally, it is not entirely clear how the D_3-VDR-PD-L1 axis and the D_3-VDR-PD-L2 axis, as well as the D_3-VDRCTLA-4 axis, play a gatekeeper role in the immunoregulation of cancer, autoimmune and allergic processes, which requires further research in this area.

4. Modern concepts of productive granulomatous inflammation and potential points of application of the D_3-VDR complex in its implementation

Immune granuloma (the most common type of granuloma) is a HRT IV involving T-helpers ($CD4^+$) and macrophage cells. First, monocytes differentiate into mature macrophages and DCs. Then, APCs (macrophages, DCs) phagocytose the pathogen, cleave it into epitopes, then bind them to MHC class II in the Bjorkman cleft and present them on their surface to naive $CD4^+$ lymphocytes. After contact with the epitope, Th0 lymphocytes differentiate into Th1 lymphocytes under the influence of macrophages synthesizing IL-12. Activated Th1 lymphocytes synthesize IFN-γ, the main cytokine of granulomatous inflammation [66].

At the cellular level, the key to the pathogenesis of granuloma is the differentiation of monocytes into mature macrophages [67]. This process can be determined histologically (a threefold increase in the size of the cell and its organelles, a corrugated cytoplasmic membrane) and microscopically (the appearance of vesicles and granules in the cytoplasm) [68], as well as immunohistochemically, since monocytes express mainly CD14 and CD16 on their cell surface, while macrophages express CD14, Cd11b, CD68, MAC-1 and MAC-3, EMR1 and Lysozyme M [69]. In addition, the D_3-VDR complex influences the differentiation of monocytes, promoting their differentiation into M2-phenotype macrophages, which express PD-L1 [70]. On the other hand, ligand-associated activation of VDR suppresses M1-phenotype macrophages, although these data are contradictory.

Theodor Langhans first described multinucleated giant cells (MGCs) in his studies of tuberculosis over 150 years ago, and these cells were posthumously named Langhans giant cells in his honor. Like epithelioid cells, MGCs can be identified histologically by their characteristic morphology: three or more nuclei of the same shape within a cell. Macrophages isolated from various tissues can differentiate into MGCs *in vitro* [71], as well as in the presence of IL-4 or IL-13, GM-CSF (granulocyte-macrophage colony stimulating factor) + IL-4, IFN-γ + IL-3, or mycobacterial glycolipids [72]. Thus, MGC formation is specific only to macrophages. On the other hand, it is completely unclear how the D_3-VDR complex promotes MGC formation in immune granuloma.

Several subpopulations of T lymphocytes can be found in immune granulomas: $CD4^+$ effector T cells, $CD4^+$ regulatory T cells, and $CD8^+$ cytotoxic T-cells. Depending on the etiology of the granuloma, different types of polarized effector $CD4^+$ cells can be identified, such as Th1, Th2, Th3, or Th17 lymphocytes (e.g., Th1 and Th2 cells are detected in tuberculosis and schistosome granulomas, respectively) [73]. In addition, T lymphocytes, along with monocytes, express VDR [74] and PD-L1 [62].

Among all cytokines, IFN-γ and TNF-α are most closely associated with granuloma formation. Both IFN-γ and TNF-α play a critical role in granuloma formation. The main function of these cytokines in tuberculous granuloma is to increase the bactericidal capacity and survival of macrophages and, thereby, to maintain the cellular integrity of the granuloma [75]. In addition, binding of PD-1 to its ligand PD-L1 and/or PD-L2 contributes to a decrease in IFN-γ production by T lymphocytes, similar to the effect of the D_3-VDR complex. At the same time, the mechanism of the relationship between ligand-associated activation of VDR and these signaling pathways is not entirely clear.

Ligand-associated activation of VDR inhibits the production of IFN-γ, lymphotoxin, IL-2 and proliferation of certain T-lymphocyte subsets [76]. *In vitro* studies have shown that 1,25(OH)2 D_3 stimulates proliferation, differentiation and transformation of monocytes into epithelioid cells [77]. On the other hand, VD inhibits the differentiation of macrophages into DCs and the maturation of the latter, while stimulating their apoptosis [78]. Thus, the effects of ligand-associated activation of VDR on granuloma development are ambiguous. In addition, to date, there are no data on the relationship between the D_3-VDR complex and immune checkpoint proteins (PD-1, PD-L, CTLA), which requires further research in this area.

CONCLUSION

Thus, according to the literature, ligand-associated activation of VDR initiates both genomic and extragenomic effects. These effects are mediated by the D_3-VDR complex. The absence, deficiency or structural defect of any of the components of the complex (D_3 or VDR) disrupts the functioning of its components and the implementation of "classical" and "non-classical" effects. Among the "non-classical" effects, special attention is paid to the action of VD on the immune system, which is manifested in general by an anti-inflammatory vector with the implementation of effects on antigen-presenting cells and lymphoid cells, including indirectly through the signaling pathways of immune checkpoints. The immunocorrective effects of calcitriol have opened up new possibilities for the therapeutic use of VD and its analogues (e.g., paricalcitol) to control autoimmune diseases associated with excessive synthesis of cytokines and the formation of autoreactive immune cells. In addition, the D_3-VDR complex stimulates cell differentiation and has antiproliferative activity, which may play a key role in inhibiting tumor processes. This is probably the result of the relationship between ligand-associated activation of VDR and immune checkpoint proteins (PD-1, PD-L, CTLA), which remains unexplored and seems to be a promising direction for further research.

Conflicts of interest

No potential conflict of interest relevant to this article reported.

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GENETICS, PROTEOMICS AND METABOLOMICS

GENETIC PRION DISEASE – FATAL FAMILIAL INSOMNIA (CLINICAL CASE)

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ABSTRACT

Background. *Fatal familial insomnia is a rare genetically determined neurodegenerative disorder from the group of prion diseases. Its main cause is the autosomal dominant D178N mutation of the PRNP gene, which leads to the synthesis of the pathological prion protein PrP.*
The aim. *Using the example of a clinical case to describe an example of the early onset of fatal familial insomnia in a teenager, a clinical example of its management.*
Materials and methods. *Female patient V., 16 years old, of hyposthenic constitution, undernourished, with negative family history (multiple sclerosis in her paternal grandmother) for the first time consulted a neurologist in Tver for the complaints of superficial sleep, shortened to 4–5 hours, unspecific pain all over the body, periodic numbness in the upper limbs. Six months later, retardation of speech and movements, changes in gait, and intentional tremor occurred; sleep was shortened to 2 hours. In the future, the teenager lost the ability to independently maintain the vertical body position, the ability to walk without assistance, speech was reduced to syllable answers to questions. In order to verify the diagnosis and to carry out differential diagnosis with other neurodegenerative diseases, the girl underwent auxiliary research methods: detection of antibodies to nuclear antigens, magnetic resonance imaging, computer electroencephalography, polyexomal genome sequencing.*
Results. *Based on the anamnesis, complaints, clinical picture and results of genetic research the final diagnosis of fatal familial insomnia was made. Due to the lack of etiological and pathogenetic therapy, the patient was subsequently provided with palliative medical care. The fatal outcome occurred 19 months after the onset of the disease.*
Conclusions. *The presented clinical case reflects the complexity of managing patients with rare genetic diseases, confirms the need for mandatory polyexomal genome sequencing in order to verify the diagnosis, which allows timely palliative care.*

Key words: *fatal familial insomnia, prion diseases, prion proteins, neurodegeneration*

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ГЕНЕТИЧЕСКОЕ ПРИОННОЕ ЗАБОЛЕВАНИЕ – ФАТАЛЬНАЯ СЕМЕЙНАЯ БЕССОННИЦА (КЛИНИЧЕСКИЙ СЛУЧАЙ)

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РЕЗЮМЕ

Обоснование. *Фатальная семейная бессонница – редкое генетически детерминированное нейродегенеративное расстройство из группы прионных заболеваний. Основной причиной его возникновения является аутосомно-доминантная мутация D178N гена PRNP, приводящая к синтезу патологического прионного белка PrP.*

Цель. *На примере клинического случая описать пример раннего дебюта фатальной семейной бессонницы у подростка, клинический пример его ведения.*

Материалы и методы. *Пациентка В., 16 лет, гипостенического телосложения, пониженного питания, с отягощённой наследственностью (у бабушки по линии отца – рассеянный склероз) впервые обратилась к неврологу г. Тверь с жалобами на поверхностный, укороченный до 4–5 часов сон, боли неспецифического характера во всём теле, периодические онемения в верхних конечностях. Спустя полгода присоединились заторможенность в речи и движениях, изменение походки, интенционный тремор, сон укорочен до 2 часов. В дальнейшем подросток утратил способность к самостоятельному поддержанию вертикального положения тела, способность ходить без посторонней помощи, речь была сведена к односложным ответам на вопросы. С целью верификации диагноза и дифференциальной диагностики с иными формами нейродегенеративных заболеваний подростку проводились вспомогательные методы исследования: определение наличия антител к ядерным антигенам, магнитно-резонансная томография, компьютерная электроэнцефалография, полиэкзомное секвенирование генома.*

Результаты. *Учитывая анамнез, жалобы подростка, многообразие клинических проявлений заболевания, а также результаты полиэкзомного секвенирования генома, был поставлен окончательный диагноз фатальной семейной бессонницы. Ввиду отсутствия этиологической и патогенетической терапии больной в дальнейшем оказывалась паллиативная медицинская помощь. Летальный исход наступил через 19 месяцев после дебюта заболевания.*

Выводы. *Рассмотренный клинический случай отражает сложность ведения пациентов с редкими генетическими заболеваниями, подтверждает необходимость обязательного проведения полиэкзомного секвенирования генома с целью верификации диагноза, что позволяет своевременно оказывать паллиативную помощь.*

Ключевые слова: *фатальная семейная бессонница, прионнные заболевания, белки-прионы, нейродегенерация*

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BACKGROUND

Prion diseases are a group of neurodegenerative diseases that result from the conversion of the normal prion protein PrPC, which has a predominantly α-helical structure, into an abnormal form of the protein called the PrPSc prion. This group of human diseases occurs in most developed countries of the world with a frequency of 1–1.5 cases per 1 million per year. Among them, fatal familial insomnia with an autosomal dominant type of inheritance is distinguished, associated with a mutation in codon 178 (D178N) of the *PRNP* gene, located on the short (p) arm of chromosome 20 at position p13 [1]. As a result of missense mutation, normal aspartic acid (Asp) is replaced by asparagine (Asn), which leads to neuronal degradation, proliferation of astrocytes and microglial cells, accumulation of abnormal prion protein mainly in the anterior ventral and mediodorsal thalamus nuclei, inferior olivary nucleus, cerebellum and entorhinal brain cortex [2, 3]. Patients with fatal familial insomnia are more common in the age group from 20 to 61 years, and it is equally common among men and women. The fundamental symptom of this pathology is insomnia, and its severity correlates with the disease progression. Vegetative dysfunction may manifest itself in attacks of high blood pressure, episodes of tachypnea, hyperhidrosis, sexual dysfunction, and persistent subfebrile body temperature. In terms of the motor system, the most common are gait ataxia and myoclonus. In the area of cognitive impairment, inhibition, decreased concentration, and loss of short-term memory are prevalent. Dysarthria and bulbar dysfunctions may appear at a later stage. As the neurological deficit increases, changes in mental status are added [4, 5].

The complexity of managing patients with fatal familial insomnia is associated with the absence of specific signs of the disease in routine diagnostic methods, international standards of clinical diagnostic criteria, and the insufficient scientific data for the development of etiological and pathogenetic therapy [6, 7].

THE AIM OF THE STUDY

To describe an example of the early onset of fatal familial insomnia in an adolescent, and a clinical case of its management.

MATERIALS AND METHODS

Female patient V., 16 years old, of hyposthenic constitution, undernourished, hereditary tainted (multiple sclerosis in her paternal grandmother) for the first time consulted a neurologist in Tver in March 2019 for the complaints of sleep disorders, periodic numbness in the upper limbs, persistent dry cough, non-specific pain all over the body. It is known from the anamnesis that the first symptoms appeared in January 2019. According to the mother, against the background of a stressful situation in the family, the adolescent's sleep duration shortened to 4–5 hours, and parasomnias associated with the REM sleep, such as nightmares and dysphoria appeared. By the end of February, the patient began to complain of sensory disturbances, manifested by episodes of numbness in the upper limbs, non-specific pain all over the body, not related to physical activity, stress, or medication, and an obsessive dry cough.

Upon examination, the patient is hyposthenic, undernourished, skin and visible mucous membranes are of physiological color and moderate humidity. Vesicular breathing occurs in all parts of the lungs. Heart sounds are clear and rhythmic. Blood pressure is 110/75 mm Hg. Pulse is symmetrical, rhythmic, of satisfactory filling and tension, 96 beats per minute. The musculoskeletal system is normal. Urination and stool are not impaired. Neurological status: in the cranial nerves – horizontal nystagmus in extreme leads. Muscle tone is slightly reduced in the upper limbs. Tendon reflexes are brisk. In the Romberg pose, there is mild static ataxia.

Neurometabolic therapy (gamma-amino-beta-phenylbutyric acid hydrochloride 250 mg) and intramuscular injections of a vitamin complex containing thiamine disulfide, pyridoxine hydrochloride, and cyanocobalamin were prescribed.

In March, the patient was admitted to the neurology department of the regional hospital to clarify the diagnosis and treatment. The previously described symptom complex is joined by non-specific, constant pain in the back of the neck, non-systemic dizziness, and persistent increase in body temperature to 37.5 °C. Magnetic resonance imaging (MRI) with a power of 1.5 T did not reveal any pathology of the brain. Examination for systemic diseases showed the presence of antibodies to nuclear antigens – 0.2 units (with the norm being 0 units); other indicators were normal. A decision was made to continue the course of neurometabolic therapy in combination with therapeutic exercise and magnetic therapy for the neck area. A consultation with a psychotherapist was conducted and the presence of a depressive disorder was diagnosed; an antidepressant (escitalopram oxalate 5 mg) was added to the treatment regimen – showing no positive clinical effect. The neurologist transferred the patient to a tranquilizer (medazepam 10 mg) and a nootropic (hopantenic acid 250 mg), but the condition remained without positive dynamics.

Five months after the onset of the disease, the adolescent's sleep was shortened to 2 hours; speech and movement retardation, changes in gait such as obsessive short stepping movements when stopping, and intention tremor were observed. A second course of medazepam (15 mg) was prescribed, which led to sleep extension to 12 hours, but upon awakening, an attack of derealization occurred: the child did not understand where she was, what day and time it was, did not recognize the surrounding environment and spoke

phrases unrelated to the environment. The neurological status includes rest and action tremor (ability to walk independently is preserved), eyelid tics, ataxia, noisy sighs and vocalizations, diplopia, poor speech, mainly monosyllabic sentences. All therapy was discontinued. The patient was referred for a psychiatrist consultation, and borderline state of dissociative personality disorder was diagnosed. Neuroleptic treatment was prescribed – quetiapine fumarate 25 mg.

Six months after the onset of the disease, clinical manifestations are supplemented by obsessive head movements, non-specific pain in the lumbar region, and acute urinary retention. The patient was hospitalized, a second MRI scan with intravenous contrast was performed, but no specific data was found to determine the cause of the disease. The patient was diagnosed with undifferentiated hereditary degenerative disease of the central nervous system. During the patient's inpatient treatment, an episode of confusion was noted, symptoms of acute psychosis with attacks of depersonalization and derealization, autoaggression, chaotic movements, echolalia, and lack of insight into her condition appeared. Psychocorrection was carried out without pharmacological support. Discharged home for outpatient supervision.

In September 2019, the child's condition worsened: the patient lost weight to 47 kg, pelvic disorders in the form of daytime and nighttime enuresis, encopresis appeared. Due to progressive degenerative symptoms, she was referred to the neurology department of a federal hospital. There are medium-swinging horizontal nystagmus in extreme abductions in the area of cranial innervation. Positive symptoms of oral automatism (proboscis and nasolabial). In the motor-reflex sphere: impaired gait, astasia-abasia, the patient walks only with support, moves in small steps, during examination involuntarily moves the upper and lower limbs. Notable muscle hypotrophy is observed. Muscle strength is reduced in the lower limbs to 4 points. Muscle tone is altered according to the plastic type, the "cogwheel" symptom is noted in the upper limbs. Tendon reflexes from the upper limbs are high, with expansion of zones without a clear difference between the sides, from the lower limbs – are not evoked. Inconstant Babinski's symptom on the right. The patient does not manage to stand in the Romberg pose, sits with support. Intention on both sides during the finger-hammer test. There are pelvic organ dysfunctions in the form of periodic urinary retention and incontinence, constipation. Hyperhidrosis is observed, facial expression is poor, the patient answers questions passively, the answers are scanty. Speech is quiet, hyperlalia with a nasal tint. Pharmacological therapy was prescribed, including a multivitamin complex, valproic acid (300 mg), levocarnitine (1 ml), phenobarbital (25 mg). Discharged for residential treatment.

Twelve months after the onset of the disease, the child's condition remained severe. The patient's weight was 35 kg, body mass index was 14 kg/m^2. Upon examination: consciousness was confused, a forced position in bed was noted, accompanied by obsessive, fanciful movements of the limbs and trunk. The skin was pale gray, clean, there were periorbital dark circles on the face. Speech was still impaired, the patient answered questions with difficulty, used monosyllabic sentences. General hyperhidrosis with accompanying distal hyperthermia was observed. She could not sit or walk independently, movement was possible only with support. Pelvic organ functions were also impaired: stool with a tendency to constipation, dysuric phenomena in the form of urinary retention.

To verify the diagnosis, the patient underwent diagnostic tests. Thus, electroencephalographic monitoring showed slow forms of theta-range activity of 6 Hz, registered in all parts of the cerebral hemispheres, without regional differences.

General blood and urine analysis revealed no pathology. Polyexome genome sequencing revealed a mutation in the second exon of the *PRNP* gene, leading to the pAsp178Asn substitution, associated with fatal familial insomnia.

RESULTS AND DISCUSSION

Taking into account the anamnesis, complaints of the adolescent, the diversity of clinical manifestations of the disease, as well as the results of polyexome genome sequencing, the final diagnosis was: fatal familial insomnia. Due to the lack of etiological and pathogenetic therapy for this disease, the patient subsequently received palliative medical care. The fatal outcome occurred 19 months after the onset of the disease.

According to literature data, patients with fatal familial insomnia are more common in the age group from 20 to 61 years, and life expectancy varies from 7 to 72 months [8]. In the described clinical case, the onset was diagnosed in a 16-year-old girl; patients with fatal familial insomnia in adolescence have not been previously reported.

The presented clinical case demonstrates the ambiguity and multifaceted nature of clinical manifestations of fatal familial insomnia, which confirms the need to use unified diagnostic criteria. Thus, a clear diagnostic hierarchy was established, which included: organic symptoms associated with sleep in the form of intractable insomnia; decreased cognitive abilities in the form of progressive dementia; the appearance of hallucinations, delusional disorders, depression; dissociative identity disorder; weight loss of more than 10 kg over the past 6 months. The determining diagnostic criteria remained a positive family history with existing insomnia and data genome from polyexome sequencing with the identified mutation of the PRNP gene [9].

CONCLUSION

The clinical case reflects the complexity of managing patients with rare genetic diseases, in particular

with fatal familial insomnia, associated with the absence of specific changes during typical diagnostic procedures (MRI, EEG) and standards of etiopathogenetic therapy, which confirms the need for mandatory polyexome genome sequencing and detection of the D178N mutation to verify the diagnosis, allowing timely provision of palliative care.

Conflicts of interest

No potential conflict of interest relevant to this article reported.

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INFECTIOUS DISEASES

LIPID PEROXIDATION – ANTIOXIDANT DEFENSE SYSTEM IN CHILDREN WITH SEASONAL INFLUENZA

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ABSTRACT

Introduction. *Influenza remains a serious viral infection in children and has consequences for the organism.*
The aim of the study. *To analyze the lipid peroxidation products and antioxidant defense (AOD) components level in children of two age groups with seasonal influenza. Materials and methods. We examined 141 children aged from 1 month to 6 years with a diagnosis of influenza (subgroup 1 – 1 month – 2.11 years (n = 78); subgroup 2 – 3–6 years (n = 63)), 47 children of control group (subgroup 3 – 1 month – 2.11 years (n = 17); subgroup 4 – 3–6 years (n = 30)). Spectrophotometric, fluorometric and statistical methods were used.*
Results. *In subgroup 1 of children with influenza, there were higher levels of compounds with double bonds ($p = 0.001$), conjugated dienes (CDs) ($p < 0.0001$), ketodienes and conjugated trienes (KD and CT) ($p = 0.004$); in subgroup 2 of children with influenza – increased values of CDs ($p < 0.0001$), KD and CT ($p < 0.0001$) and thiobarbituric acid reactants ($p < 0.0001$) compared to the control. The AOD system in subgroup 1 was characterized by a decrease in the level of α-tocopherol ($p < 0.0001$), retinol ($p < 0.0001$) and higher oxidized glutathione (GSSG) values ($p = 0.002$) compared to the control. Children of subgroup 2 had lower values of the level of α-tocopherol ($p < 0.001$), retinol ($p = 0.012$) and total antioxidant activity ($p < 0.0001$) and higher values of GSSG ($p = 0.035$) compared to the control.*
Conclusion. *In children with influenza, regardless of age, there is a higher level of production of lipid peroxidation indicators, a lack of fat-soluble vitamins and higher values of oxidized glutathione than in healthy children.*

Key words: *influenza, children, lipid peroxidation, antioxidant defense*

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ПЕРЕКИСНОЕ ОКИСЛЕНИЕ ЛИПИДОВ – СИСТЕМА АНТИОКСИДАНТНОЙ ЗАЩИТЫ У ДЕТЕЙ С СЕЗОННЫМ ГРИППОМ

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РЕЗЮМЕ

Введение. *Грипп остаётся серьёзной вирусной инфекцией у детей и имеет последствия для здоровья.*

Цель исследования. *Проанализировать уровень продуктов перекисного окисления липидов и компонентов антиоксидантной защиты (АОЗ) у детей двух возрастных групп, больных сезонным гриппом.*

Материалы и методы. *Обследован 141 ребёнок в возрасте от 1 месяца до 6 лет с диагнозом грипп (1-я подгруппа – от 1 месяца до 2,11 года (n = 78); 2-я подгруппа – 3–6 лет (n = 63)); 47 детей контрольной группы (3-я подгруппа – от 1 месяца до 2,11 года (n = 17); 4-я подгруппа – 3–6 лет (n = 30)). Использовались спектрофотометрические, флуорометрические и статистические методы.*

Результаты. *В 1-й подгруппе детей с гриппом отмечались более высокие уровни соединений с двойными связями (p = 0,001), диеновых конъюгатов (ДК) (p < 0,0001), кетодиенов и сопряжённых триенов (КД и СТ) (p = 0,004); во 2-й подгруппе детей с гриппом – повышенные значения ДК (p < 0,0001), КД и СТ (p < 0,0001) и ТБК-активных продуктов (p < 0,0001) в сравнении с контролем. Система АОЗ в 1-й подгруппе характеризовалась снижением уровня α-токоферола (p < 0,0001), ретинола (p < 0,0001) и более высокими значениями окисленного глутатиона (GSSG) (p = 0,002) по отношению к контролю. У детей 2-й подгруппы отмечались более низкие значения уровня α-токоферола (p < 0,001), ретинола (p = 0,012), общей антиокислительной активности (p < 0,0001) и повышенные значения GSSG (p = 0,035) в сравнении с контролем.*

Заключение. *У детей при гриппе независимо от возраста отмечаются более высокий, чем у здоровых детей, уровень продуктов липопероксидации, недостаток жирорастворимых витаминов и повышенные значения окисленного глутатиона.*

Ключевые слова: *грипп, дети, перекисное окисление липидов, антиоксидантная защита*

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ABBREVIATIONS

AOD – antioxidant defence
CDs – conjugated dienes
DB – double bounds
GSH – reduced glutathione
GSSG – oxidized glutathione
KD and CT – ketodienes and conjugated trienes
LPO – lipid peroxidation
OS – oxidative stress
SB – Schiff basis
SOD – superoxide dismutase
TAA – total antioxidant activity
TBARs – thiobarbituric acid reactants

INTRODUCTION

Influenza remains a pressing acute respiratory viral infection, which causes significant social and economic damage to public health [1]. Influenza and influenza-like infections are comparable in scale of damage to traumas, cardiovascular diseases and malignant neoplasms [2]. The influenza virus is characterized by seasonal circulation; small children, the elderly, people with weakened immune system and people with chronic diseases are at risk and have a high susceptibility to this disease [3]. The number of sick children during the epidemic period exceeds 30 %, the probability of illness and the risk of severe course in children is 1.5–3 times higher than in adults [4]. The influenza virus has significant genetic variability, which reduces the effectiveness of existing vaccines and limits the therapeutic possibilities due to the emergence of new strains resistant to standard therapy [3]. The emergence of a new coronavirus infection caused by SARS-CoV-2 requires new diagnostic and therapeutic options, especially in risk groups, since influenza viruses and SARS-CoV-2 are both RNA viruses [2]. In the pathogenetic mechanisms of the infectious process during influenza, of great importance is the nonspecific defense system: lipid peroxidation-antioxidant defense (LPO-AOD) [5].

The role of LPO has been proven in the phagocytosis and destruction of microorganisms, in the metabolism of some xenobiotics, and in the synthesis of some biologically active substances, in particular prostaglandins [6–8]. Because hydroperoxides act as primary stable products in the oxidation of unsaturated fatty acids of phospholipids, this process is called peroxidation. Subsequently, peroxidative degradation of phospholipid molecules occurs, which changes the conformation of the cell membrane and lipoproteins [9]. Excessive synthesis of lipid peroxidation products is prevented by the antioxidant defense system, the main elements of which are antioxidants – substances that inhibit or reduce the intensity of free radical oxidation, neutralizing free radicals, exchanging their hydrogen atom for the oxygen of free radicals [10, 11]. Currently, the study of this process is very relevant and the role of oxidative stress in children of different ages and in various pathological conditions (arterial hypertension, chronic gastroduodenitis, influenza, cholelithiasis, pyelonephritis, metabolic syndrome, type 1 diabetes mellitus) has been proven [12, 13]. Despite the available research, there are very little data on changes in this system in children with influenza in a comparative age aspect.

THE AIM OF THE STUDY

To analyze the lipid peroxidation products and antioxidant defense components level in children of two age groups with seasonal influenza.

MATERIALS AND METHODS

We examined 141 children diagnosed with influenza who were hospitalized at the Irkutsk Regional Infectious Diseases Clinical Hospital from 2018 to 2019; the average age of the patients was 2.87 ± 0.9 years. In the control group (n = 47), the average age of patients was 3.13 ± 1.1 years. In the main group, patients were divided into subgroups: subgroup 1 – 1 month – 2.11 years (n = 78); subgroup 2 – 3–6 years (n = 63)), control group: subgroup 3 – 1 month – 2.11 years (n = 17), subgroup 4 – 3–6 years (n = 30).

When working with case histories, the following were assessed: gender, age of a child, duration of hospitalization, main diagnosis, concomitant diagnosis, presence of complications, nature and duration of the main clinical symptoms, general and biochemical blood tests, verification of the influenza virus by polymerase chain reaction.

Gender structure of patients with influenza: 70 (52 %) boys and 64 (48 %) girls; average age – 3 years. In the structure of influenza incidence, influenza A (H1N1sw2009) serotype predominated – in 76 % of cases, influenza A (H3N2) made 16 %. In the structure of concomitant diagnoses, a significant place belongs to acute intestinal infection – 16 % (of rotavirus and noravirus etiology), atopic dermatitis was notes in 2 % of cases, hypochromic anemia – in 6 %, enterobiasis was observed in isolated cases.

The structure of complications of the underlying disease: acetonemic vomiting syndrome or ketoacidosis syndrome was observed in 12 % of cases, pneumonia was detected in 16 %, obstructive bronchitis – in 2 %. The average length of hospitalization was 5 ± 1.6 days.

The clinical picture of seasonal influenza had the following characteristics: 66 % of children had a runny nose, 84 % had dry cough, 92 % had fever, and 38 % of children had complaints of intoxication. Apathy and drowsiness were observed in 44 % of patients, weakness was observed in 34 %, decreased appetite in 26 %, headaches bothered 8 % of children, pain in muscles and joints was not observed in anyone. Sore throat and pain when swallowing were noted by 6 % of children, abdominal pain and diarrhea were observed in 8 % of children, vomiting – in 28 %, convulsions – in 4 %, ear pain and dizziness – in 1 % of children.

The nature of rhinitis in 72 % of cases was mucous, the duration of the runny nose was 5 ± 2.48 days. The nature of the cough in 84 % of cases was dry; 6 % of children had wet cough, duration – 5 ± 1.5 days. The median body temperature during fever was 38.5 ± 0.68 °C, the duration of fever was 2 ± 1.45 days. Pharyngeal hyperemia was observed in 96 % of cases.

The study was conducted in accordance with the Declaration of Helsinki of the World Medical Association (1964, ed. 2013) and approved by the Biomedical Ethics Committee at the Scientific Centre for Family Health and Human Reproduction Problems (Extract from meeting No. 8.4 of November 2, 2018).

The research materials were plasma and serum. The S-monovette dipotassium ethylene diamine tetraacetic acid (K3-EDTA) blood collection system (Sarstedt, Germany) was used for venous blood collection (10 mL). The analysis was conducted after overnight rest of subjects, blood was taken on an empty stomach, between 8.00 and 9.00 a. m. Immediately after collection, blood was centrifuged at 1500× g for 10 min to separate the plasma from the erythrocytes. Plasma was taken, and the erythrocytes were washed three times in cold saline solution (0.9 % NaCl, w/v). Then, the erythrocytes were hemolyzed by adding 9 volumes of cold 50 mM phosphate buffer of pH = 7.4 (v:v). Samples were kept frozen at the temperature of –40 °C until use.

The intensity of the lipid peroxidation processes was assessed by the content of unsaturated double bonds (DB), primary products – conjugated dienes (CDs), and secondary – ketodienes and conjugated trienes (KD and CT) products by the method of I.A. Volchegorskiy et al. (1989) [14], based on the intensive absorption of conjugated diene structures by lipid hydroperoxides in the range 220, 232 and 278 nm. The content of end products – thiobarbituric acid reactive substances (TBARs) and Schiff basis (SB) – was determined in the reaction with thiobarbituric acid in the range 532 and 440 nm using the fluorimetric method of V.B. Gavrilov et al. (1987) [15]. Total antioxidant activity (TAA) was evaluated by the method of G.I. Klebanov et al. (1988) [16]. To evaluate TAA, a model system, which is a lipoprotein suspension of chicken egg yolk that allows to evaluate the ability of blood serum to inhibit the accumulation of TBARs in suspension, was used. LPO was induced by adding $FeSO_4*7H_2O$. The content of reduced and oxidized glutathione (GSH and GSSG) was determined by P.J. Hissin, R. Hilf (1976) [17], the activity of superoxide dismutase (SOD) was measured using the method of H.P. Misra, I. Fridovich (1972) [18]. Concentrations of α-tocopherol and retinol were determined using the method of R.Ch. Chernyauskene et al. (1984) [19]. The method provides for the removal of substances that prevent determination by saponification of samples in the presence of large amounts of ascorbic acid and extraction of unsaponifiable lipids with hexane, followed by fluorometric determination of the content of α-tocopherol and retinol. At this, α-tocopherol has intense fluorescence with maximum of excitation at λ = 294 nm and radiation at λ = 330 nm; retinol at λ = 335 and λ = 460 nm.

Plasma was analyzed to determine the levels of lipid peroxidation products (DB, CDs, KD and CT, TBARs, SB) and antioxidant defense factors (TAA, α-tocopherol, retinol). SOD, GSH, and GSSG were estimated in erythrocytes.

The measurements were carried out using a spectrophotometer SF-2000 (Russia), a spectrofluorophotometer BTS-350 (Spain) and fluorate 02 ABFF-T (Russia).

The work was carried out using the equipment of the Center for the Development of Advanced Personalized Health Technologies of the Scientific Centre for Family Health and Human Reproduction Problems, Irkutsk.

To analyze the obtained data, the statistical package Statistica 10.0 (StatSoft Inc., USA) was used. To determine the proximity to the normal law of distribution of quantitative characteristics, a visual-graphical method and Kolmogorov – Smirnov agreement criteria with the Lilliefors and Shapiro – Wilk correction were used. Equality of common variances was tested using Fisher's test (F-test). The nonparametric Mann – Whitney test was used to analyze intergroup differences for independent samples. The critical significance level was set at 5 % (0.05).

RESULTS AND DISCUSSION

The obtained data are presented in Table 1.

Data analysis in children with seasonal influenza in age group 1 showed statistically significantly higher values of compounds with unsaturated DB, CDs, KD and CT compared to the corresponding control (Table 1).

In the group of children of age group 2 with influenza, the differences concerned increased values of CDs, KD and CT and TBARs (Table 1).

The AOD system in age group 1 of children with influenza was characterized by lower levels of α-tocopherol, retinol and higher values of GSSG (Table 1).

Children of age group 2 had lower values of α-tocopherol, retinol, total AOA and higher values of GSSG (Table 1).

No statistically significant differences were obtained between different age groups of children with influenza.

In the formation of homeostasis of any living organisms, LPO-AOP processes play an important role, which has been proven by numerous scientific studies [7, 20]. In a healthy body, two processes occur in parallel in all cells and membranes: on the one hand, it is LPO and the formation of free radical metabolic products; on the other hand, it is a powerful antioxidant defense [20]. Hyperactivation of these processes leads to the formation of oxidative stress, which is of significant importance in the pathogenesis of infectious diseases [21]. LPO metabolites lead to proliferation of cells of the immune system, accumulation of neutrophils at the site of inflammation, change surface receptors in macrophages, and increase the expression of adhesion molecules on endothelial cells [7]. The death of the infectious agent in the pathogenesis of inflammatory reactions is possible only if one's own tissues are damaged at the site of injury. As a result of free radical oxidation, damage to mitochondrial membranes and nuclear structures of cells occurs, this in turn leads to the destruction of blood vessels and barrier mechanisms at the cellular level, resulting in the development of a disease with in-

TABLE 1

LEVELS OF LPO PRODUCTS AND AOD COMPONENTS IN CHILDREN OF TWO AGE GROUPS WITH SEASONAL INFLUENZA, ME (Q1; Q3)

Parameters	Control (1 month – 2.11 years) (n = 17)	Influenza (1 month – 2.11 years) (n = 78)	Control (3–6 years) (n = 30)	Influenza (3–6 years) (n = 63)	p
	1	2	3	4	
DB, units	1.54 (1.22; 1.80)	2.46 (1.68; 3.18)	1.94 (1.68; 2.18)	2.23 (1.48; 3.01)	$p_{1-2} = 0{,}001$
CDs, μmol/L	1.19 (0.96; 1.38)	2.30 (1.68; 3.25)	1.00 (0.82; 1.38)	2.33 (1.44; 3.12)	$p_{1-2} < 0{,}0001$ $p_{3-4} < 0{,}0001$
KD and CT, units	0.47 (0.28; 0.80)	0.76 (0.48; 1.36)	0.37 (0.24; 0.66)	0.74 (0.42; 1.14)	$p_{1-2} = 0{,}004$ $p_{3-4} < 0{,}0001$
TBARs, μmol/L	1.03 (0.92; 1.33)	1.47 (0.83; 2.01)	0.82 (0.62; 1.13)	1.55 (1.08; 2.06)	$p_{3-4} < 0{,}0001$
SB, units	0.04 (0.03; 0.05)	0.05 (0.03; 0.09)	0.05 (0.04; 0.06)	0.07 (0.03; 0.12)	
retinol, μmol/L	1.75 (1.63; 1.97)	1.23 (1.08; 1.58)	1.32 (1.14; 1.88)	1.22 (0.95; 1.51)	$p_{1-2} < 0{,}0001$ $p_{3-4} = 0{,}012$
α-tocopherol, μmol/L	10.00 (9.47; 11.74)	6.28 (5.30; 7.46)	8.67 (6.35; 10.22)	6.27 (5.05; 7.47)	$p_{1-2} < 0{,}0001$ $p_{3-4} < 0{,}001$
TAA, units	16.43 (8.87; 26.28)	12.15 (8.62; 15.94)	18.01 (14.68; 26.81)	12.20 (8.27; 15.76)	$p_{3-4} < 0{,}0001$
GSSG, mmol/L	1.86 (1.56; 2.22)	2.33 (2.23; 2.57)	1.96 (1.69; 2.46)	2.32 (2.12; 2.55)	$p_{1-2} = 0{,}002$ $p_{3-4} = 0{,}035$
GSH, mmol/L	2.42 (2.11; 2.65)	2.34 (2.24; 2.52)	2.52 (2.24; 2.66)	2.33 (2.27; 2.58)	
SOD activity, units	1.56 (1.55; 1.60)	1.61 (1.57; 1.68)	1.56 (1.52; 1.59)	1.60 (1.55; 1.64)	

Note. p – statistically significant differences between groups with influenza and control groups.

flammatory, toxic and autoimmune mechanisms in pathogenesis [22, 23]. The synthesis of various other metabolites leads to persistent changes in the composition of biomembranes, the development of a serious imbalance in the LPO-AOD system, and they further have a damaging effect on healthy cells of the body.

We have shown an increase in the content of lipid peroxidation products in children with seasonal influenza, regardless of the child's age. It is known that the end products of lipid peroxidation can change the functional activity of phagocytes, inhibit the biosynthesis of superoxide radicals by neutrophils, slow down phagocytosis in monocytes and neutrophils, having pronounced chemotactic activity [24]. The described changes are possible under conditions of a significant deficiency of antioxidant substrates. In our study, we found a pronounced deficiency of fat-soluble vitamins in children with seasonal influenza, regardless of age. At the same time, in young children there was an increase in the oxidized form of glutathione, and at the age of 3–6 years – a decrease in the total antioxidant activity of blood serum. Fat-soluble vitamins (tocopherols and retinol) are present in the fat layer of cell membranes and are able to neutralize free radicals [19]. Alpha tocopherol is the biologically most active of the tocopherols; it limits free radical reactions, being a donor of hydrogen ions, like ascorbate. Due to its lipophilicity, the tocopherol molecule has the ability to integrate into the lipid layer of cell membranes, providing membrane protective and membrane stabilizing effects. Alpha-tocopherol can maintain the functional integrity of the outer plasma membrane of cells, can participate in the mechanisms of tissue respiration in mitochondria, regulate the functioning of cell enzyme systems, thereby preventing LPO activity [24]. The greatest antioxidant properties among retinol precursors has beta-carotene; it has conjugated double bonds and is capable of rapid oxidation using free radical oxidation mechanisms [25]. When antioxidant defense is suppressed, free radical damage occurs to various cell structures and tissues [26, 27].

The results of our study are confirmed by literature data. It is known that in uncomplicated forms of influenza A among pediatric patients, there is a decrease in AOD and a significant increase in LPO processes, depending on the severity of the clinical course of the viral infection. In patients with influenza A who had complications in the form of viral-bacterial pneumonia, AOD is signifi-

cantly weakened, and LPO processes are very active [26]. It was previously studied that influenza produces reactive oxygen species of mitochondrial origin, which destroys lung epithelial cells, leading to their histopathological damage. Pore-forming toxins produced as a result of necroptosis further aggravate the damage to lung tissues and structures, while creating a breeding ground for the spread of pathogenic bacteria [27]. The use of drugs with antioxidant properties in the treatment of viral infections, in particular influenza in children, can lead to the elimination of necroptosis and reduce the severity of the disease complicated by a secondary bacterial infection [27, 28].

Thus, the study confirms the importance of lipid peroxidation processes in the pathogenesis of infectious pathology, including the formation of complicated and uncomplicated forms of seasonal influenza in children.

CONCLUSION

In conclusion, we would like to note that there are higher rates of primary, secondary and end products of lipid peroxidation, and a pronounced lack of fat-soluble vitamins in children with seasonal influenza, regardless of age. In young children, higher levels of the oxidized form of glutathione were observed, in 3–6 years old children – reduced production of antioxidant factors. In connection with the changes in the LPO-AOD system identified in our study, we recommend implementing measures aimed at correcting the above indicators, namely, prescribing medications with antioxidant properties for the treatment of children with seasonal influenza.

Conflict of interests

The authors declare that they have no competing interests.

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DRUG MONITORING OF ANTIRETROVIRAL DRUGS IN CHILDREN WITH PERINATAL HIV INFECTION

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ABSTRACT

Therapeutic drug monitoring is the practice of measuring the concentration of a drug in patient's biological fluids to assess the effectiveness and safety of drug therapy. The results of determining the drug level in biological fluids can also indicate noncompliance of therapy regimen and low adherence to therapy.

The aim. *To compare the concentrations of some antiretroviral drugs (lopinavir, ritonavir, lamivudine, abacavir, zidovudine) in children living with HIV infection of different age groups.*

Methods. *We examined 184 children with perinatal HIV infection who underwent therapeutic drug monitoring of nucleoside reverse transcriptase inhibitors (lamivudine, abacavir, zidovudine) and protease inhibitors (lopinavir, ritonavir). Children were divided into four age groups. Group 1 included children 1–2 years old (n = 7); group 2 – children 3–5 years old (n = 14); group 3 – children 6–11 years old (n = 78); group 4 – children 12–17 years old (n = 85). The concentration of antiretroviral drugs in blood plasma was determined using high-performance liquid chromatography with mass selective detection.*

Results. *The lowest lopinavir concentration was found in children 12–17 years old (3782 [2117–5046] ng/ml), which was statistically significantly different from the similar values in children 6–11 years old (5614 [3521–7264] ng/ml; p = 0.011). For other antiretroviral drugs, no statistically significant differences in blood plasma concentrations were found in children of different age groups.*

Conclusion. *The lowest lopinavir concentrations are detected in children older than 11 years. For the other studied antiretroviral drugs, this pattern was not revealed.*

Key words: *antiretroviral therapy, HIV infection, therapeutic drug monitoring, children and adolescents, adherence*

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ЛЕКАРСТВЕННЫЙ МОНИТОРИНГ АНТИРЕТРОВИРУСНЫХ ПРЕПАРАТОВ У ДЕТЕЙ С ПЕРИНАТАЛЬНОЙ ВИЧ-ИНФЕКЦИЕЙ

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РЕЗЮМЕ

Терапевтический лекарственный мониторинг – практика измерения концентрации лекарственного препарата в биологических жидкостях пациента для оценки эффективности и безопасности лекарственной терапии.
Результаты определения уровня лекарственного препарата в биологических жидкостях также могут указывать на несоблюдение режима лечения и низкую приверженность к терапии.
Цель исследования. *Сравнить концентрации некоторых антиретровирусных препаратов (лопинавир, ритонавир, ламивудин, абакавир, зидовудин) у детей, живущих с ВИЧ-инфекцией, в разных возрастных группах.*
Методы. *Обследовано 184 ребёнка с перинатальной ВИЧ-инфекцией, которым проведён терапевтический лекарственный мониторинг нуклеозидных ингибиторов обратной транскриптазы (НИОТ) (ламивудин, абакавир, зидовудин) и ингибиторов протеазы (лопинавир, ритонавир). Дети разделены на 4 возрастные группы. В первую группу включены дети 1–2 лет (n = 7); во вторую – дети 3–5 лет (n = 14); в третью – дети 6–11 лет (n = 78); в четвёртую – дети 12–17 лет (n = 85). Концентрацию антиретровирусных препаратов в плазме крови определяли методом высокоэффективной жидкостной хроматографии с масс-селективной детекцией.*
Результаты. *Самая низкая концентрация лопинавира выявлена у детей 12–17 лет (3782 [2117–5046] нг/мл), которая статистически значимо отличалась от аналогичного показателя детей 6–11 лет (5614 [3521–7264] нг/ мл; p = 0.011). Для других антиретровирусных препаратов не выявлено статистически значимых различий концентраций в плазме крови у детей разных возрастных групп.*
Заключение. *Самые низкие концентрации лопинавира детектируются у детей старше 11 лет. Для других изучаемых антиретровирусных препаратов данной закономерности не выявлено.*

Ключевые слова: *антиретровирусная терапия, ВИЧ-инфекция, терапевтический лекарственный мониторинг, дети и подростки, приверженность*

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INTRODUCTION

According to estimates by the Joint United Nations Programme on HIV/AIDS (UNAIDS); in 2022 there were 1.5 million children under 15 years of age living with HIV worldwide [1]. The World Health Organization (WHO) has recommended combination antiretroviral therapy (ART), a combination of three drugs that leads to long-term suppression of the virus, which has made it possible to significantly reduce mortality and increase life expectancy in children with HIV infection [2].

Early initiation and high adherence to antiretroviral therapy provide maximum suppression of HIV replication, reduce viral accumulation in cells, and help maintain immunological function that supports normal growth and development in children living with HIV [3–7]. Poor adherence and non-adherence to the ART regimen for just a few weeks can lead to loss of efficacy of the regimen and selection of strains resistant not only to the given drug combination, but also, possibly, to other antiretroviral drugs (ARVs) that the patient was not prescribed (cross-resistance) [8].

A highly informative method for assessing adherence is measuring the concentration of drugs and/or their metabolites in the blood, urine and other biological fluids, i.e. therapeutic drug monitoring (TDM). In people living with HIV infection, TDM is a laboratory method for monitoring the effectiveness and toxicity of ARVs, and involves the analysis of failures in the absence of viral resistance and non-compliance with the pharmacotherapy regimen. Moreover, the identification of suboptimal ARVs concentrations allows for timely correction of therapy, thereby avoiding the formation of drug-resistant strains of the virus [3].

Thus, TDM as a method for assessing adherence and evaluating the characteristics of ARV metabolism helps prevent the progression of HIV infection.

THE AIM OF THE STUDY

To compare the concentrations of antiretroviral drugs (lopinavir, ritonavir, lamivudine, abacavir, zidovudine) in children of different age groups living with HIV infection.

MATERIALS AND METHODS

The study included children and adolescents aged 1 to 18 years with perinatal HIV infection. A total of 184 patients registered at the Irkutsk Regional Center for the Prevention and Control of AIDS and Infectious Diseases (IOC AIDS) were examined from January 2019 to March 2022. A more detailed description of the study groups and data collection is published in our previous article [9].

According to the clinical guidelines for the treatment of HIV infection in children (2020), the drug dose was calculated taking into account the child's weight or body surface area using the Mosteller formula (mg/m^2). All children and adolescents were divided into four age groups depending on the preferred, according to the clinical guidelines "HIV infection in children" (2020), age-specific antiretroviral drug regimens [10]. In the first age group of children (≥ 1 year and < 3 years), the ART regimen included lopinavir (n = 5), ritonavir (n = 5), lamivudine (n = 7), abacavir (n = 5), zidovudine (n = 1). Children in the second age group (≥ 3 years and < 6 years) were prescribed lopinavir (n = 11), ritonavir (n = 11), lamivudine (n = 14), abacavir (n = 9), and zidovudine (n = 1). The ART regimen for children in the third age group (≥ 6 years and < 12 years) included lopinavir (n = 54), ritonavir (n = 54), lamivudine (n = 70), abacavir (n = 53), and zidovudine (n = 16). Children in the older age group (≥ 12 years) were prescribed ART regimens including lopinavir (n = 38), ritonavir (n = 38), lamivudine (n = 59), abacavir (n = 25), and zidovudine (n = 28). Information on ARVs and the duration of their use is presented in table 1.

The frequency of prescribing the studied ARVs did not differ in boys and girls. The viral load in children of different age groups taking the analyzed antiretroviral drugs also did not have statistically significant differences. A comparative analysis of the duration of ARVs intake revealed regular trends of increasing the duration of intake with increasing age of children. The shortest period of taking antiretroviral drugs (in months) was recorded in the youngest age group.

The study was conducted in accordance with the principles of the World Medical Association's Declaration of Helsinki. Written informed consent was obtained from the parents or legal representatives of all participants under 15 years of age, as well as directly from adolescents aged 15 years and older. The study protocol was approved by the local Ethics Committee of the Scientific Centre for Family Health and Human Reproduction Problems (protocol No. 3 dated April 07, 2021).

Quantitation of antiretroviral drugs in plasma

Blood sampling for determination of concentration of antiretroviral drugs in blood plasma was performed once during the next scheduled appointment. Patients and their legal representatives were not informed in advance about the upcoming blood sampling. To determine the concentration of ARVs, a method of multiplex concentration assessment using high-performance liquid chromatography with mass-selective detection was developed. The analytical standards of ritonavir, lopinavir, abacavir, lamivudine and zidovudine prepared in accordance with the American Formulary (United States Pharmacopeia) and the European Pharmacopoeia were used as compounds for identification of substances. All organic solvents were of MS-grade/extra pure. Water for preparation of eluent was prepared using the Arium Mini Plus water purification system (Sartorious AG,

TABLE 1

CHARACTERISTICS OF PATIENTS AT THE TIME OF DETERMINING THE CONCENTRATION OF ANTIRETROVIRAL DRUGS IN BLOOD PLASMA

Characteristics	Lopinavir	Ritonavir	Lamivudine	Abacavir	Zidovudine
Total (n = 184)	108	108	150	92	82
	incl. girls, n (%)				
≥ 1 year and < 3 years (n = 7)	3 (60 %)	3 (60 %)	4 (57 %)	3 (60 %)	0
≥ 3 years and < 6 years (n = 14)	7 (64 %)	7 (64 %)	9 (64 %)	4 (44 %)	1 (100 %)
≥ 6 years and < 12 years (n = 78)	25 (46 %)	25 (46 %)	35 (50 %)	24 (45 %)	8 (50 %)
≥ 12 years (n = 85)	18 (47 %)	18 (47 %)	33 (56 %)	13 (52 %)	18 (64 %)
Total girls, n (%)	53 (49.1 %)	53 (49.1 %)	81 (54 %)	44 (47.8 %)	27 (32.9 %)
p	0.7095	0.7095	0.7617	0.8849	0.3940
	Duration of therapy (months), Me [Q1–Q3]				
≥ 1 year and < 3 years (n = 7)	10 [6–11]	10 [6–11]	10 [7.5–13.5]	10 [6–11]	16
≥ 3 years and < 6 years (n = 14)	34 [21–52]	34 [21–52]	42.5 [26.2–53.8]	53 [47–57]	34
≥ 6 years and < 12 years (n = 78)	68.5 [48–92]	68.5 [48–92]	68 [48.2–86.8]	66 [48–83]	69 [47.5–104]
≥ 12 years (n = 85)	65 [31–116]	65 [31–116]	36 [12–92]	47 [11–94]	40.5 [26.2–110]
p	0.00027*	0.00027*	0.04771*	0.00766*	0.3171
	Viral load ($\log_{10}$ copies/ml), Me [Q1–Q3]				
≥ 1 year and < 3 years (n = 7)	0 [0–0]	0 [0–0]	0 [0–1.3]	0 [0–0]	2.6
≥ 3 years and < 6 years (n = 14)	0 [0–2.15]	0 [0–2.15]	0.95 [0–2.75]	0 [0–2,3]	5.6
≥ 6 years and < 12 years (n = 78)	0 [0–1.2]	0 [0–1.2]	0 [0–0]	0 [0–0]	0 [0–2.03]
≥ 12 years (n = 85)	0 [0–2.3]	0 [0–2.3]	0 [0–2.5]	0 [0–2,3]	0 [0–2.4]
p	0.5718	0.5718	0.0895	0.5207	0.1668

Note. The χ^2 test was used to compare groups by gender; for other parameters, the Kruskal – Wallis test was used; * – $p < 0.05$.

Germany). The chemical reagents used in the work met the standard requirements for bioanalytical studies. To prepare control and calibration solutions, a precisely dosed amount of a solution of a mixture of analytical standards was placed into a "blank" biological material (pooled K2EDTA human blood plasma containing no analyzed drugs). During the preparation of model mixtures, the original biomaterial was diluted by no more than 5 % of its volume so that the resulting mixture reflected the composition of real human blood as accurately as possible. Chromatographic separation was performed on a Shimadzu Nexera X2 system (Japan) with two high-pressure pumps and gradient creation on the high-pressure side. Analyte detection was carried out using a LCMS-8060 triple-quadrupole tandem mass spectrometer (Shimadzu, Japan) in the positive ionization mode with a hybrid dual ionization source (DUIS) and the use of multiple reaction monitoring (MRM).

Statistical analysis

Statistical analysis was performed using R studio, a free and open-source software development environment for the R programming language, designed for statistical data processing and graphics.

The χ^2 criterion is used to evaluate qualitative variables. The Kruskal – Wallis criterion was used to evaluate differences in quantitative variables. The difference was considered statistically significant at $p < 0.05$. When statistically significant differences were detected between groups, a posteriori pairwise comparison was additionally performed using the Mann – Whitney criterion. The difference was considered statistically significant with the Bonferroni correction ($p < 0.0125$).

RESULTS

A comparative analysis of the concentrations of the studied antiretroviral drugs in different age groups is presented in figure 1.

When comparing the concentration of lamivudine in children of different age groups taking this drug as part of a combination ARV regimen, no statistically significant differences were found ($p > 0.05$). Thus, the median and interquartile range (Me [Q1–Q3]) of the lamivudine concentration were 198 [186–444] ng/ml in the first age group, 276 [142–1664] ng/ml in the second, 400 [162–1708] ng/ml in the third, and 494 [134–1279] ng/ml in the fourth. No statistically significant differences were found when comparing the concentration of another nucleoside reverse transcriptase inhibitor, abacavir: 16 [0–31], 1018 [58–1914], 128 [42.1–688], and 70.4 [6–402] ng/ml in the first, second, third, and fourth age groups, respectively. A similar trend was found for zidovudine in blood plasma. The first and second age groups for zidovudine included one patient each, so it is impossible to calculate the median and interquartile range for them. For the third group, the zidovudine concentration was 10.5 [0–80.5] ng/ml, for the fourth – 33.1 [3–115] ng/ml.

A different pattern was demonstrated for protease inhibitor ARVs: the lowest median lopinavir concentration was observed in the fourth age group (in children over 11 years old) and amounted to 3782 [2117–5046] ng/ml, which is statistically significantly lower than the same indicator in the third age group (≥ 6 years and < 12 years) – 5614 [3521–7264] ng/ml ($p = 0.011$). The median lopinavir concentration in the first group was 4872 [4765–5809] ng/ml, which is lower than in the second group – 8024 [6339–9370] ng/ml, although the difference did not reach statistical significance ($p > 0.05$).

The lowest concentrations of ritonavir, as well as lopinavir, were found in children of the fourth age group – 212 [119–489] ng/ml, then in ascending order: in the second group – 451 [223–797] ng/ml, in the first group – 326 [311–510] ng/ml, in the third group – 290 [151–745] ng/ml ($p > 0.05$).

Next, we analyzed the frequency of occurrence of undetectable concentrations of the studied ARVs in children; the prescription regimen included the drugs

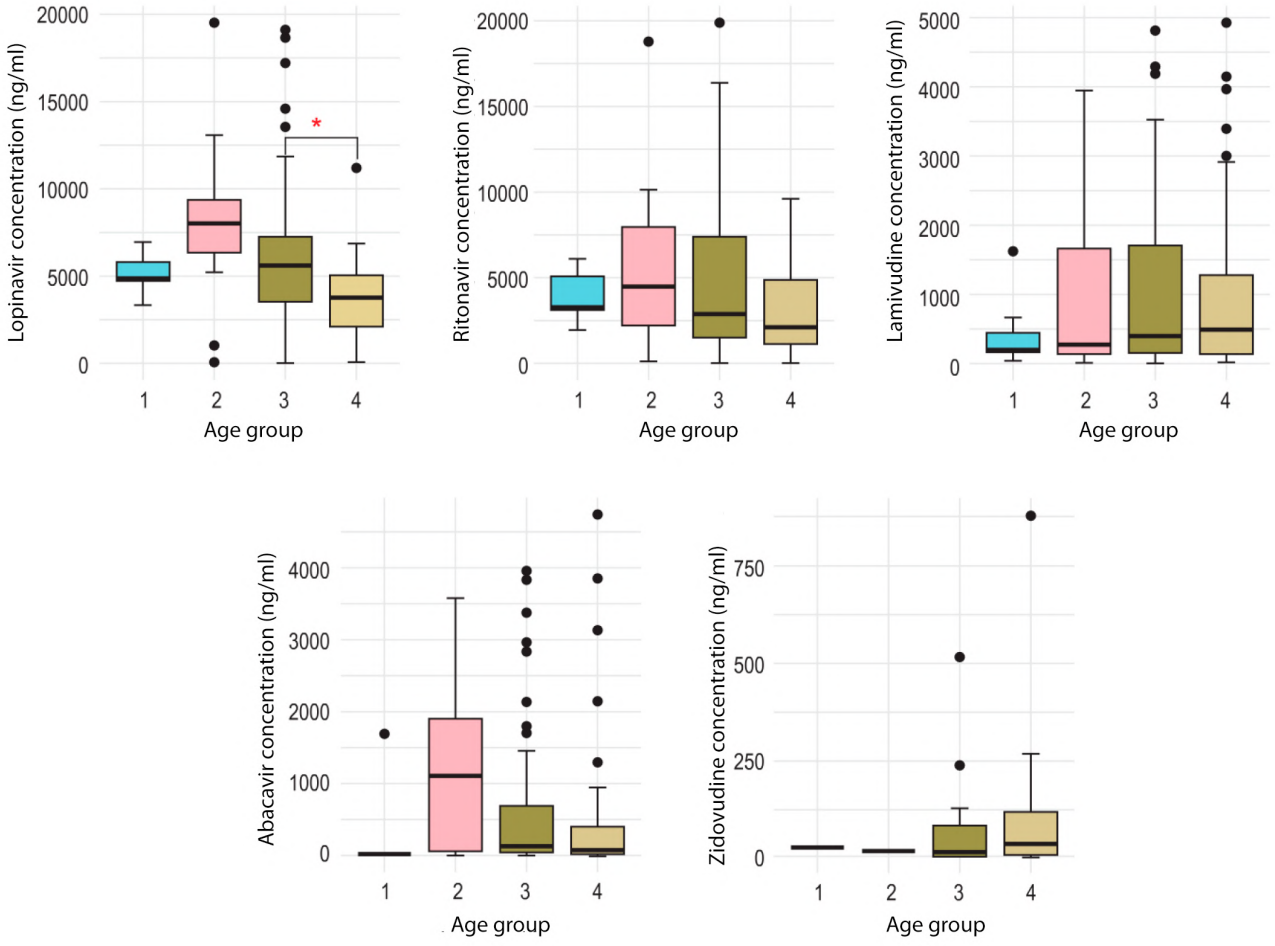

FIG. 1.
*Comparison of antiretroviral drug concentrations in different age groups using pairwise method by the Mann – Whitney test; statistical significance with Bonferroni correction: * – p < 0.0125*

lopinavir, ritonavir, lamivudine, abacavir, and zidovudine. The results are presented in table 2.

Of the ARVs analyzed, only 4.7 % of children had undetectable plasma lamivudine concentrations, which may indicate greater adherence to this drug. The highest frequency of children with undetectable concentrations was found for zidovudine, which may indicate that children living with HIV infection may have low adherence to zidovudine.

In age groups 1 and 2, no children were found with undetectable concentrations of nucleoside reverse transcriptase inhibitors (lamivudine, zidovudine) and protease inhibitors (lopinavir with ritonavir booster). Undetectable concentrations of abacavir were found in children in all analyzed age groups and with a higher frequency in children of older age groups.

At the next stage, a comparative analysis of the concentration of five antiretroviral drugs in children of different sexes was carried out, and the results are presented in table 3.

Thus, when comparing ARVs concentrations between samples of boys and girls, we did not find any statistically significant differences.

DISCUSSION

To date, of the possible objective methods for laboratory assessment of patient adherence to antiretroviral therapy, the most widely used are viral load assessment and monitoring of CD3 and CD4 counts [10]. However, according to M.V. Akulova (2016), unsatisfactory viral load and immune status indicators may indicate both patient non-adherence and an incorrectly selected treatment regimen and the presence of viral resistance to certain ARVs. Measuring the concentration level of drugs is more objective, since it confirms the fact of taking the drug, but not its regularity. Moreover, according to the author, the method is technically complex and has a high cost [11].

TABLE 2

NUMBER OF CHILDREN WHO HAD UNDETECTABLE CONCENTRATIONS OF ANTI-RETROVIRAL DRUGS, *n* (%)

Groups	Lopinavir (*n* = 108)	Ritonavir (*n* = 108)	Lamivudine (*n* = 150)	Abacavir (*n* = 92)	Zidovudine (*n* = 46)
≥ 1 year and < 3 years	0	0	0	2 (2.17 %)	0
≥ 3 years and < 6 years	0	0	0	1 (1.08 %)	0
≥ 6 years and < 12 years	5 (4.63 %)	6 (5.55 %)	4 (2.67 %)	6 (6.52 %)	6 (7.3 %)
≥ 12 years	6 (5.55 %)	6 (5.55 %)	3 (2 %)	5 (6.43 %)	7 (8.54 %)
Total	11 (10.19 %)	12 (11.11 %)	7 (4.67 %)	14 (15.22 %)	13 (28.26 %)
p	0.704	0.558	0.748	0.313	0.658

Note. The χ^2 test was used to compare groups; differences are statistically significant at $p < 0.05$.

TABLE 3

COMPARATIVE ANALYSIS OF THE ANTIRETROVIRAL DRUGS CONCENTRATIONS IN CHILDREN OF DIFFERENT SEXES (NG/ML)

Sex	Lopinavir Me [Q1–Q3], *n*	*p*	Ritonavir Me [Q1–Q3], *n*	*p*	Lamivudine Me [Q1–Q3], *n*	*p*	Abacavir Me [Q1–Q3], *n*	*p*	Zidovudine Me [Q1–Q3], *n*	*p*
Male	4957 [2167–6804] *n* = 64	0.3443	228 [88–525] *n* = 65	0.7769	387 [114–1327] *n* = 71	0.7976	208 [19.5–1764] *n* = 51	0.3544	26,1 [3–158] *n* = 20	0.324
Female	4765 [454–7025] *n* = 71		283 [20.5–625] *n* = 71		398 [112–1622] *n* = 89		99,6 [32.2–674] *n* = 46		60,1 [8.5–376] *n* = 35	

Note. The Kruskal – Wallis test was used for comparison; differences are statistically significant at $p < 0.05$.

However, previous studies highlight the utility of therapeutic drug monitoring in assessing adherence, determining dosage, and predicting ARVs efficacy in children living with HIV [12–16]. A 2002 review of 17 clinical trials examining factors that contribute to ART success or failure in children showed higher rates of virologic efficacy (60–90 %) in patients who had adherence assessed using TDM [17]. Other studies have questioned the value of routine use of therapeutic drug monitoring of ART in children and adolescents living with HIV. Other studies have questioned the value of routine use of therapeutic drug monitoring of ARVs in children and adolescents living with HIV. A.E. Engelbrecht et al. retrospectively reviewed indications for antiretroviral TDM requests at Tygerberg Children's Hospital (Cape Town, South Africa) from January 2012 to June 2017 and found that the majority of TDM requests were in children with suspected lopinavir non-adherence (83 % – 53 of 64 children), therapy inconsistency, and lopinavir-rifampicin drug interactions. Plasma lopinavir concentrations, although expected to be low for these indications, were highly variable and were detected in the main therapeutic range (> 1000 ng/ml) [18].

Lopinavir 1000 ng/ml has been shown to provide adequate lopinavir exposure in ART-naive patients, and this level is used as a target for TDM in both adults and children [19]. Lopinavir/ritonavir (LPV/r) is licensed for use in children aged 14 days. Due to cardiac and metabolic toxicity, and the risk of adrenal insufficiency, LPV/r should not be used in children aged < 14 days. Lopinavir can be administered as a liquid or tablet formulation (for children weighing > 15 kg). LPV/r doses of 230/57.5 mg/m^2 and 300/75 mg/m^2 are consistent with US Food and Drug Administration (FDA) guidelines [20] and have shown adequate efficacy and acceptable toxicity in randomized trials [21]. However, concerns have been raised about the established lopinavir exposure levels achieved with the 230/57.5 mg/m^2 dose due to the risk of viral resistance, particularly in children < 2 years of age who have increased lopinavir clearance. Because of these concerns, the 300/75 mg/m^2 LPV/r dose is recommended for treatment-experienced children of all ages, and the 230/57.5 mg/m^2 dose may be used in treatment-naive children > 1 year of age. Furthermore, N. Rakhmanina et al. concluded that the current LPV/r dosing strategy for the ART-experienced appears adequate for treatment of children infected with wild-type virus but is unlikely to be sufficient for viruses with lopinavir resistance. Therefore, the authors conclude that in such cases, patients would benefit from TDM and viral resistance testing [22]. In a modeling study, J. Yang et al. from the School of Pharmacy and Pharmaceutical Sciences at the University of California observed the effects of tablet formulations and aging on the effects of lopinavir. A sharp decrease in clearance with the onset was noted in the first 2 years of life, and an increase in bioavailability was observed when children were switched from the liquid to the tablet form, indicating the need to reassess the current dose of lopinavir [23].

We conducted TDM in children and adolescents living with HIV infection. Statistically significant differences in lopinavir concentrations were shown between children from age groups 3 and 4: the lowest lopinavir concentration was detected in children over 11 years old and amounted to 3782 [2117–5046] ng/ml versus 5614 [3521–7264] ng/ml in the third group ($p = 0.011$).

We did not detect any differences in nucleoside reverse transcriptase inhibitors (lamivudine, abacavir, zidovudine) concentrations in the different age groups analyzed. Intracellular concentrations of some nucleoside reverse transcriptase inhibitors are known to correlate with markers of therapeutic efficacy, such as viral load reduction and CD4 increase [24]. Measuring intracellular concentrations of the active compound is expensive and labor-intensive, and therefore these concentrations are not usually targeted for TDM except for research purposes. Unfortunately, the dose or plasma concentration of the parent compound correlates poorly with the intracellular concentration of the active form of the drug in the target cell [25].

It is known that adherence to ART in children and adolescents depends largely on a number of factors, including age, gender of the child, disclosure of information about HIV status, attitude to treatment, complexity of treatment regimens, mental health (of the child and caregiver), family status (e.g., orphan status), timely visits to the clinic, as well as residence in a rural or urban area [7].

CONCLUSION

Increasing adherence to ART is a relevant and global task that allows reducing the further spread of HIV infection. One of the methods for assessing adherence to ART is therapeutic drug monitoring, and its use in the presented study made it possible to identify some factors influencing adherence to therapy in children living with HIV infection. Such factors include the patient's age and antiretroviral drug. Thus, it was shown that the highest frequency of undetectable concentrations was found for zidovudine, and the lowest – for lamivudine. Adolescents have statistically significantly lower concentrations of lopinavir than children of other age groups. We believe that the comprehensive use of adherence assessment, including therapeutic drug monitoring, will support efforts to develop simplified safe and effective dosages of ARVs in the pediatric population.

Conflicts of interest

No potential conflict of interest relevant to this article reported.

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CARDIOLOGY

CONTEMPORARY ALGORITHMS FOR DIAGNOSING OBSTRUCTIVE CORONARY ARTERY DISEASE IN REAL CLINICAL PRACTICE

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ABSTRACT

Background. *Despite the high evidence level of the currently existing international recommendations on stable coronary heart disease (CHD) and chronic coronary syndrome, their implementation in domestic clinical practice is insufficient.*
The aim of the work. *To analyze the choice of diagnostic tactics (non-invasive and invasive) in patients with suspected obstructive coronary heart disease in real clinical practice.*
Methods. *The study included outpatients with suspected obstructive CHD, in whom the pre-test probability (PTP) of obstructive CHD was determined; if PTP = 5–15 %, clinical probability was assessed based on CHD risk factors. Based on the results of coronary angiography, the following groups were identified: group I – obstructive lesion of the coronary arteries (≥ 70 %) (n = 50); group II – non-obstructive lesion of the coronary arteries (< 70 %) (n = 32); group III – intact coronary arteries (n = 40).*
Results. *According to the results of coronary angiography, the frequency of detection of obstructive lesion of the coronary arteries was 42 % (in patients without past medical history of myocardial infarction – 31 %). Before performing coronary angiography, non-invasive tests were performed in 2.5 % of cases. Pain in the chest was represented by typical angina in 74 % of patients, with no difference in frequency in all groups. PTP values were statistically significantly higher in the group with obstructive CHD (median – 32 %), however, in the other two groups, PTP values corresponded to a high risk of obstructive CHD (median – 27 % and 21 %, respectively). PTP was an independent predictor for obstructive CHD and subsequent myocardial revascularization.*
Conclusion. *In the cohort of outpatients with suspected coronary heart disease we examined during invasive coronary angiography, the frequency of obstructive lesion of the coronary arteries remains low. Non-invasive tests were performed in isolated cases, while PTP was an independent predictor for obstructive CHD and subsequent myocardial revascularization. To increase the frequency of detection of obstructive coronary heart disease, we should adhere to the diagnostic algorithms of the European Society of Cardiology and make wider use of non-invasive imaging tests.*

Key words: *chronic coronary syndrome, pre-test probability, clinical probability, coronary angiography, clinical practice, diagnostic algorithm*

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СОВРЕМЕННЫЕ АЛГОРИТМЫ ДИАГНОСТИКИ ОБСТРУКТИВНОЙ ИШЕМИЧЕСКОЙ БОЛЕЗНИ СЕРДЦА В РЕАЛЬНОЙ КЛИНИЧЕСКОЙ ПРАКТИКЕ

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РЕЗЮМЕ

Обоснование. *Несмотря на высокий доказательный уровень существующих на сегодняшний день международных рекомендаций по стабильной ишемической болезни сердца (ИБС) и хроническому коронарному синдрому, их внедрение в отечественную клиническую практику является недостаточным.*

Цель работы. *Провести анализ выбора диагностической тактики (неинвазивной и инвазивной) у пациентов с подозрением на обструктивную ишемическую болезнь сердца в реальной клинической практике.*

Методы. *В исследование включены амбулаторные пациенты с подозрением на обструктивную ИБС, у которых определяли предтестовую вероятность (ПТВ) обструктивной ИБС; при ПТВ = 5–15 % оценивали клиническую вероятность на основе факторов риска ИБС. По результатам коронароангиографии (КАГ) были выделены: группа I – обструктивное поражение коронарных артерий (КА) (≥ 70 %) (n = 50); группа II – необструктивное поражение КА (< 70 %) (n = 32); группа III – интактные КА (n = 40).*

Результаты. *По данным КАГ частота выявления обструктивных поражений КА составила 42 % (у больных без инфаркта миокарда в анамнезе – 31 %). Перед проведением КАГ неинвазивные тесты выполнены в 2,5 % случаев. Болевой синдром в грудной клетке был представлен типичной стенокардией у 74 % больных, не различаясь по частоте во всех группах. Значения ПТВ были статистически значимо выше в группе обструктивной ИБС (медиана – 32 %), однако и в двух других группах значения ПТВ соответствовали высокому риску наличия обструктивной ИБС (медиана – 27 % и 21 % соответственно). ПТВ была независимым предиктором обструктивной ИБС и последующей реваскуляризации миокарда.*

Заключение. *В обследованной нами когорте амбулаторных больных с подозрением на ИБС при проведении инвазивной КАГ частота выявления обструктивных поражений коронарных артерий остаётся невысокой. Неинвазивные тесты были проведены в единичных случаях, в то же время ПТВ была независимым предиктором обструктивной ИБС и последующей реваскуляризации миокарда. Для увеличения частоты выявления обструктивной ИБС следует придерживаться диагностических алгоритмов Европейского общества кардиологов, шире использовать неинвазивные визуализирующие тесты.*

Ключевые слова: *хронический коронарный синдром, предтестовая вероятность, клиническая вероятность, коронарная ангиография, клиническая практика, диагностический алгоритм*

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INTRODUCTION

In the diagnostics of coronary heart disease (CHD), the following contradiction is currently unresolved: on the one hand, early detection of obstructive lesions of the coronary arteries (CA) is possible for carrying out possible myocardial revascularization procedures; on the other hand, with invasive coronary angiography (ICA) in patients with stable CHD, the frequency of detection of such lesions remains low [1–4]. Thus, according to data from a foreign national registry study, obstructive lesions of the CA were detected in 37.6 % of cases [1], while in domestic publications the results vary greatly depending on the cohort of patients examined [2] and the criteria for coronary obstruction [5]. In general, throughout Russia, only about 50 % of ICAs end in myocardial revascularization [6], but it should be taken into account that both emergency and planned ICAs are considered, therefore the diagnostic effectiveness of planned ICA in domestic centers apparently does not differ from foreign data [2]. At the same time, in foreign countries, diagnostic algorithms in real clinical practice differed (wide use of non-invasive tests – both functional, with detection of myocardial ischemia, and anatomical) from those used in most Russian clinics (clinical assessment and electrocardiographic (ECG) stress test). Although attempts were made in Russian centers to expand the range of non-invasive assessment in patients with suspected coronary heart disease, they were not very successful, and the frequency of such examinations remained low [7, 8].

Apparently, the adoption of a new diagnostic algorithm for CHD, presented in the 2019 European Society of Cardiology (ESC) guidelines [9], can change this situation. In any case, the recently published results of the EURECA (European Registry of Cardiac Arrest) showed that when these recommendations are followed, the frequency of detection of obstructive coronary artery lesions increases [10]. Several Russian centers also took part in this study, but these data have not yet been published. Meanwhile, it is interesting to compare the extent to which new diagnostic algorithms are currently used. This served as the basis for this study, and the purpose was to analyze the choice of diagnostic tactics (non-invasive and invasive) in patients with suspected obstructive coronary heart disease in real clinical practice.

MATERIALS AND METHODS

In this cohort retrospective study, 133 patients with suspected obstructive coronary artery disease were selected using the continuous sampling method. They visited the outpatient clinic of the Research Institute for Complex Issues of Cardiovascular Diseases in 2022 and were selected by cardiologists for ICA.

All patients underwent pain assessment (presence of typical, atypical angina or non-anginal pain), as well as dyspnea, assessed as equivalent to angina. Based on the results of pain assessment, age and gender, pretest probability (PTP) of obstructive coronary artery disease was determined in patients according to the table proposed by the ESC experts [9]. In case of intermediate PTP values (5–15 %), clinical probability of obstructive coronary artery disease was assessed in patients based on the presence or absence of coronary artery disease risk factors (dyslipidemia, arterial hypertension, diabetes mellitus, smoking, heredity) [11]. Some patients underwent non-invasive tests (stress echocardiography (stress ECHO) with physical activity or computed tomography (CT) angiography of the coronary artery) at the discretion of the attending physician.

Ten patients (7.2 %) refused ICA; in one case (0.7 %) the patient died before ICA. As a result, ICA was performed in 122 patients, which constituted 91.7 % of the entire sample. All patients underwent invasive assessment of the coronary bed using an INNOVA 3100 angiographic apparatus (USA) using the standard technique. Based on the ICA results, three groups of patients were identified depending on the hemodynamic significance of the coronary artery lesion: group I – obstructive coronary artery lesion (≥ 70 %; n = 50 – 36.0 %); group II – non-obstructive coronary artery lesion (< 70 %; n = 32 – 23.0 %); group III – intact coronary arteries (n = 40 – 28.8 %).

The choice of surgical treatment tactics was carried out by a multidisciplinary team (cardiovascular surgeon, endovascular surgeon, cardiologist) based on clinical and instrumental data, the significance of coronary atherosclerosis, existing clinical guidelines and internal algorithms of the institution.

The groups were compared based on initial clinical and anamnestic data, the nature of chest pain syndrome, the results of PTP assessment, and clinical probability. We also studied the chosen tactics of surgical treatment of obstructive coronary artery lesions and/or other cardiac pathology.

The study is conducted in accordance with the fundamental topic of the Research Institute for Complex Issues of Cardiovascular Diseases "Development of innovative models for managing the risk of developing diseases of the circulatory system taking into account comorbidity based on the study of fundamental, clinical, epidemiological mechanisms and organizational technologies of medical care in the industrial region of Siberia" (state registration No. 122012000364-5 dated January 20, 2022). The study was carried out in compliance with the principles of the World Medical Association Declaration of Helsinki; the study protocol and the informed consent form were approved by the local Ethics Committee (LEC) of the institution (LEC protocol No. 20191121 dated November 21, 2019).

Statistical processing of the material was performed using the Statistica 10.0 software package (StatSoft Inc., USA). The distribution of quantitative data was checked using the Shapiro – Wilk criterion. Considering that the distribution of all quantitative features differed from normal, they are presented as median,

upper and lower quartiles (Me (Q25; Q75). The Kruskal – Wallace, Mann – Whitney, and χ^2 criteria were used to compare groups. With a small number of observations, Fisher's exact test with Yates' correction was used. The Bonferroni correction was used to solve the problem of multiple comparisons. Multiple logistic regression analysis (Forward Stepwise LR method) was performed to assess the factors associated with the presence of obstructive coronary artery disease and subsequent myocardial revascularization. The independent variables included in the models were risk factors, gender, age, and chest pain symptoms, presence of comorbid pathology, PTP values, and noninvasive test data. Calculations were performed both for the entire examined cohort and separately for patients without myocardial infarction in medical history. The ability of various indicators to predict the presence of obstructive coronary lesions was assessed using ROC-analysis. The level of statistical significance (p) was taken to be 0.05.

RESULTS

When assessing the clinical and demographic parameters (table 1), the groups were comparable in age (p = 0.798), prevalence of arterial hypertension, presence of hypercholesterolemia, obesity, and stroke in medical history. In group I (obstructive coronary artery disease), men prevailed (72 %), in group III (intact coronary arteries), there were more women (67.5 %), and in group II (non-obstructive coronary artery disease), men accounted for 53.1 % ($p < 0.001$ for trend).

Patients with obstructive coronary artery disease were statistically significantly more likely to smoke (50 %) compared to patients in groups II (34.4 %) and III (25.6 %), but this difference did not reach statistical significance (p = 0.065). Patients in group I were more likely to have myocardial infarction in medical history (46 %; $p < 0.001$ for trend), but in the other two groups (non-obstructive coronary artery disease and intact

TABLE 1

COMPARATIVE CHARACTERISTICS OF THE MAIN CLINICAL AND DEMOGRAPHIC INDICATORS IN THE STUDIED GROUPS

Indicators	Group I: obstructive CA (*n = 50*)	Group II: non-obstructive CA (*n = 32*)	Group III: intact CA (*n = 40*)	*p*
Age (years), Me (LQ; UQ)	66.0 (61.0; 71.0)	67.0 (61.0; 73.0)	66.0 (60.5; 72.0)	0.798
Men, *n* (%)	32 (72.0)	17 (53.1)*	13 (32.5)*; #	< 0.001
Smoking, *n* (%)	25 (50.0)	11 (34.38)	9 (25.7)	0.065
Obesity, *n* (%)	14 (28.0)	10 (31.3)	13 (32.5)	0.891
Hypercholesterolemia, *n* (%)	50 (100.0)	30 (93.75)	38 (95.0)	0.227
PIC in medical history, *n* (%)	23 (46.0)	8 (25.0)*	3 (7.5)*; #	< 0.001
AH in medical history, *n* (%)	50 (100.0)	32 (100.0)	38 (95.0)	0.124
CVA in medical history, *n* (%)	8 (16.0)	5 (15.6)	4 (10.0)	0.681
DM in medical history, *n* (%)	5 (10.2)	6 (19.4)	10 (25.0)	0.179
PCI in medical history, *n* (%)	12 (24.0)	8 (25.0)	0	0.032
CABG in medical history, *n* (%)	4 (8.0)	3 (9.4)	0	0.157
CE in medical history, *n* (%)	1 (2.0)	0	0	0.483
AFib in medical history, *n* (%)	8 (16.0)	7 (21.9)	15 (37.5)	0.057
CHFpEF in medical history, *n* (%)	2 (4.0)	3 (3.13)	0	0.458
CKD in medical history, *n* (%)	3 (6.0)	2 (6.25)	0	0.279
AL BCA in medical history, *n* (%)	10 (20.0)	5 (15.6)	5 (12.5)	0.627
MFA in medical history, *n* (%)	10 (20.0)	5 (15.6)	0	0.013

Note. PIC – postinfarction cardiosclerosis; AH – arterial hypertension; CVA – acute cerebrovascular accident; DM – diabetes mellitus; PCI – percutaneous coronary intervention; CABG – coronary artery bypass grafting; CE – carotid endarterectomy; AFib – atrial fibrillation; CHFpEF – chronic heart failure with preserved ejection fraction; CKD – chronic kidney disease; AL BCA – atherosclerotic lesion of the brachiocephalic arteries; MFA – multifocal atherosclerosis; * – $p < 0.05$ compared to group I; # – $p < 0.05$ compared to group II.

coronary arteries), postinfarction cardiosclerosis was also noted (in 25 % and 7.5 % of cases, respectively).

According to clinical symptoms (table 2), typical angina was more often present in patients of group I with obstructive coronary artery disease (in 90 % of cases), while in groups II and III – only in 75 % of cases. Atypical pain prevailed in groups II and III (in 18.8 % and 17.5 % of cases, respectively), while in group I – only in 9.0 % of patients. Nevertheless, the differences between the groups in the nature of the pain syndrome did not reach statistical significance. Patients with dyspnea were equally common in all groups of subjects.

The analysis of clinical probability (table 3) did not show statistically significant differences between the groups, and it was 14.0 in each group. The pre-test probability of CHD was higher in group I with obstructive coronary artery disease and averaged 32 %; in the group with non-obstructive coronary artery disease – 27 %, in the group with intact coronary arteries – 21 % ($p < 0.001$ for trend).

Patients with PTP less than 5 % were not referred for invasive examination – there were no such patients in our cohort; there were also no patients with PTP from 5 % to 15 % in group I. Statistically significantly more patients with PTP = 5 – 15 % were in the group of intact coronary arteries (20 %) than in the group of non-obstructive coronary artery disease (6.25 %; $p = 0.002$). PTP was more than 15 % in all patients with obstructive coronary artery disease, in 93.75 % of patients with non-obstructive coronary artery disease, and in 80 % of patients with intact coronary arteries ($p = 0.0024$). Non-invasive tests before ICA were performed only in 2.5 % of patients; in these patients, despite the positive test, the coronary arteries were intact.

Percutaneous coronary intervention was performed in 76 % of patients with obstructive coronary lesions (table 4); coronary artery bypass grafting was not performed in the examined patients. Carotid endarterectomy was performed in 3 (6.0 %) patients from the group with obstructive coronary lesions. Radiofrequency

TABLE 2

CLINICAL SYMPTOMS IN COMPARISON WITH THE RESULTS OF CORONARY ANGIOGRAPHY

Indicators	Group I: obstructive CA (*n* = 50)	Group II: non-obstructive CA (*n* = 32)	Group III: intact CA (n = 40)	*p*
Typical pain, *n* (%)	45 (90.0)	24.0 (75.0)	30 (75.0)	0.125
Atypical pain, *n* (%)	3 (6.0)	6 (18.8)	7 (17.5)	0.071
Non-anginal pain, *n* (%)	3 (6.0)	1 (3.13)	2 (5.0)	0.923
Dyspnea, *n* (%)	2 (4.0)	1 (3.13)	1 (2.5)	0.953

TABLE 3

ASSESSMENT OF THE PRE-TEST PROBABILITY OF CORONARY HEART DISEASE DEPENDING ON THE DEGREE OF CORONARY ARTERY STENOSIS

Indicators	Group I: obstructive CA (*n* = 50)	Group II: non-obstructive CA (*n* = 32)	Group III: intact CA (*n* = 40)	*p*
PTP (%), Me (LQ; UQ)	32.0 (27.0; 44.0)	27.0 (18.0; 44.0)*	21.0 (16.0; 27.0)*,#	< 0.001
PTP < 5 %, *n* (%)	0	0	0	–
PTP = 5–15 %, *n* (%)	0	2 (6.25)	8 (20.0)#	0.002
PTP > 15 %, *n* (%)	50 (100.0)	30 (93.75)*	32 (80.0)*,#	0.0024
Clinical probability (%), Me (LQ; UQ)	14.0 (11.0; 14.0)	14.0 (14.0; 19.0)	14.0 (10.0; 14.0)	0.551
Non-invasive tests, *n* (%)	0	0	1 (2.5)	0.355

Note. * – $p < 0.05$ compared to group I; # – $p < 0.05$ compared to group II.

catheter ablation was performed in 2 (4.0 %) patients from the obstructive coronary lesions group and in 1 (3.13 %) patient in the non-obstructive coronary lesions group. A pacemaker was installed only in 1 (3.13 %) patient in the non-obstructive coronary lesions group.

In the binary logistic regression model, the independent factors associated with the presence of obstructive coronary lesions in the entire cohort of patients examined were myocardial infarction in medical history and PTP values (table 5). This logistic regression model was statistically significant ($\chi^2(2) = 24.936$;

TABLE 4

TACTICS OF SURGICAL TREATMENT DEPENDING ON THE DEGREE OF CORONARY ARTERY STENOSIS

Indicators	Group I: obstructive CA (*n* = 50)	Group II: non-obstructive CA (*n* = 32)	Group III: intact CA (n = 40)	*p*
PCI, *n* (%)	38 (76.0)	0	0	< 0.001
CABG, *n* (%)	0	0	0	–
CE, *n* (%)	3 (6.0)	0	0	0.109
RFCA, *n* (%)	2 (4.0)	1 (3.13)	0	0.457
PM, *n* (%)	0	1 (3.13)	0	0.242
Heart valve replacement, *n* (%)	0	1 (3.13)	0	0.242

Note. PCI – percutaneous coronary intervention; CABG – coronary artery bypass grafting; CE – carotid endarterectomy; RFCA – radiofrequency catheter ablation; PM – pacemaker.

TABLE 5

FACTORS ASSOCIATED WITH OBSTRUCTIVE CORONARY HEART DISEASE AND SUBSEQUENT MYOCARDIAL REVASCULARIZATION ACCORDING TO BINARY LOGISTIC REGRESSION ANALYSIS (FORWARD STEPWISE LR METHOD)

Factors	B	S.E.	Wald	df	Sig.	Exp(B)
Obstructive coronary artery disease (entire cohort of subjects)						
Myocardial infarction in medical history	1.454	0.469	9.612	1	0.002	4.280
PTP	0.057	0.019	9.429	1	0.002	1.059
Constant	–2.504	0.629	15.866	1	0.000	0.082
Obstructive coronary artery disease (patients without myocardial infarction in medical history)						
PTP	0.088	0.025	12.322	1	0.000	1.092
Carotid artery atherosclerosis	1.672	0.711	5.537	1	0.019	5.324
Constant	–3.749	0.872	18.504	1	0.000	0.024
Conducting myocardial revascularization after ICA (the entire cohort of subjects examined)						
Myocardial infarction in medical history	1.242	0.451	7.591	1	0.006	3.463
PTP	0.071	0.019	13.452	1	0.000	1.073
Constant	–3.658	0.713	26.353	1	0.000	0.026
Conducting myocardial revascularization after ICA (patients without myocardial infarction in medical history)						
Obesity	1.850	0.690	7.191	1	0.007	6.360
PTP	0.101	0.030	11.258	1	0.001	1.107
Multifocal atherosclerosis	2.769	1.228	5.084	1	0.024	15.947
Constant	–5.629	1.256	20.074	1	0.000	0.004

$p < 0.001$). The model explained 26.4 % (Nagelkerke R2) of the variance in obstructive coronary lesions and correctly classified 71.1 % of cases. In the cohort of patients without myocardial infarction in medical history, PTP and the presence of carotid artery atherosclerosis were precursors of obstructive coronary lesions ($\chi^2(2) = 20.646$; $p < 0.001$; Nagelkerke R2 = 31.7 %; correct classification in 74.1 % of cases). In patients undergoing myocardial revascularization, predictors of obstructive coronary artery disease were PTP, obesity, and multifocal atherosclerosis ($\chi^2(3) = 24.878$; $p < 0.001$; Nagelkerke R2 = 37.3%; correct classification in 87.2 % of cases).

According to the ROC analysis (fig. 1), in patients without CHD in medical history, the PTP values to the greatest extent predicted the presence of obstructive coronary artery disease (AUC = 0.747), in contrast to other studied indicators (gender, age, nature of chest pain, risk factors).

DISCUSSION

The present study yielded several interesting results. First, the frequency of detection of obstructive coronary lesions remains low (42 % overall among all examined patients and 31 % in patients without CHD in medical history). Second, non-invasive tests are still performed episodically. Third, the determination of PTP is used in the practice of cardiologists, which indicates the absence of patients with PTP < 5 % among those referred for ICA. In addition, PTP assessment remains the most informative indicator in predicting the presence of obstructive CHD.

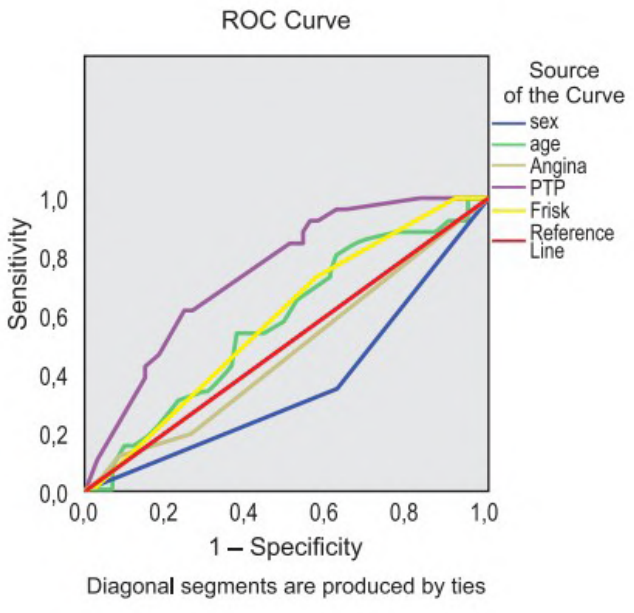

FIG. 1.
ROC-analysis: using various indicators in the detection of obstructive lesion of the coronary arteries in coronary angiography

According to the KUGH registry (South Korea), over a ten-year period (2004–2014), obstructive CHD was detected in 41.4 % of cases during ICA [4]. Obstructive coronary artery lesions were detected somewhat more frequently in the study by M. Gonçalves et al. [3] – in 46 % of cases. However, this work had some peculiarities in referring patients for ICA (only patients with angina were included, excluding possible angina equivalents, changes in resting ECG or ECHO were not considered as a positive test), which contributed to the formation of a more selective sample of patients. In the EURECA, the overall incidence of obstructive CHD in the examined cohort of patients during ICA was 45 % (the percentage was increased due to the fact that the examination algorithm proposed by the ESC was followed during the examination of some patients). According to our clinic, the frequency of detection of obstructive CHD in the specified registry was 43.3 %, which is quite comparable with the data of the present study.

However, the implementation of non-invasive tests for the diagnosis of CHD in our country differs significantly from foreign countries. Thus, in the EURECA, stress ECG was performed in 32 % of patients, and most often it was performed in patients with PTP ≤ 5 % (in 50 % of cases), progressively decreasing in groups with higher PTP, with left ventricular ejection fraction (LVEF) < 50 % and with previous CHD. Among non-invasive imaging tests, CT angiography of the CA was performed in 24 % of patients, more often in patients with normal left ventricular function and without previous CHD. Stress imaging was performed in 41 % of patients, mainly using single-photon emission CT (SPECT) or echocardiography (23 % and 16 %, respectively), and its use gradually increased from groups with lower PTP to groups with higher PTP, LVEF < 50 %, and/or previous CHD. ICA was performed in 29 % of patients, and in a significant proportion (17 %) as the first imaging test [10]. The choice of diagnostic tests was determined by several factors (PTP, EF, and CHD in medical history), and based on these factors optimal diagnostic algorithms were proposed according to previously proposed guidelines [9]. Only 20 % of patients did not undergo imaging tests (non-invasive or invasive). According to our clinic, in this registry, bicycle ergometry was performed in 7.5 % of patients, stress ECHO in 1 %, SPECT in 11.5 %, and CT angiography of the CA in 0.5 %. When analyzing the registries of patients who underwent hospital examination and treatment in 2017–2019 for CHD in several large cardiac surgery centers of the Russian Federation, the frequency of stress ECG was 4.88 %, stress ECHO in 0.19 %, and CT angiography in 0.05 % [8]. It can be noted that in domestic registry studies, non-invasive tests before coronary artery disease are rather an exception, while in foreign countries, on the contrary, such an exception is the failure to perform non-invasive tests.

It is obvious that in domestic clinical practice it is necessary to focus on clinical symptoms and assessment of PTP [8, 12]. In the present study it was shown

that the nature of the pain syndrome did not differ in groups with different degrees of coronary artery disease. At the same time, the median PTP value was statistically significantly higher in the obstructive CHD group compared to the other two groups; PTP also had an independent association with the detection of obstructive CHD. Nevertheless, focusing only on PTP in the diagnosis of CHD has its limitations. As can be seen from our data, the median PTP values in groups with insignificant coronary artery disease and their absence was within the high risk of the presence of CHD. As a result, the overall frequency of obstructive CHD detection turned out to be relatively low. However, a paradox is revealed: despite the minimal number of non-invasive tests in domestic studies, the frequency of detection of obstructive coronary artery lesions is comparable with the data of foreign studies, where these tests are carried out in the overwhelming majority of cases. Therefore, a logical question arises: how then is it possible to increase the productivity of ICA in detecting obstructive CHD?

A recent analysis showed that among non-invasive imaging methods for detecting myocardial ischemia, stress magnetic resonance imaging and positron emission tomography were the most effective [13]. However, the widespread use of these methods in routine clinical practice will not happen anytime soon. At the same time, the results of the EURECA study showed that following the algorithm proposed in the ESC recommendations [9] allows achieving the following results: a decrease in the number of ICAs performed (from 48 % in patients not following the diagnostic algorithm to 15 % in the group following it) and an increase in the number of detected obstructive changes in the coronary arteries (from 39 % to 60 %, respectively) and subsequent myocardial revascularizations (from 37 % to 54 %, respectively) [10]. However, adherence to the recommendations varies greatly depending on the region. Thus, in Western European countries it was 87 %, and in Eastern European countries – 48 % [10]. According to our clinic, which also participated in the study, adherence to the algorithm was only 23.4 % (Shcheglova A.V., report at the Congress of the Russian Society of Cardiology, 2021). Therefore, one of the ways to improve the diagnosis of obstructive CHD is to increase adherence to the proposed diagnostic scenarios. Apparently, multispiral computed tomography CA should be used more widely. Firstly, the assessment of the calcium index allows more than half of patients to be classified in the low clinical risk category in the CACS-CL (Coronary Artery Calcium Score – Clinical Likelihood) model, which does not require further non-invasive or invasive examination [11]. Secondly, the use of CT angiography of the CA allows to reduce the number of ICA [14], while significantly improving the detection rate of obstructive coronary lesions [14, 15]. Interestingly, in the article by J.R. Weir-McCall et al. [14] the results of the implementation in the UK of the diagnostic approach proposed in the NICE (National Institute for Health and Care Excellence) CG95 guidelines were analyzed [16]. The essence of this algorithm is to refuse to assess the PTP, and in patients with typical and atypical angina, CT coronary angiography should be immediately performed, and if it is uninformative, non-invasive functional tests should be immediately performed [17]. That is, at the moment, real clinical practice has confirmed the effectiveness of two algorithms for diagnosing obstructive CHD (European Society of Cardiology 2019 and NICE CG95) [18]. Therefore, a prospective randomized study OPERATE is currently planned in China, which plans to compare the ability of these algorithms to identify low-risk patients (which will be additionally verified by CT angiography data) [19].

In addition, the diagnostic algorithms should take into account the results of the ISCHEMIA study [20, 21], which did not demonstrate an improvement in prognosis from the primary invasive strategy for patients with stable CHD and moderate to high risk stress test results compared with optimal drug therapy. As a result, there are proposals not to use non-invasive tests, in particular, which showed their ineffectiveness as a "gatekeeper" in the recent study by J. Jo et al. [22]. When analyzing these results, it is proposed to discuss the following tactics for managing patients with suspected CHD: starting not with the choice of tests, but with the treatment of modifiable risk factors, including consideration of the appointment of antiplatelet therapy and statins. The decision on the need for testing or its postponement is made after assessing the effectiveness of drug therapy. Changes in angina symptoms, quality of life, and coronary artery disease test results will allow the clinician to modify drug therapy and consider the need for revascularization [23]. Without a doubt, the application of this paradigm is possible only after comparing it with the traditional approach in randomized clinical trials.

This study has a number of limitations that should be taken into account when interpreting its results. First, the study included a small number of patients. Nevertheless, despite this, we were able to obtain statistically significant results. In addition, our data are quite consistent with the results of previous studies in our center [2], as well as large multi-thousand registries – both domestic [8] and foreign [1, 4, 10]. Secondly, this study is a single-center study, so its results cannot be translated into the CHD diagnostics in other regions. Nevertheless, we tried to level out this limitation by analyzing our experience of participation in the international EURECA. Thirdly, we included patients referred for ICA in the analysis. In the case of assessing coronary artery disease using coronary artery CT angiography, the frequency of detection of obstructive lesions could be significantly lower, as shown by recent studies [24].

CONCLUSION

In the cohort of outpatients with suspected CHD, the frequency of detection of obstructive coronary

artery lesions during ICA remains low. Non-invasive tests were performed in isolated cases, while PTP was an independent predictor of obstructive CHD and subsequent myocardial revascularization. To increase the frequency of detection of obstructive coronary artery disease, it is necessary to adhere to the diagnostic algorithms of the European Society of Cardiology and to use non-invasive imaging tests more widely.

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Conflicts of interest

No potential conflict of interest relevant to this article reported.

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MICROBIOLOGY AND VIROLOGY

ANTAGONISTIC ACTIVITY OF MONOCULTURES AND CONSORTIA OF LACTOBACILLI AGAINST MULTIDRUG-RESISTANT ISOLATES OF OPPORTUNISTIC BACTERIA AS A SCREENING OF THEIR PROBIOTIC POTENTIAL

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ABSTRACT

Background. *In recent years, special attention has been paid to the studying the consortia of probiotic bacteria. In these associations, the properties of individual microorganisms can be enhanced, in particular, their antagonistic activity which is an effective indicator for screening of probiotic potential. The development of probiotics based on such consortia with antibacterial properties is critical in the light of the growing problem of drug resistance in microorganisms.*

The aim of the work. *To study the antagonistic activity of monocultures and consortia of lactobacilli against multidrug-resistant isolates of opportunistic bacteria.*

Materials and methods. *The antagonistic activity of lactobacilli monocultures and their consortia was assessed simultaneously by two methods: the cross streak method and the well diffusion method.*

Results. *All strains of lactobacilli and their consortia, depending on the research method, had varying degrees of antagonistic activity. Five consortia had stronger antagonism to test cultures as compared to monocultures, while in one consortium, the effect of antagonistic activity was reduced compared to monocultures. The results of studying the antagonistic activity of two consortia (Limosilactobacillus fermentum 44/1 and Lacticaseibacillus rhamnosus 12L, Latilactobacillus curvatus LCR-111-1 and Lactiplantibacillus plantarum 8PAZ) contradict data on the biocompatibility of strains in these consortia. Differences in the degree of antagonistic effects of lactobacilli on gram-positive and gram-negative species of opportunistic bacteria were revealed.*

Conclusion. *The study showed that both the biocompatibility of the probiotic strains and the antagonistic activity of the consortium are the important requirements for creating a probiotic consortium with effective probiotic potential. To study the antagonistic properties of lactobacilli, the number of isolates of target gram-positive and gram-negative bacteria and normobiota should be increased. This will allow us to determine effective strategies for using probiotics in conditions of the spread of drug resistance of microorganisms.*

Key words: *lactobacilli, probiotic consortia, probiotic potential, antagonistic activity, opportunistic bacteria, multidrug-resistant isolates*

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АНТАГОНИСТИЧЕСКАЯ АКТИВНОСТЬ МОНОКУЛЬТУР И КОНСОРЦИУМОВ ЛАКТОБАЦИЛЛ В ОТНОШЕНИИ ПОЛИРЕЗИСТЕНТНЫХ ИЗОЛЯТОВ УСЛОВНО-ПАТОГЕННЫХ БАКТЕРИЙ КАК СКРИНИНГ ИХ ПРОБИОТИЧЕСКОГО ПОТЕНЦИАЛА

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РЕЗЮМЕ

Актуальность. *В последние годы особое внимание уделяется изучению консорциумов пробиотических бактерий. В этих ассоциациях свойства отдельных микроорганизмов могут усиливаться, в частности их антагонистическая активность, которая является эффективным показателем для скринига пробиотического потенциала. Разработка пробиотиков на основе таких консорциумов с антибактериальными свойствами имеет решающее значение в свете растущей проблемы лекарственной устойчивости микроорганизмов.*
Цель работы. *Изучение антагонистической активности монокультур и консорциумов лактобацилл в отношении полирезистентных изолятов условно-патогенных бактерий.*
Материалы и методы. *Антагонистическую активность монокультур лактобацилл и их консорциумов оценивали параллельно двумя методами: методом перпендикулярных штрихов и методом лунок.*
Результаты. *Все штаммы лактобацилл и их консорциумы в зависимости от метода исследования обладали разной степенью антагонистической активности. В пяти консорциумах антагонизм к тестовым культурам был сильнее, чем в монокультурах, в то время как в одном консорциуме эффект антагонистической активности снизился по сравнению с монокультурами. Результаты исследования антагонистической активности двух консорциумов (Limosilactobacillus fermentum 44/1 и Lacticaseibacillus rhamnosus 12L, Latilactobacillus curvatus LCR-111-1 и Lactiplantibacillus plantarum 8PA3) противоречат данным о биосовместимости штаммов в этих консорциумах. Выявлены различия в степени антагонистического воздействия лактобацилл на грамположительные и грамотрицательные виды условно-патогенных бактерий.*
Заключение. *Исследование показало, что важными требованиями для создания пробиотического консорциума с эффективным пробиотическим потенциалом являются как биосовместимость пробиотических штаммов, так и антагонистическая активность консорциума. Для изучения антагонистических свойств лактобацилл следует увеличить количество изолятов целевых грамположительных и грамотрицательных бактерий и нормобиоты. Это позволит определить эффективные стратегии применения пробиотиков в условиях распространения лекарственной устойчивости микроорганизмов.*

Ключевые слова: *лактобациллы, пробиотические консорциумы, пробиотический потенциал, антагонистическая активность, условно-патогенные бактерии, полирезистентные изоляты*

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BACKGROUND

Recently, the human body has been considered as a system of symbiotic relationships with the community of microorganisms that inhabit it. This community includes bacteria, archaea, viruses, fungi, and protozoa and is called microbiota [1, 2]. Research shows that an imbalance in the intestinal microbiota not only leads to problems with the digestive system, but also increases the likelihood of cardiovascular and endocrine diseases and causes disturbances in the psychoemotional state [3–5].

Probiotics are an effective means of restoring a healthy balance of intestinal microbiota [2]. Today, special emphasis is placed on the study of associations (consortia) of probiotic microorganisms, in which the diversity of strains and species of bacteria determines the range of positive effects on the body [6]. The scientific literature indicates that one of the important reasons for the inconsistent clinical efficacy of multi-strain probiotic drugs is the lack of consideration of the biocompatibility of microorganisms when creating a consortium. This leads to a decrease in the viability of microorganisms and the loss of significant properties. In addition, it is reported that consortia of microorganisms are often unstable [6]. Therefore, the main goal of developing a complex probiotic is the selection of microorganisms with biocompatibility and similar biological and technological properties and maintaining a constant composition of these strains. The biocompatibility of strains is assessed using the direct co-cultivation method, taking into account the ability of lactobacilli to produce bacteriocins and other biologically active substances that determine the degree of strain antagonism in relation to representatives of its genus and affect the nature of interstrain interactions [7–9].

Modern methods, including next-generation sequencing (genomic, proteomic and metabolomic studies), are used to characterize the fundamental mechanisms of the bacteria probiotic effect on various functions of the macroorganism. New scientific technologies make it possible to assess the role of normal human microbiota and identify the subtle mechanisms of its response to various stressful environmental influences, and determine the factors that maintain the biochemical, metabolic and immunological balance necessary for stable relationships between the macroorganism and symbiotic microorganisms [10, 11].

However, the characterization based on genetic mechanisms and their potential for implementation should be investigated both *in vivo* and *in vitro*. Requirements for probiotic strains include resistance to low pH of gastric juice and bile acids, antagonism to opportunistic and pathogenic microorganisms, stability of composition and viability of bacteria during long-term storage. In addition, the safety of probiotic use should be confirmed in *in vitro* and *in vivo* animal studies and in the first phase of clinical trials [12].

Studying the biocompatibility of strains and the biotechnological potential of the consortium *in vitro* is an initial but very important stage in the development of an effective probiotic drug based on the consortium.

A correctly selected probiotic consortium may produce a synergistic effect, a phenomenon in which the combined action of several factors produces a more significant effect than the action of each of them separately. The synergistic effect allows the consortium to form a single system capable of resisting the effects of other microorganisms. The protective properties of the consortium are due to the antagonistic activity of bacteria and the synthesis of a number of biologically active substances [13].

Antagonistic activity against opportunistic and pathogenic microorganisms is not only one of the classic characteristics of probiotic bacteria, but also an important indicator of the efficacy and safety of a probiotic product, determined *in vitro* [14]. As knowledge of the structure and functions of the intestinal microbiota develops, it is becoming increasingly clear that, in addition to a large number of external factors causing microbial imbalance, antibiotics have a significantly more harmful effect on the microbiota. The resistance of opportunistic microorganisms to antimicrobial drugs, which is growing every year, is one of the global health problems worldwide [15]. In this regard, the development of effective consortia of probiotic strains with antagonism to multiresistant opportunistic microorganisms is extremely necessary.

THE AIM OF THE WORK

To study the antagonistic activity of monocultures and consortia of lactobacilli against multidrug-resistant isolates of opportunistic bacteria.

METHODS

Research objects

Gram-positive facultative anaerobic and/or microaerophilic bacteria of the family Lactobacillaceae

Lactobacillus strains and their consortia were obtained as part of the research work on the topic of "Obtaining microecological agents based on different lactobacillus strains" from the East Siberian State University of Technology and Management (ESSUTM) (agreement No. 6 dated May 10, 2023).

The following strains were obtained: *Lactobacillus curvatus* (*Latilactobacillus curvatus*) LCR-111-1, *Lactobacillus fermentum* (*Limosilactobacillus fermentum*) 44/1, *Lactobacillus acidophilus* 100ASH, *Lactobacillus rhamnosus* (*Lacticaseibacillus rhamnosus*) 12L, *Lactobacillus paracasei* (*Lacticaseibacillus paracasei*) k-406, *Lactobacillus plantarum* (*Lactiplantibacillus plantarum*) 8PAZ *Lactobacillus casei* (*Lacticaseibacillus casei*) MDP-1 [16].

The following consortia were received: *L. fermentum* 44/1 *и L. acidophilus* 100ASH (+); *L. fermentum* 44/1

и *L. rhamnosus* 12L (±); *L. curvatus* LCR111-1 и *L. fermentum* 44/1 (+); *L. curvatus* LCR-111-1 и *L. acidophilus* 100ASH (+); *L. curvatus* LCR-111-1 и *L. plantarum* 8-RA-3 (+); *L. curvatus* LCR-111-1 и *L. casei* MDP-1 (+); *L. acidophilus* 100ASH и *L. rhamnosus* 12L (+); *L. acidophilus* 100ASH и *L. casei* MDP-1 (+) [16].

The biocompatibility of the strains is indicated in brackets: "+" – the strains are compatible; "–" – the strains are incompatible; "±" – moderate antagonism is observed ("exit to the top" of one of the cultures) [16].

Normobiota and polyresistant isolates of opportunistic bacteria

Seven bacterial isolates were used as test cultures for testing antagonistic activity, including one strain belonging to the intestinal normobiota and six isolates of opportunistic bacteria with multiple antibiotic resistance (ABR) included in the "Human Microbiota Collection of the Irkutsk Region" of the Scientific Center for Family Health and Human Reproduction Problems [15]. The species composition of the bacterial test cultures is presented in Table 1.

Research methods

Cultivation of test cultures for in vitro experiments

The test culture strains were plated on the surface of meat-peptone agar (Research Center for Pharmacotherapy LLC, Russia) in a Petri dish and cultured at 37°C until the exponential growth phase was reached. Under sterile conditions, colonies of the exponential culture of the test strain were selected and suspensions were prepared in 10 ml of physiological solution, which were brought to the turbidity of the 0.5 McFarland standard.

Determination of antagonistic activity by the perpendicular streak method

The studied strain/consortium of lactobacilli was streaked on the surface of the Bifidum agar medium (State Research Center for Applied Biotechnology and Microbiology, Russia) in a Petri dish and incubated in an anaerobic jar with gas-generating bags (Anaerogas GasPak, Russia) at 37 °C for 48 h to allow the formation and diffusion of inhibitory compounds into the agar. Then, an exponential culture of the test strain was streaked perpendicularly from the edge of the dish to the streak of the grown culture/consortium of lactobacilli. The dish was incubated again, but under conditions favorable for the growth of the test culture: 37 °C without an anaerobic jar for 24 h. The experiment was repeated 3–4 times.

The presence and width of growth inhibition zones of microorganism test cultures were taken into account. Cultures that formed growth inhibition zones of the indicator strain from 4 to 9 mm were considered weak in antagonistic relation to lactobacilli; cultures that formed a zone from 9 to 14 mm were considered medium in antagonistic relation to microorganisms; cultures that formed a zone from 14 mm and more were considered highly active antagonists [14].

Determination of antagonist activity by the well method

The test cultures were inoculated with three-way streaking movements onto the Müller-Hinton medium (HiMedia Laboratories, India). No later than 15 min later, 10 mm diameter wells were cut in the agar layer containing the test strain using a cork drill and 0.1 ml of the lactobacillus monoculture/consortium suspension (cell count of at least 109 CFU/cm^3) were placed into each well. The cultures were incubated in a thermostat at 37 °C for 48 h. The experiment was repeated 2–3 times. The presence of growth inhibition zones and the diameters of the zones were taken into account with an accuracy of 1 mm, taking into account the diameter of the well itself. Weak antagonists include lactobacilli, the metabolites of which form growth inhibition zones for test cultures from 10 to 15 mm, medium antagonists – from 15 to 20 mm, and strong antagonists – more than 20 mm [14].

Statistical methods

Data on growth inhibition zones are presented as the arithmetic mean of the diameters of growth inhibition zones of test cultures (M) and the standard deviation (m).

Statistical data processing was performed using the PAST v. 4.03 program (Sweden). A nonparametric

TABLE 1

SPECIES COMPOSITION OF BACTERIAL TEST CULTURES

Type of microorganism	Number of isolates, abs.	Labeling of isolates
Human normobiota		
Escherichia coli	1	No. 10
Opportunistic bacteria with multiple resistance to antimicrobial drugs		
Enterobacter hormaechei	1	No. 2
Klebsiella pneumoniae	2	No. 9, No. 12
Pseudomonas aeruginosa	2	No. 34, No. 38
Staphylococcus aureus	1	No. 19

criterion for assessing statistical significance (Mann – Whitney U-test) was calculated for data on the antagonistic activity of consortia and individual lactobacilli strains included in their composition. Differences in statistical indicators were considered significant at $p < 0.05$.

The work was carried out using the equipment of the Collective Usage Center "Center for the Development of Progressive Personalized Health Technologies" and the Scientific Research Institution "Human Microbiota Collection of the Irkutsk Region" of the Scientific Center for Family Health and Human Reproduction Problems (Irkutsk), as well as the equipment of the Collective Usage Center "Progress" and the Biotechnology Center of ESSUTM (Ulan-Ude).

RESULTS AND DISCUSSION

When studying lactobacilli monocultures using the perpendicular streak method, 5 strains showed antagonistic activity against test bacterial cultures: *L. curvatus* LCR-111-1, *L. rhamnosus* 12L, *L. plantarum* 8PAZ, *L. casei* MDP-1 and *L. paracasei* k-406 (Table 2).

According to the experiment results, weak antagonists are strains of *L. plantarum* 8PAZ, *L. rhamnosus* 12L and *L. paracasei* k-406 in relation to the gram-positive isolate of *S. aureus* No. 19. In relation to the gram-negative isolates of *E. hormaechei* No. 2, *K. pneumoniae* No. 9, *K. pneumoniae* No. 12, *P. aeruginosa* No. 34, *P. aeruginosa* No. 38 and *E. coli* No. 10, all 5 strains of lactobacilli showed moderate antagonism.

In a well-based study, two strains showed antagonistic activity: *L. acidophilus* 100ASH and *L. fermentum* 44/1, against all isolates of the test cultures, while the other five lactobacilli strains had no effect. At the same time, the strain *L. acidophilus* 100ASH was a weak antagonist against the gram-positive isolate *S. aureus* No. 19 and a highly active antagonist, along with *L. fermentum* 44/1, against gram-negative isolates No. 2, No. 9, No. 12, No. 34, No. 38, and No. 10.

It should be noted that the effect of antagonistic activity of lactobacilli may be different depending on the type of opportunistic microorganisms with which they interact. This is probably due to the fact that gram-positive and gram-negative bacteria have different cell wall structures and mechanisms of interaction

TABLE 2

ANTAGONISTIC ACTIVITY OF LACTOBACILLI MONOCULTURES AGAINST MULTIDRUG-RESISTANT ISOLATES OF OPPORTUNISTIC BACTERIA AND *E. COLI*

Labeling of test cultures	Growth inhibition zones (M ± m)						
	***L. curvatus* LCR-111-1**	***L. acidophilus* 100ASH**	***L. rhamnosus* 12L**	***L. plantarum* 8PAZ**	***L. casei* MDP-1**	***L. fermentum* 44/1**	***L. paracasei* k-406**
Perpendicular streak method							
No. 2	10.5 ± 2.0	0	10.0 ± 0.2	10.0 ± 1.4	9.5 ± 0.2	0	9.5 ± 2.0
No. 9	12.0 ± 1.4	0	10.0 ± 0.1	12.0 ± 0.4	12.0 ± 0.8	0	11.5 ± 0.7
No. 12	12.5 ± 0.7	0	12.5 ± 0.7	12.0 ± 1.4	11.5 ± 0.7	0	12.0 ± 0.2
No. 19	10.0 ± 0.3	0	8.0 ± 1.4	8.8 ± 0.6	8.6 ± 0.3	0	8.5 ± 1.4
No. 34	10.0 ± 0.1	0	10.0 ± 0.3	10.0 ± 0.3	11.0 ± 1.4	0	10.0 ± 1.4
No. 38	11.5 ± 2.0	0	13.0 ± 0.2	11.0 ± 1.4	16.0 ± 5.6	0	12.5 ± 0.7
No. 10	10.0 ± 4.0	0	12.0 ± 1.4	11.0 ± 1.4	11.5 ± 0.7	0	11.0 ± 1.4
Well method							
No. 2	0	22.0 ± 0.8	0	0	0	23.2 ± 0.6	0
No. 9	0	23.5 ± 0.4	0	0	0	23.3 ± 0.5	0
No. 12	0	22.5 ± 0.7	0	0	0	22.0 ± 0.8	0
No. 19	0	18.5 ± 0.6	0	0	0	23.0 ± 0.8	0
No. 34	0	24.3 ± 1.2	0	0	0	24.5 ± 0.7	0
No. 38	0	24.0 ± 0.8	0	0	0	22.3 ± 1.2	0
No. 10	0	22.7 ± 0.5	0	0	0	22.3 ± 0.9	0

Note. No. 2 – *E. hormaechei;* No. 9 – *K. pneumoniae;* No. 12 – *K. pneumoniae;* No. 19 – *S. aureus;* No. 34 – *P. aeruginosa;* No. 38 – *P. aeruginosa;* No. 10 – *E. coli.*

with other bacteria. In addition, different isolates (No. 34 and No. 38) of the same species – *P. aeruginosa* – showed different results of antagonistic activity (different growth inhibition zones). This may indicate that the more isolates of the same species are tested, the more effectively the antagonistic properties of lactobacilli will be studied.

Among the consortia, antagonistic activity in the study using the perpendicular streak method was noted in two (table 3):

• *L. curvatus* LCR-111-1 and *L. plantarum* 8PAZ, which showed weak antagonism towards isolates No. 2, No. 9, No. 19, No. 34 and moderate antagonism towards isolates No. 12, No. 38 and No. 10. Moreover, the antagonism of the consortium was statistically significantly weaker than the antagonism of individual strains of lactobacilli included in its composition ($p = 0.002$);

• *L. curvatus* LCR-111-1 and *L. casei* MDP-1, which showed weak antagonism towards isolate No. 2 and moderate antagonism towards isolates No. 9, No. 12, No. 19, No. 34, No. 38 and No. 10.

Unlike lactobacilli monocultures, which exhibit different antagonistic activity towards gram-positive and gram-negative isolates of opportunistic microorganisms, such differences are not observed in consortia: according to the degree of antagonistic effect, isolates are combined regardless of the type of cell wall or any specific mechanisms of interaction.

In a well-based study, 6 consortia (highly active antagonists) containing *L. acidophilus* 100ASH and/or *L. fermentum* 44/1 strains showed antagonistic activity, with five of them having statistically significantly more pronounced antagonism than individual strains:

TABLE 3

ANTAGONISTIC ACTIVITY OF LACTOBACILLI CONSORTIUMS AGAINST MULTIDRUG-RESISTANT ISOLATES OF OPPORTUNISTIC BACTERIA AND *E. COLI*

Labeling of test cultures	Growth inhibition zones (M ± m)							
	L. fermentum 44/1 and *L. acidophilus* 100ASH	*L. fermentum* 44/1 and *L. rhamnosus* 12L	*L. curvatus* LCR-111-1 and *L. fermentum* 44/1	*L. curvatus* LCR-111-1 and *L. acidophilus* 100ASH	*L. curvatus* LCR-111-1 and *L. plantarum* 8PAZ	*L. curvatus* LCR-111-1 and *L. casei* MDP-1	*L. acidophilus* 100ASH and *L. rhamnosus* 12L	*L. acidophilus* 100ASH and *L. casei* MDP-1
Perpendicular streak method								
No. 2	0	0	0	0	7.0 ± 0.1*	8.5 ± 0.7	0	0
No. 9	0	0	0	0	8.0 ± 0.3*	9.5 ± 0.7	0	0
No. 12	0	0	0	0	9.0 ± 0.2*	11 ± 0.3	0	0
No. 19	0	0	0	0	6.0 ± 0.3*	9.5 ± 0.7	0	0
No. 34	0	0	0	0	8.0 ± 1.4*	9.0 ± 1.4	0	0
No. 38	0	0	0	0	9.5 ± 2.0*	10 ± 0.2	0	0
No. 10	0	0	0	0	9.5 ± 2.0*	10.5 ± 0.7	0	0
Well method								
No. 2	24.5 ± 0.7*	24.3 ± 0.9*	23.7 ± 1.2*	22.8 ± 1.4*	0	0	23.3 ± 1.2*	19.0 ± 6.3
No. 9	24.3 ± 0.6*	24.3 ± 0.9*	24.3 ± 0.5*	23.3 ± 0.5*	0	0	23 ± 0.8*	23.3 ± 0.5
No. 12	23.5 ± 1.5*	23.3 ± 0.5*	23.3 ± 1.2*	24.7 ± 0.5*	0	0	23.7 ± 0.5*	23.0 ± 0.8
No. 19	23.3 ± 0.9*	23.7 ± 1.2*	24.0 ± 0.3*	24.3 ± 0.6*	0	0	24.7 ± 1.5*	24.2 ± 1.2
No. 34	25.5 ± 1.0*	24.3 ± 0.9*	25.3 ± 1.2*	23.2 ± 1.6*	0	0	23.2 ± 1.4*	22.2 ± 6.7
No. 38	26.5 ± 1.8*	26.3 ± 1.7*	26.5 ± 1.0*	24.7 ± 1.2*	0	0	24.7 ± 1.2*	23.7 ± 0.9
No. 10	23.6 ± 2.0*	23.3 ± 1.2*	23.5 ± 1.9*	24.3 ± 2.0*	0	0	25.7 ± 1.2*	23.3 ± 2.4

Note. No. 2 – *E. hormaechei;* No. 9 – *K. pneumoniae;* No. 12 – *K. pneumoniae;* No. 19 – *S. aureus*; No. 34 – *P. aeruginosa;* No. 38 – *P. aeruginosa;* No. 10 – *E. coli;* * – statistical significance of differences between the consortium and individual strains of lactobacilli included in its composition ($p < 0.05$).

• *L. fermentum* 44/1 and *L. acidophilus* 100ASH ($p = 0.002$);

• *L. fermentum* 44/1 and *L. rhamnosus* 12L ($p = 0.001$);

• *L. curvatus* LCR-111-1 and *L. fermentum* 44/1 ($p = 0.002$);

• *L. curvatus* LCR-111-1 and *L. acidophilus* 100ASH ($p = 0.002$);

• *L. acidophilus* 100ASH and *L. rhamnosus* 12L ($p = 0.002$);

• *L. acidophilus* 100ASH and *L. casei* MDP-1 ($p = 0.198$).

The studied lactobacillus strains in the declared consortia had high biotechnological potential and biocompatibility level, with the exception of the consortium based on *L. fermentum* 44/1 and *L. rhamnosus* 12L strains, which showed moderate antagonism (±) [16]. Comparison of the results of strain biocompatibility and consortia antagonistic activity revealed the following features:

1. In a consortium based on the strains *L. curvatus* LCR111-1 and *L. plantarum* 8PAZ, which was characterized by a high level of biocompatibility (+), a statistically significant decrease in the effect of antagonistic activity was revealed in comparison with the activity of monocultures.

2. In a consortium based on *L. fermentum* 44/1 and *L. rhamnosus* 12L strains, in which moderate antagonism (±) was demonstrated between the strains, the antagonistic activity against opportunistic microorganisms was statistically significantly more pronounced than that of individual strains.

The study showed that a high degree of compatibility of lactobacilli strains does not guarantee a synergistic effect. In the context of lactobacilli, synergy can be expressed as an increase or decrease in antagonistic activity against pathogenic microorganisms, affecting the probiotic potential of the consortium. Therefore, key aspects for the creation of an effective probiotic consortium are both the biocompatibility of probiotic strains and the antagonistic activity of the consortium. A probiotic product developed taking into account these criteria can demonstrate increased efficacy and a wider range of beneficial properties for the body.

The antagonistic activity of probiotic strains is studied using various methods: at the first stage, *in vitro* methods are used (diffusion methods, analysis in liquid nutrient media, etc.), at the second stage, *in vivo* methods (reception of antagonist live culture by a person or experimental animals with subsequent analysis of changes in the intestinal microbiota). All these methods differ in the degree of complexity of implementation, efficiency, the possibility of comparison and the accuracy of the results obtained [17, 18]. For example, the results obtained by two classical methods (the perpendicular streak method and the well method), based on the diffusion of components produced by lactobacilli in the thickness of agar, are difficult to compare. Thus, the perpendicular streak method gives an advantage to individual strains producing inhibitory compounds of small molecular weight. The well method is convenient, in turn, for testing the antagonistic activity of not only monocultures, but also consortia, since a ready-made suspension of bacteria, including their metabolites, is placed in the well. Thus, both of these methods complement each other and should be used in combination, as they provide a more complete picture of the antagonistic activity of lactobacilli and their consortia, which is one of the characteristics of probiotic potential.

CONCLUSION

The results of the study showed that all lactobacilli and their consortia, depending on the study method, had different degrees of antagonistic activity against multiresistant isolates of opportunistic bacteria. In five studied consortia, the antagonistic effect against test cultures was more pronounced than that of individual strains, while one consortium, on the contrary, showed a decrease in the effect of antagonistic activity compared to monocultures. The obtained results of the study of the antagonistic activity of two consortia (*L. fermentum* 44/1 and *L. rhamnosus* 12L, *L. curvatus* LCR111-1 and *L. plantarum* 8PAZ) are not consistent with the data on the biocompatibility of strains in these consortia. Consequently, the compatibility of strains does not always lead to a positive synergistic effect, which can manifest itself in increased antagonistic activity. Thus, the creation of probiotic consortia requires fine-tuning and selection of bacterial strains, taking into account both the biocompatibility of the strains and the antagonistic properties of the consortium to ensure effective and safe action to improve human health.

The detected antagonistic activity against the *E. coli* isolate may be due to the same mechanism of lactobacilli action on both opportunistic bacteria and normobiota. The analysis of the obtained results revealed differences in the degree of lactobacilli antagonistic action on gram-positive and gram-negative bacterial species. Moreover, such differences may also be observed in different isolates of the same species. Given the identified features, a wider range of both gram-positive bacteria and normobiota isolates should be included in the experiment for a more detailed study of the lactobacilli antagonistic properties. In addition, it would be advisable to use not different species, but different isolates of the same species: *E. coli* and target intestinal opportunistic bacteria, for example, *K. pneumonia*, one of the most dangerous types of opportunistic pathogens. This will help to determine more effective strategies for the probiotics use in the context of the mass spread of drug resistance of opportunistic microorganisms.

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Conflicts of interest

No potential conflict of interest relevant to this article reported.

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NEUROLOGY AND NEUROSURGERY

AGE-RELATED PARAMETERS OF P300 AUDITORY EVOKED POTENTIALS IN ELDERLY PERSONS IN THE CONTEXT OF COGNITIVE HEALTH: A STUDY IN THE EUROPEAN NORTH OF RUSSIA

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ABSTRACT

Background. *Setting of norms for the parameters of P300 cognitive auditory evoked potentials (EP) in elderly people with intact cognitive functions considering their residence in certain climatic and geographical regions is an urgent problem.*
The aim of the study. *To determine age-related parameters of P300 cognitive auditory evoked potentials in elderly people aged 60–69 and 70–74 years, living in the European North of Russia (using the example of Arkhangelsk).*
Methods. *The parameters of P300 auditory EP were determined in randomly selected urban residents in the age groups of 60–69 years (n = 284) and 70–74 years (n = 115) with normal scores on the Montreal Cognitive Assessment Scale (MoCA), without depression (according to Beck Depression Inventory), with preserved ability to work and/or social functions. We calculated the 5th–95th percentile values (P5–P95) of the P300 EP parameters and assessed the relationships of these parameters with socio-demographic characteristics, lifestyle and the results on the MoCA scale and Beck Depression Inventory.*
Results. *Statistically significant differences in latency indicators of P300 EP were determined between groups of 60–69 and 70–74 years (P25–P90) in all studied brain regions (frontal, central). In the group of 60–69 years, the range of P25–P75 values of P300 EP latencies was 342.5–401 ms, in the group of 70–74 years – 358.5–443 ms. Age differences in P300 EP amplitudes were minimal with an interquartile range of 4–13 μV in the total sample. Participants who smoked had higher latency scores and lower amplitude scores; former smokers had higher latency scores compared to never-smokers.*
Conclusion. *Latency above 400 ms at the age of 60–65 years and above 443 ms at 70–74 years can be considered as a criterion for reduced cognitive reserve and an increased risk of developing cognitive disorders in elderly people living in the European North of Russia.*

Key words: *cognitive function, cognitive evoked potentials, North, healthy aging, cognitive health*

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ВОЗРАСТНЫЕ ОСОБЕННОСТИ ПАРАМЕТРОВ СЛУХОВЫХ ВЫЗВАННЫХ ПОТЕНЦИАЛОВ P300 У ПОЖИЛЫХ ЛЮДЕЙ В КОНТЕКСТЕ КОГНИТИВНОГО ЗДОРОВЬЯ: ИССЛЕДОВАНИЕ НА ЕВРОПЕЙСКОМ СЕВЕРЕ РОССИИ

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РЕЗЮМЕ

Обоснование. *Нормирование параметров когнитивных слуховых вызванных потенциалов (ВП) P300 у пожилых людей с сохранными когнитивными функциями с учётом проживания в определённых климатогеографических условиях является актуальной проблемой.*

Цель исследования*. Определение возрастных особенностей параметров когнитивных слуховых вызванных потенциалов P300 у пожилых людей 60–69 и 70–74 лет, жителей Европейского Севера России (на примере г. Архангельска).*

Методы. *Определены параметры слуховых ВП P300 у случайно отобранных городских жителей в возрастных группах 60–69 лет (n = 284) и 70–74 лет (n = 115) с нормальными показателями по Монреальской шкале оценки когнитивных функций (MoCA, Montreal Cognitive Assessment), отсутствием депрессии (по шкале депрессии Бека), сохранной трудоспособностью и/или социальными функциями. Рассчитаны 5–95-е процентильные значения (P5–P95) параметров ВП P300 и оценены связи этих параметров с социально-демографическими характеристиками, образом жизни и результатами по шкале MoCA и шкале депрессии Бека.*

Результаты. *Определены статистически значимые различия показателей латентности ВП P300 между группами 60–69 и 70–74 лет (P25–P90) во всех изучаемых мозговых отделах (лобных, центральных). В группе 60–69 лет диапазон значений P25–P75 латентностей ВП P300 составил 342,5–401 мс, в группе 70–74 лет – 358,5–443 мс. Возрастные различия амплитуд ВП P300 были минимальными при межквартильном диапазоне 4–13 мкВ в общей выборке. Курящие участники имели более высокие показатели латентности и более низкие показатели амплитуды, курившие в прошлом – более высокие показатели латентности в сравнении с никогда не курившими.*

Заключение. *Латентность выше 400 мс в возрасте 60–65 лет и выше 443 мс в 70–74 года может рассматриваться в качестве критерия сниженного когнитивного резерва и повышенного риска развития когнитивных нарушений у пожилых людей, проживающих на Европейском Севере России.*

Ключевые слова: *когнитивные функции, когнитивные вызванные потенциалы, Север, здоровое старение, когнитивное здоровье*

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BACKGROUND

Maintaining higher mental functions at an optimal level in old age ensures the active participation of elderly people in public life. Successful cognitive activity in elderly people is also necessary for the effective transfer of accumulated work experience to the younger generation, which also ensures the stability of society. However, the individual viability of elderly people decreases, primarily due to a decrease in the indicators of the cognitive domain [1]. Modern psychodiagnostic approaches make it possible to assess the risks of cognitive impairment in elderly people, but neurophysiological tests are not always sensitive to the detection of early, pre-dementia disorders.

Parameters of event-related bioelectric potentials of the brain (evoked potentials (EP)) are promising electroneurophysiological correlates of cognitive functions both in norm and in pathology [2, 3]. At the same time, the problem of identifying the limits of fluctuations in the values of cognitive evoked potentials in elderly individuals in the context of healthy aging remains relevant. The natural age-related change in the EP P300 parameters occurs both against the background of a decrease in cognitive functions reflected in the data of neuropsychological testing, and against the background of preserved cognitive and social functions. This complicates the interpretation of the EP P300 parameters in elderly individuals in the context of the need to differentiate the characteristics of the age norm and electrophysiological correlates of the risk of developing cognitive impairment [4]. Lifestyle, his activity in society significantly determines the neurophysiological status of an elderly person.

Age-related decrease in the speed of information processing in the form of increased latency and decreased amplitude of the P300 EP is traditionally considered in 10-year periods [2]. The age of 70 as an important age limit is often used to predict various aspects of the lives of elderly people. For example, the age of 70 is used to calculate survival predictors (socioeconomic status, lifestyle, cognitive functions, etc.) [5]. Therefore, age-related features of the P300 EP parameters and their relationship with social and psychological characteristics seem important to consider taking into account the division of old age into periods before and after 70 years.

According to the concept of the "aging curve" proposed in the 1970s–90s based on the age-related dynamics of the P300 EP latency, with age the average latency increases according to the formula: Latency P300 = 1.25 × age + 285 ms [2]. However, the maximum values of the P300 EP latency may differ significantly from the calculated values. That is, in older age groups, the ranges of values of the P300 latency indicators may be quite wide.

The amplitude of the P300 EP is also traditionally considered within the framework of regression models, according to which the amplitude decreases with age (P300 EP Amplitude = 11.9 μV – 0.09 × Age) [6]. In this case, the spread of amplitude values in age samples can be quite wide, and the data presentation is limited to the values of the arithmetic mean for the sample and the standard deviation. There is also information on a slight age-related decrease in the P300 EP amplitude with aging [7].

Age-related changes in physiological parameters, including cognitive functions, may have regional characteristics. Thus, in a random sample of elderly people visiting outpatient clinics in one of the regions of the Arctic zone of the Russian Federation (Arkhangelsk), pre-dementia disorders according to neuropsychological testing were detected in half of the visitors [8]. In general, this is comparable with the results of assessing cognitive impairment among individuals from the all-Russian sample, when mild and moderate (pre-dementia) disorders were detected in 46.6 % of visitors to the I.M. Sechenov University of the Ministry of Health of the Russian Federation (Moscow) among individuals with an average age of 66.0 ± 15.7 years [9]. However, it is noted that when recording visual EP P300 in elderly women in Arkhangelsk, the greatest decrease in amplitude and prolongation of latency of visual EP P300 were detected in the 70–74 year age group [10].

Determination of normative ranges of the P300 EP latency and amplitude parameters taking into account the data of neuropsychological testing, as well as information on the socioeconomic status, labor and social activity of elderly people would ensure their applicability both for assessing the parameters of healthy aging and for identifying electrophysiological criteria for the risk of developing cognitive impairment in elderly people. Taking into account the nonparametric distribution of these parameters, the presentation of their normative ranges using percentile corridors (from P5 to P95) would also contribute to increasing their validity. On these grounds, the aim of this study was to determine age-related parameters of P300 cognitive auditory evoked potentials in elderly people aged 60–69 and 70–74 years, living in the European North of Russia (using the example of Arkhangelsk).

METHODS

In 2023, study participants were recruited from among residents of Arkhangelsk who had previously been included in the random population sample of the "Know Your Heart" study [11], formed on the basis of an anonymized database of the territorial compulsory health insurance fund. Random addresses were selected for visiting, and elderly men and women (according to age periodization – aged 60-74 years) living at them were invited to participate in the study. The survey was conducted at the consultative and diagnostic clinic of the Northern State Medical University of the Ministry of Health of the Russian Federation (NSMU) and the biorhythmology laboratory of the N. Laverov Federal Center for Integrated Arctic Research

of the Ural Branch of the Russian Academy of Sciences (FECIAR UrB RAS). All participants provided written informed consent for the examination, which was approved by the local Ethics Committee of NSMU (protocol No. 03/04-23 dated April 26, 2023) and carried out in compliance with the principles of the Helsinki Declaration of the World Medical Association.

The inclusion criteria for the sample of this study were age 60–74 years; residence in the Arkhangelsk region for 10 years; availability of signed informed consent for the study.

The exclusion criteria were the presence of an acute infectious disease at the time of the study; exacerbation of a chronic disease; history of mental illness.

A total of 605 people aged 60–74 years took part in the study. They were offered to undergo a full examination, including a medical examination and questionnaire (stage one), as well as cognitive-psychological testing and recording of the parameters of P300 cognitive auditory EP (stage two). Additional exclusion criteria for assessing the parameters of P300 cognitive auditory EP were the presence of epilepsy, traumatic brain injury with damage to the bones of the skull, cerebrovascular accidents accompanied by hemiparesis, and hearing loss above grade I. A total of 529 people completed both stages. The remaining participants met the exclusion criteria or refused to complete the second stage.

The questionnaire provided data on the participants' ability to work (currently employed or not employed, but could work if desired or necessary), level of education, marital status, length of residence in Arkhangelsk, financial situation, smoking and alcohol consumption, performance of functions in the family (1 – financial support of family and relatives; 2 – housekeeping; 3 – managing a summer cottage or garden plot; 4 – raising children and grandchildren; 5 – caring for elderly and/or sick relatives) and socially significant activities (volunteer work, participation in the work of political parties, public organizations, in community work at least once a year).

Cognitive functions were tested using the Montreal Cognitive Assessment (MoCA) [12]. A total score of 26 or higher was considered normal on this scale. The level of depression was determined using the Beck Depression Inventory [13]. The absence of depressive symptoms was considered at a level of less than 14 scores. In addition, the Age Is No Barrier test was used to determine signs of frailty [14, 15].

The P300 EP auditory was assessed using the Neuron-Spectrum electroencephalograph (Neurosoft LLC, Russia); the P300 EP parameters were recorded in standard electroencephalogram (EEG) leads using the international 10-20 electrode placement system with an ear referent in the frontal (F3, F4) and central (C3, C4) regions of the brain. The conditions of binaural nonverbal acoustic stimulation in the oddball paradigm with button pressing included: stimulus duration – 50 ms; intensity – 80 dB; inter-stimuli period – 1 s; tone frequency – 2000 Hz with 30 % occurrence of a significant stimulus, 1000 Hz with 70 % occurrence of an insignificant stimulus. The P300 EP latencies (ms) and the N2-P300 interpeak amplitude (μV) were determined [2, 6].

Subsequently, to examine the age ranges of the P300 EP indicators, a selection of individuals with normal cognitive function indicators according to the MoCA scale (26 scores or more), without signs of depression (less than 14 scores according to the Beck Depression Inventory), preserved ability to work, performing two or more work functions in the family and/or performing socially significant activities at least once a month, was made.

In statistical analysis, categorical variables are presented as absolute values (Abs) and percentages (%). Continuous variables are presented as arithmetic means (M) and standard deviations (SD) or medians (Me) with 25% and 75% percentiles [P25; P75]. Comparisons of groups by categorical variables were performed using the Pearson`s chi-square (χ^2) test, and by continuous variables – using the Mann – Whitney test. Normative values of the P300 cognitive auditory evoked potential for the 60–69 and 70–74 age groups are presented as percentile values (P5, P10, P25, P50, P75, P90, P95) modeled using multiple quantile regressions with age group, gender, and education as covariates, with the condition of uniform distribution of age groups by gender and education. Differences between the corresponding percentile values in the age groups were assessed based on the statistical significance of the regression coefficients for the age group variable in the described quantile regression models. The relationships between the P300 EP indices and socio-demographic characteristics, lifestyle (smoking and alcohol consumption) and the results on the MoCA scale and the Beck Depression Inventory in the analyzed sample of elderly people with normal MoCA indices, without signs of depression (on the Beck Depression Inventory) and with preserved working capacity and/or social functions were estimated using multiple linear regressions with adjustments for gender and age. The conditions for the applicability of multiple linear regression models were estimated by visual assessment of the distribution of residuals. Regression coefficients and differences between groups were considered statistically significant at $p < 0.05$. STATA 18.0 (Stata Corp., USA) was used for data analysis.

RESULTS

As a result of applying the selection criteria for participants with normal scores on the MoCA scale and the Beck Depression Inventory, with preserved working capacity and/or social functions, a group of 399 people (284 people aged 60–69 years and 115 people aged 70–74 years) was formed for the analysis of age-related characteristics of the ranges of the P300 EP parameters, which amounted to 75.4 % of the total sample size (605 people) (table 1).

According to the "Age Is No Barrier" questionnaire, the group included: 310 (77.7 %) people for whom

frailty is unlikely (0–2 points); 84 (21 %) people for whom pre-asthenia is probable (3–4 points); 5 (1.3 %) people with probable frailty (5–7 points). Taking into account the selection of participants with normal indicators on the MoCA scale [16, 17], the presence of signs of pre-asthenia and asthenia in the participants was considered to be due to a decrease in physical functions.

The study participants aged 60–69 and 70–74 were comparable ($p > 0.05$) in terms of the ratio of men to women, time of residence in Arkhangelsk, performance of family functions (except for the proportion of those working on summer cottages and garden plots, which was higher among individuals aged 70–74), participation in socially useful activities, and frequency of alcohol consumption (table 2). There were more unemployed people and those reporting that they would not be able to work if they wanted to or had to, aged 70–74 ($p < 0.001$), and there was a higher proportion of people with no more than secondary education at this age ($p = 0.037$). There were more married people aged 60–69 ($p = 0.011$), and there were more people living alone aged 70–74 ($p = 0.046$). There were more smokers among those aged 60–69 ($p = 0.005$). Income levels were lower in the 70–74 year old group due to a higher proportion of individuals reporting financial constraints on purchasing major household appliances. Participants aged 70–74 also had a lower mean score on the MoCA scale. Scores on the Beck Depression Inventory were not statistically significant between participants aged 60–69 and 70–74.

Analysis of the ranges of the P300 EP values showed an increase in the P300 EP latency values with age in all the studied EEG leads (table 3). Taking into account the maximum spread of values in the four considered EEG leads, in the 60–69 year old group, the range of P300 EP latency values at the level of average percentile values (P25–P75), reflecting the conditional "average norm", was 342.5–400.9 ms, in the 70–74 year old group – 358.5–442.9 ms. In all the considered EEG leads (C3, C4, F3, F4), higher percentile values in the P10–P95 range were determined in the 70–74 year old group compared to the 60–69 year old group. At the P90 level, the P300 EP latency increased significantly compared to the value at P75 in each age group, especially in the 70–74 age group – more than 40 ms.

The range of the conditional "average" norm (P25–P75) of the P300 EP amplitudes in the 60–69 year old group, taking into account the spread of values in the EEG leads, was 4.2–12.6 μV, in the 70–74 year old group – 3.5–12.3 μV. The differences in the P300 EP amplitudes between the age groups were insignificant and reached statistical significance only at the level of low percentiles (P5–P25) for lead C4.

In the analyzed group of participants, consisting of individuals without cognitive and psychological impairments, with preserved working capacity and/or social functions, the analyzed P300 EP indices had no statistically significant relationships with the level of education, employment, or income. Compared with those who had never smoked, current smokers had higher latency indices (lead C4: Me – 394.0 vs. 374.0 ms, $p = 0.024$; lead F4: Me – 393.4 vs. 370.1 ms, $p = 0.008$; lead F3: Me – 393.3 vs. 366.5 ms, $p = 0.004$) and lower amplitude indices (lead C3: Me – 6.5 vs. 8.5 μV; $p = 0.043$); former smokers had higher latency values (lead F3: Me – 384.8 vs. 366.5 μV; $p = 0.019$). The frequency of alcohol consumption had no statistically significant relationship with the P300 EP values.

When examining the same sample, no relationships were determined between the latency indicators of the P300 EP amplitude and the results on the MoCA scale, limited to a range of 26 to 30 points (fig. 1, 2), as well as with the results on the Beck Depression Inventory scale, considered in a range of 0 to 13 points (fig. 3, 4).

TABLE 1

RESULTS OF SELECTING STUDY PARTICIPANTS WITH NORMAL MOCA SCORES, NO SIGNS OF DEPRESSION AND PRESERVED ABILITY TO WORK AND/OR SOCIAL FUNCTIONS

Age groups	Total ($n = 529$)	60–69 years ($n = 354$)	70–74 years ($n = 175$)	p*
	Abs (%)			
No cognitive impairment (MoCA score ≥ 26)	441 (84.2)	306 (87.2)	135 (78.0)	0.007
No depression (Beck Depression Inventory score < 14)	477 (90.7)	328 (93.2)	149 (85.6)	0.005
Maintained ability to work (a person works or could work if desired and/or necessary)	347 (65.6)	260 (73.5)	87 (49.7)	< 0.001
Maintained social functions (≥ 2 functions in the family and/or socially useful activities at least once a month)	498 (94.1)	334 (94.4)	164 (93.7)	0.770
No cognitive and psychological impairments and preservation of working capacity and/or social functions	399 (76.2)	284 (80.9)	115 (66.5)	< 0.001

Note. * – p-value estimated by the Pearson`s chi-square (χ^2) test.

TABLE 2

SOCIODEMOGRAPHIC, BEHAVIOURAL, COGNITIVE, PSYCHOLOGICAL AND NEUROPSYCHOLOGICAL CHARACTERISTICS OF STUDY PARTICIPANTS AGED 60–69 AND 70–74 YEARS

Characteristics	60–69 years (*n* = 284)	70–74 years (*n* = 115)	*p**
	Abs (%)		
Socio-demographic			
Gender:			
– male	112 (39.4)	35 (30.4)	0.091
– female	172 (60.6)	80 (69.6)	
Residence in Arkhangelsk:			
– since birth	140 (49.3)	58 (50.4)	0.837
– more than 10 years	144 (50.7)	57 (49.6)	
Education:			
– higher education	98 (34.5)	38 (33.0)	
– secondary specialized education	158 (55.6)	55 (47.8)	0.037
– secondary and lower education	28 (9.9)	22 (19.1)	
Employment:			
– currently working	116 (40.9)	14 (12.2)	
– does not work, but could work if desired and/or necessary	100 (35.2)	54 (47.0)	< 0.001
– does not work and could not work if desired and/or necessary	68 (23.9)	47 (40.9)	
Income level:			
– not enough money for food/clothes	21 (7.4)	7 (6.1)	0.046
– enough for food/clothes, buying large household appliances is difficult	124 (43.8)	68 (59.1)	
– a person can buy large household appliances, but buying a car is difficult	106 (37.5)	29 (25.2)	
– there are no financial difficulties, it is possible to buy a car, an apartment	32 (11.3)	11 (9.6)	
Marital status:			
– married	172 (60.6)	60 (52.2)	
– divorced	42 (14.8)	13 (11.3)	0.011
– widower/widow	45 (15.9)	35 (30.4)	
– never been married	25 (8.8)	7 (6.1)	
Living alone	73 (25.7)	41 (35.7)	0.046
Functions in the family:			
– financial support of family and relatives	204 (71.8)	88 (76.5)	0.338
– housekeeping	265 (93.3)	107 (93.0)	0.924
– managing a summer cottage or garden plot	180 (63.4)	86 (74.8)	0.029
– raising children and grandchildren	186 (65.5)	78 (67.8)	0.656
– caring for elderly and/or sick relatives	72 (25.4)	29 (25.2)	0.978
Socially useful activity or volunteer work:			
– rarely or never	263 (92.6)	105 (1.3)	0.660
– at least once a month	21 (7.4)	10 (8.7)	
Lifestyle			
Smoking:			
– never	169 (59.5)	86 (74.8)	0.005
– in the past	68 (23.9)	22 (19.1)	
– in the present	47 (16.6)	7 (6.1)	

TABLE 2 (*continued*)

Alcohol consumption:			
– once a month or less	212 (74.6)	89 (77.4)	
– 2-4 times a month	59 (20.7)	16 (13.9)	0.103
– 2-3 times a week or more often	13 (4.6)	10 (8.7)	
Mental health, Me [P25; P75]			
MoCA scale, scores	28 [27; 29]	28 [26; 29]	0.001
Beck Depression Inventory, scores	4 [1; 7]	5 [2; 7]	0.060

Note. * – for quantitative characteristics, the *p*-value is estimated by the *t*-test for independent samples or the Mann – Whitney test, for categorical characteristics – by the Pearson`s test (χ^2).

TABLE 3

NORMATIVE VALUES FOR THE PARAMETERS OF P300 COGNITIVE AUDITORY EVOKED POTENTIAL IN INDIVIDUALS AGED 60–69 (*n* = 284) AND 70–74 YEARS (*n* = 115) WITH NORMAL MOCA SCORES, NO SIGNS OF DEPRESSION AND PRESERVED ABILITY TO WORK AND/OR SOCIAL FUNCTIONS

Groups	Mean	SD	P5	P10	P25	P50	P75	P90	P95
P300 EP latency, ms									
C4									
60–69 years	378.0	46.2	319.2	330.7	349.2	370.3	395.3	430.1	453.1
70–74 years	403.0	53.9	328.2	345.7*	367.2*	392.3*	440.3*	488.1*	512.1*
C3									
60–69 years	379.7	42.4	322.1	331.2	351.5	374.6	400.9	434.0	465.8
70–74 years	406.4	55.9	332.1	343.7*	371.5*	392.6*	442.9*	482.0*	514.8*
F4									
60–69 years	374.5	45.7	317.9	328.0	344.0	368.1	396.4	426.4	454.1
70–74 years	398.5	53.6	324.9	338.0	360.0*	390.1*	422.4*	469.4*	503.1*
F3									
60–69 years	374.7	45.2	317.1	326.1	342.5	369.3	395.6	429.3	467.3
70–74 years	399.2	54.7	330.1*	337.1	358.5*	384.3*	427.6*	479.3*	506.3
P300 EP amplitude, μV									
C4									
60–69 years	9.5	6.0	2.1	3.1	5.7	8.4	12.1	18.0	21.3
70–74 years	8.3	5.2	0.9*	1.4*	4.3*	8.1	11.4	15.3	18.2
C3									
60–69 years	9.9	6.2	1.9	3.2	6.2	9.0	12.6	17.0	21.1
70–74 years	9.2	4.8	2.9	3.7	5.6	8.3	12.3	16.7	19.3
F4									
60–69 years	8.5	6.3	0.9	2.1	4.3	7.4	10.5	16.0	21.5
70–74 years	8.3	6.1	0.7	1.7	3.5	7.2	12.3	17.3	21.6
F3									
60–69 years	8.4	6.5	1.1	2.2	4.2	7.2	10.9	15.2	20.2
70–74 years	7.9	5.2	1.7	2.3	4.1	6.5	11.2	14.8	19.0

Note. Percentile values were modeled and differences between them across age groups were estimated using multiple quantile regressions with age group, sex, and education as covariates, assuming that age groups were equally distributed across sex and education; * – $p < 0.05$ for differences between corresponding percentile values.

DISCUSSION

The obtained data reflect statistically significant age differences in the P300 EP latencies in elderly people in the 60–69 and 70–74 year old groups, to a greater extent in the P25–P90 percentile range, when the differences are reflected in all EEG leads, and minimal age differences in the P300 EP amplitudes.

Socio-demographic factors, as well as parameter variations within the normative ranges according to the MoCA and Beck Depression Inventory data, had no connection with the P300 EP parameters in individuals of the group with normal MoCA scores, no depression, preserved working capacity and/or social functions. This indicates that the principles of study participant selection for the formation of normative values of the P300 EP parameters were chosen correctly, as far as the survey capabilities allowed. Nevertheless, the smoking factor, even past smoking (in those who quit smoking by the time of the study), was associated with both the latency and the amplitude of the P300 EP. The obtained data are consistent with studies where, in representative samples of mentally healthy people who were smoking at the time of the study and had never smoked, the electrophysiological phenotype of a smoker with reduced P300 EP amplitude was shown, with a possible dose-response relationship [18, 19]. The latency prolongation of the auditory P300 EP is associated with the prolongation of its sensory component N1, when there is a decrease in the perception of the auditory signal due to the chronic effect of nicotine on the neuronal pathways from the inner ear to the auditory zone of the cerebral cortex [20].

Despite the fact that the issue of creating normative databases of P300 EP parameters has been raised for a long time, for more than 30 years [21], comparison of the obtained results with literature data is difficult. This is due to the different number of participants in the samples and different statistical approaches. The most commonly used method is to evaluate P300 EP indicators taking into account the average value and standard deviation (sigma) [2]. Nevertheless, focusing on the data of the P300 EP norm in the central EEG leads with pressing a button in the group of 60–69 years (383 ± 40 ms), given in the work of V.V. Gnezditsky et al. [2], it can be concluded that in our study both the average values (374.5–380 ms) and standard deviations (42.4–46.2 ms) in the 60–69 year old group are

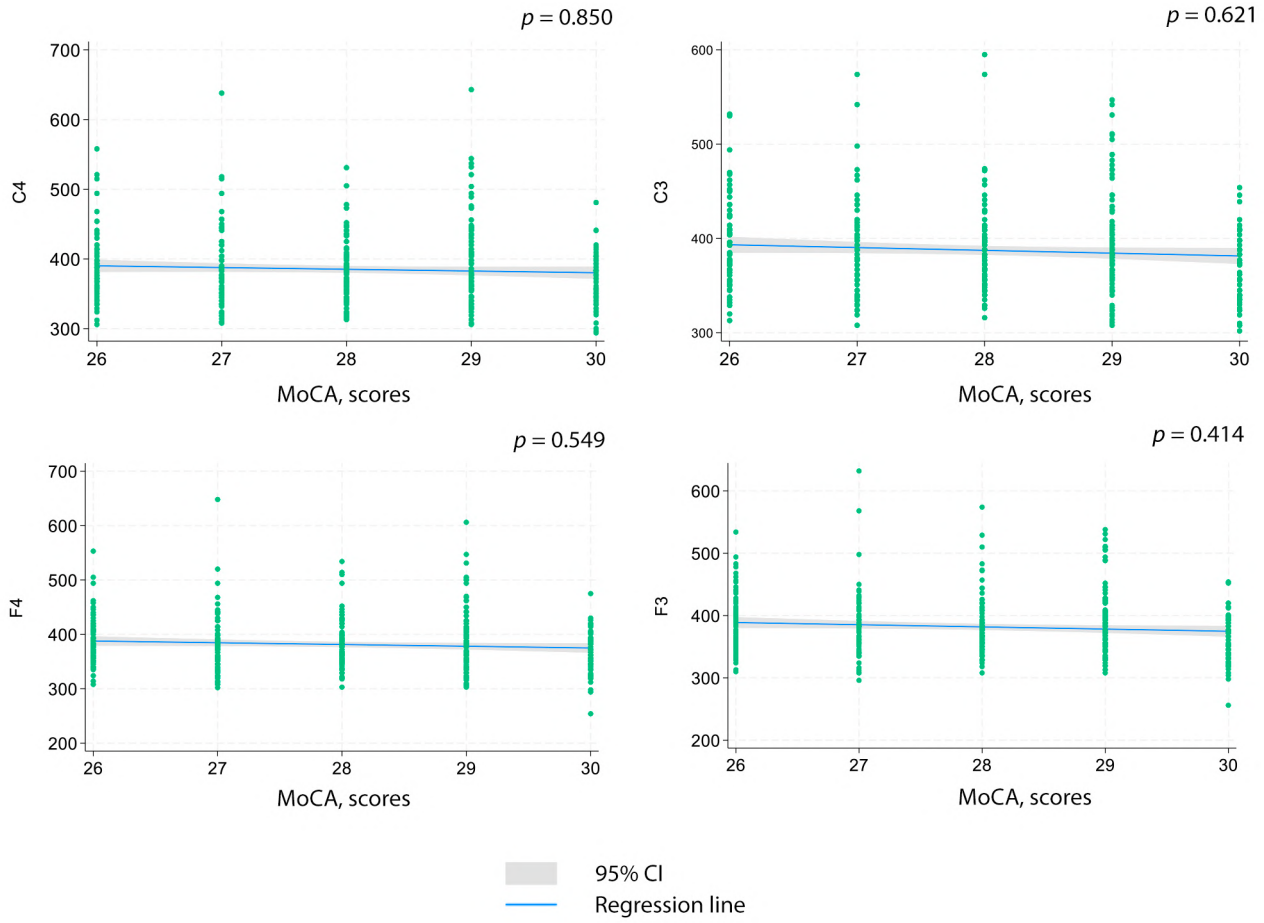

FIG. 1.
Results of multiple regression analysis showing the relationship between P300 evoked potential latency and MoCA scores ranging from 26 to 30 points in individuals with normative values of P300 evoked potential parameters: regression lines and relationships between variables are defined with correction for gender and age

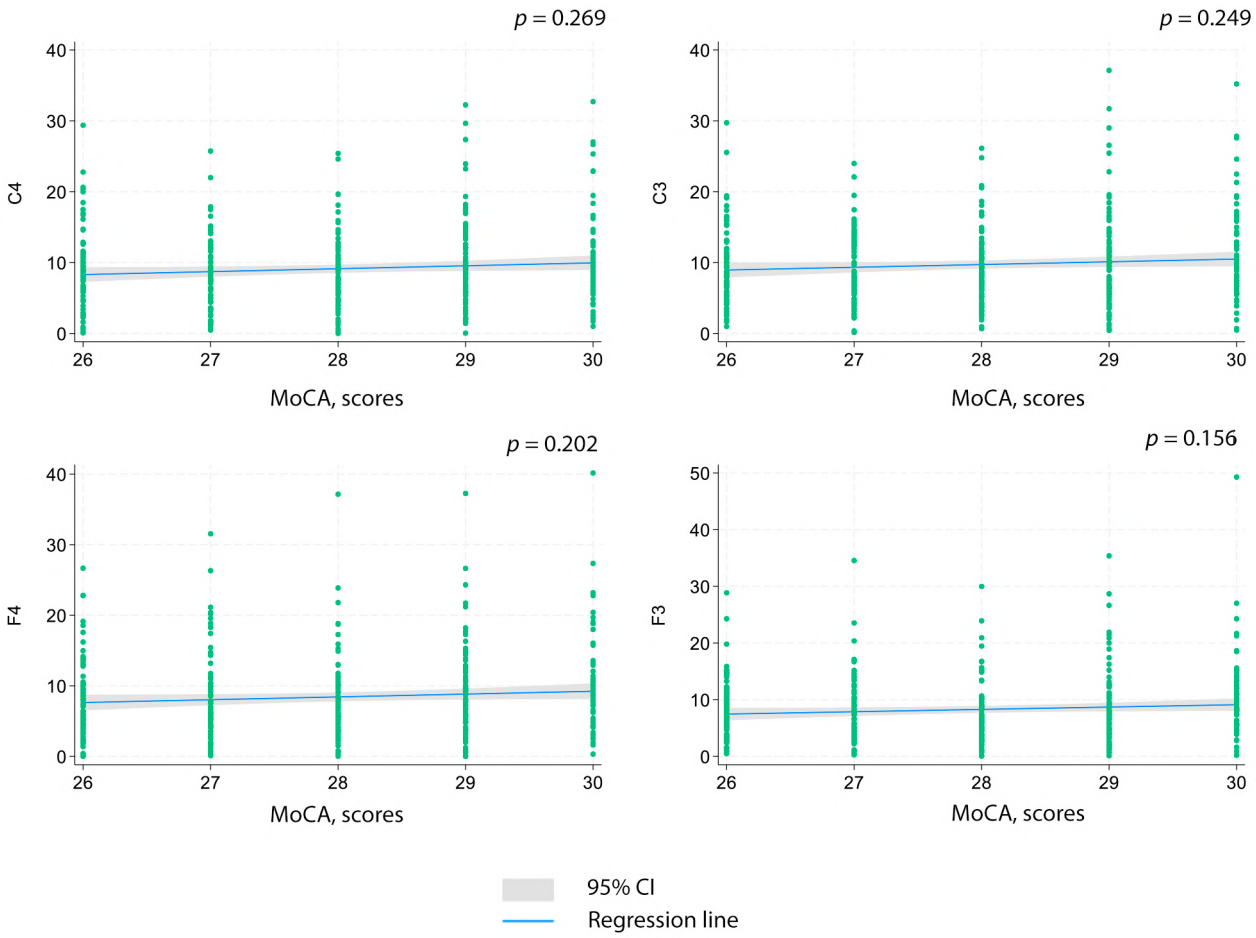

FIG. 2.
Results of multiple regression analysis showing the relationship between P300 evoked potential amplitude and MoCA scores ranging from 26 to 30 points in individuals with normative values of P300 evoked potential parameters: regression lines and relationships between variables are defined with correction for gender and age

generally comparable with the data of the above-mentioned authors. In the 70–74 year old group, the P300 EP latency in our study appears to be more prolonged (average – 399–406 ms, sigma – 54–56 ms; according to V.V. Gnezditsky et al. [2] – 392 ± 46 ms).

In another study, also conducted using auditory P300 EPs [22] in healthy elderly individuals in South Korea with an average age of 75.8 years, the P300 EP latency was 362.5 ± 44.2 ms in the median frontal EEG lead, 362.2 ± 43.5 ms in the central EEG lead (M ± sigma). In a study conducted by the authors from Japan in healthy individuals with an average age of 62.7 years, the P300 EP latency was 377–406 ms [23].

Acknowledging that a comparison of the obtained data with the data presented in the literature is only conditionally possible (differences in the electrode placement schemes, different characteristics of the samples) [4], we can state in general the correspondence of the ranges of auditory P300 EP latencies in our study with the literature data for individuals aged 60–69 years (especially at the level of P25–P75), but there is a more pronounced shift towards an increase in the P300 EP latency in a group of Arkhangelsk residents aged 70–74 years.

Despite the recognition by various authors of the presence of extended P300 EP latencies in individuals without identified cognitive impairment, the P300 EP latency of about 400 ms is considered a threshold above which the risk of cognitive impairment and a decrease in the volume of working memory statistically significantly increases, and in individuals with both neurodegenerative and neurovascular pathology [2]. There is evidence that the P300 EP latency of more than 400 ms can be associated with cerebrovascular insufficiency and signs of dementia according to neuropsychological testing [24]. Thus, it can be stated that in elderly people of Arkhangelsk aged 60–69 years, the limit of the conditional "average" norm (P25–P75) of the P300 EP latency in all the EEG leads under consideration corresponds to 400 ms.

The results showed that age differences in the P300 EP amplitudes in the age range of 60–74 years were minimal. According to the "aging curve" calculations [6], for the age of up to 74 years, the amplitude norm

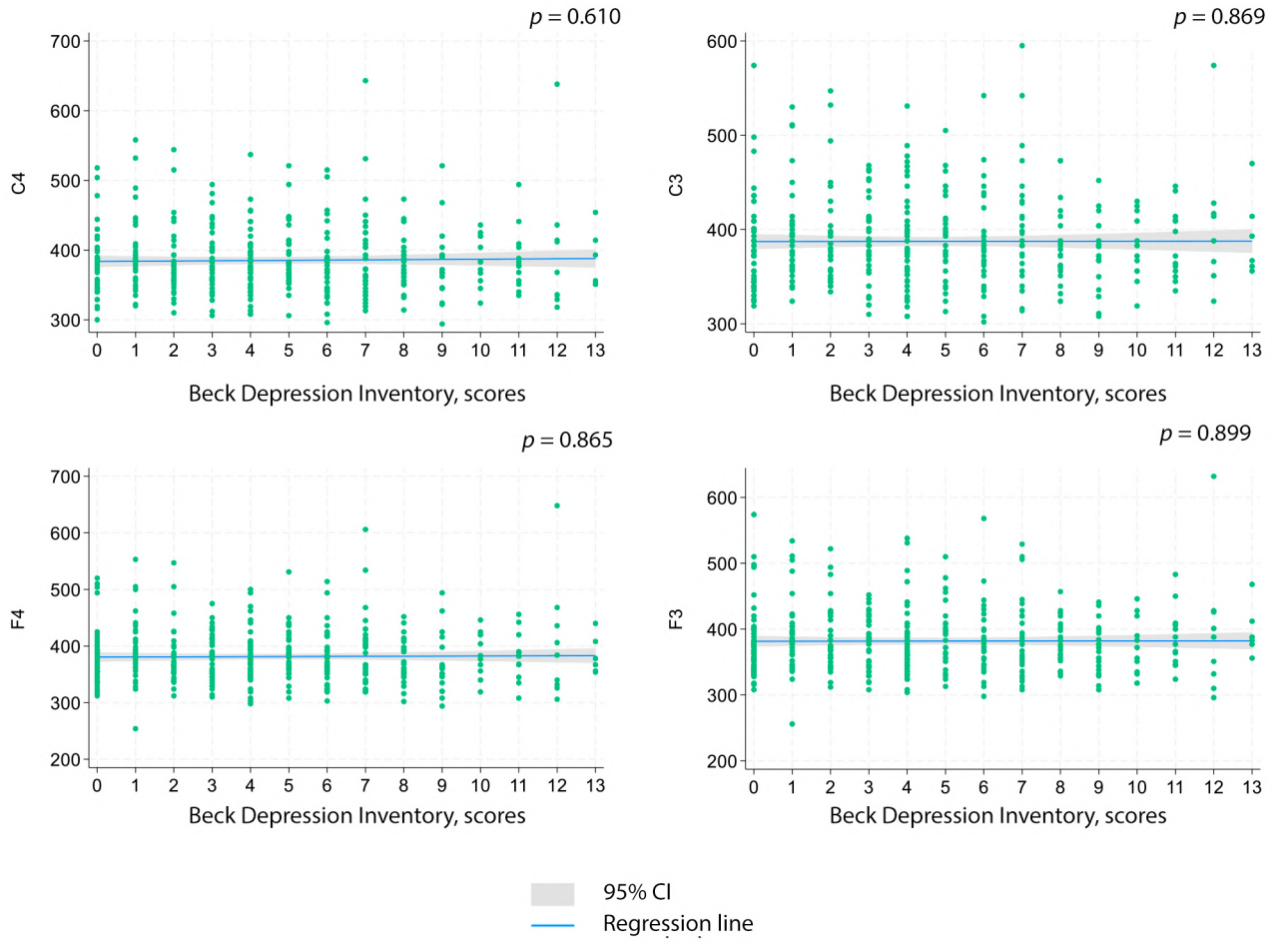

FIG. 3.
Results of multiple regression analysis showing the relationship between P300 evoked potential latency and Beck Depression Inventory scores ranging from 0 to 13 points in individuals with normative values of P300 evoked potential parameters: regression lines and relationships between variables are defined with correction for gender and age

is expected to be more than 5 μV. In our study, the lower limit of the average norm (P25) is presented slightly lower – at the level of 5 μV (4 μV), but at P10 it is significantly lower (from 1.4 to 4 μV) depending on the EEG lead. It is worth considering that the calculations of regression models for predicting the amplitude depending on age presented in the literature were carried out taking into account a large range of ages (from 18 to 80 years) and on small samples of people.

If we rely on the works devoted to the study of the auditory P300 EP parameters in elderly people, then the relationship between age and the P300 amplitude is not obvious, as in our study. In the previously presented work, in healthy individuals with an average age of 75.8 years, the amplitude in the median frontal lead was 6.5 ± 5.5 μV, in the median central lead – 4.0 ± 2.8 μV (M ± sigma) [22]. According to other data, in individuals with an average age of 62.7 years, the amplitudes were 5.2 ± 1.9 μV (M ± sigma) [25]. Some authors associate the decrease in the P300 EP amplitudes with aging not with age, but with a lower level of education and gender differences [26], which was not confirmed by the data of our study.

Thus, the average values of the P300 EP amplitudes, as well as their ranges, do not always have a statistically significant dependence on age in elderly individuals. In the study by C.F. Kügler et al. [7], a slight age-related decrease in the P300 EP amplitudes with aging was also indicated. Apparently, a change in the perception of auditory information due to an increasing neurosensory deficit in sound recognition can cause compensatory activation of various cortical areas to maintain the process of recognizing sensory information, which can contribute to the expansion of the normal range of the P300 EP amplitude, characteristic of healthy aging. It can be stated that in our study, the P300 EP amplitude is represented by a wide range of values – both towards low and towards fairly high amplitudes, especially at the upper percentiles (P75–P95 – up to 18–22 μV).

The prolongation of the P300 EP latency in individuals with intact cognitive functions according to neuropsychological testing data can be considered

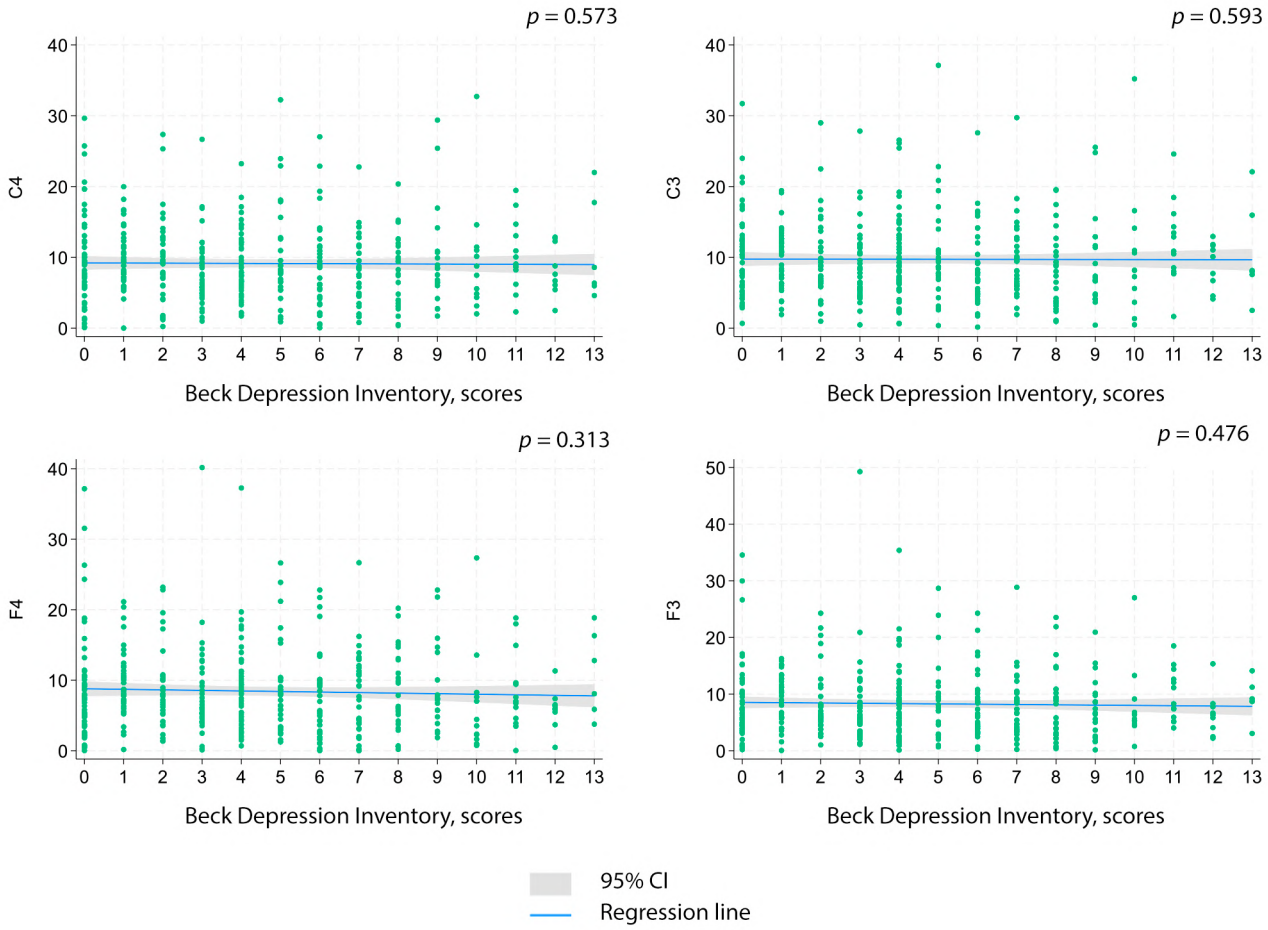

FIG. 3.
Results of multiple regression analysis showing the relationship between P300 evoked potential amplitude and Beck Depression Inventory scores ranging from 0 to 13 points in individuals with normative values of P300 evoked potential parameters: regression lines and relationships between variables are defined with correction for gender and age

from the standpoint of a decrease in cognitive reserve. The concept of cognitive reserve includes the ability of the brain to optimize or maximize its performance due to a differentiated set of neural connections that allows finding alternative cognitive strategies when making a decision [27, 28]. Therefore, the optimal time for making a decision when recognizing a significant sensory stimulus, reflected in the auditory P300 EP latency, may reflect the process of effective adjustment of the brain neural network in the decision-making process.

Based on the experience of previous researchers and taking into account our own results, in individuals aged 60–69 years, the P300 EP latency values higher than P75 (in individuals aged 60–69 years – more than 400 ms, in individuals aged 70–74 years – more than 443 ms) can be considered from the position of a decrease in cognitive reserve and a prognostically unfavorable risk criterion for the cognitive impairment development in elderly people living in a certain climatic and geographic region (Arkhangelsk). For subsequent analysis of the reasons for the significant representation of the extended P300 EP latency (in percentiles, P75 and above), dynamic observations are necessary, as well as a more in-depth study of cognitive functions, neuroimaging data on morphofunctional changes in the brain, in order to differentiate groups with "normal" aging and "pathological" aging with the risk of developing various pathophysiological variants of dementia (vascular, neurodegenerative nature) [29].

It is also important to further study the role of endocrine-metabolic factors that directly or indirectly determine changes in the rate of brain neuronal activity during decision-making, for example, the state of the thyroid system, metabolic parameters (carbohydrate, fat, protein). These endocrine-metabolic factors can affect both the formation of the normal ranges of the P300 EP parameters and the effectiveness of cognitive functions in residents of the Arctic zone of the Russian Federation, primarily memory functions [30].

Based on the fact that with age the values of the conditional norm of the P300 EP latency increase, the preservation of the P300 EP latency within the age

norm or even at the level of the previous age decade can be considered as an electroneurophysiological reflection of the cognitive reserve preservation for an elderly person, the basis of his cognitive longevity.

The P300 EP amplitude below the P25 value can also be considered in the context of a decrease in cognitive reserve. However, its minimal changes in the age aspect give reason to believe that during healthy aging, the P300 EP amplitude may not undergo significant changes. A pronounced decrease in the P300 EP amplitude in this case will be more associated not with physiological, but with pathological vascular, metabolic changes in brain functions, primarily associated with the risk of developing neurodegeneration [13].

The limitation of the presented study may be associated with the insufficient analysis of possible gender differences, age-related changes in the studied parameters taking into account cognitive impairment according to neuropsychological testing, and the technical complexity of assessing morphofunctional changes in the brain in all participants of the population study using neuroimaging methods. Such an analysis would be more correct in a comparative analysis with the inclusion of younger age groups (up to 60 years), which is planned for the next stage of the study. Thus, the subsequent development of the research topic is planned in the context of the analysis of the role of socio-demographic, behavioral factors, cognitive decline (according to the expanded neuropsychological study) and the severity of depression, which have the greatest impact on the age ranges of the P300 EP parameter values in the elderly, as well as in comparison with individuals of the previous age group (average age).

Another possible limitation of the study may be the inclusion in the analyzed group of 84 (21 %) participants with probable preasthenia and 5 (1.3 %) participants with probable frailty, who got 3–4 and 5–7 scores, respectively, on the Age Is No Barrier test. According to the questionnaire requirements, it was necessary to check the cognitive functions of individuals with 3 points or more using the Mini-Cog questionnaire [14, 15], which was not done. However, the results obtained on the MoCA scale, which is more informative than the Mini-Cog screening test [16, 17], demonstrated normal values according to the MoCA scale, which served as the basis for considering probable preasthenia and asthenia in these individuals to be due to a decrease in physical functions, and not the presence of cognitive decline.

CONCLUSION

For the first time, the normative values of the parameters of cognitive auditory evoked potentials P300 in residents of the European North of Russia aged 60–74 years in the range from 5-95 percentile are presented. In the group of 60–69 years, the range of P300 EP latencies at the level of the "average" norm (P25–P75) was 343–401 ms, and in the group of 70–74 years – 359–443 ms. Age differences in the P300 EP amplitudes were minimal, the range on average in the total sample was 4–13 μV (P25–P75). Latency above 400 ms (60–65 years) or above 443 ms (70–74 years) in individuals with preserved working capacity and/or social functions and normal indicators on the MoCA scale and the absence of depression (according to the Beck Depression Inventory) is proposed to be considered from the position of a decrease in cognitive reserve and a prognostically unfavorable criterion for the risk of developing cognitive impairment in elderly people living in the European North of Russia.

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Conflicts of interest

No potential conflict of interest relevant to this article reported.

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PREDICTORS OF THE DYNAMICS OF CHANGES IN COGNITIVE FUNCTIONS IN PATIENTS 6 MONTHS AFTER CAROTID ENDARTERECTOMY

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ABSTRACT

Background. *Carotid atherosclerosis is one of the urgent problems due to the high risk of developing ischemic stroke and cognitive impairment. The dynamics of clinical disorders in patients with carotid stenosis is determined by a complex of neuro-physiological, angiological, tissue and biomolecular reactions, the characteristics of which can act as predictors of the course of the pathology.*
The aim of the work. *To determine the neurophysiological parameters and predictors of cognitive dysfunction in patients who underwent carotid endarterectomy.*
Materials and methods. *The study included 59 people with carotid atherosclerotic disease. All included patients underwent carotid endarterectomy. We assessed the degree of stenosis of the internal carotid artery and cognitive status using the FAB (Frontal Assessment Battery) scale and MoCA (Montreal Cognitive Assessment) Test and recorded electroencephalogram (EEG), P300 cognitive evoked potentials and heart rate variability in patients at various terms (before surgery, 6 months after the surgery). Patients were divided into groups based on the dynamics of cognitive tests using cluster analysis (k-means) with identification of elements included in the clusters: patients of cluster 1 had a "preserved" profile of cognitive status; patients of cluster 2 – moderate cognitive dysfunction.*
Results. *Patients of cluster 1 had a higher power of beta oscillations in the frontal lead, a higher amplitude of the P3 component of the P300 potential, and a greater variability of R-R intervals in terms of the total indicator and high-frequency power. We proposed a model that allows us to classify patients into groups according to the dynamics of cognitive function scores. According to the data obtained, the most significant predictors of the dynamics of cognitive status were the initial characteristics of the EEG and the P300 cognitive evoked potential.*
Conclusions. *We determined the clinical and neurophysiological correlates of cognitive dysfunction: an association with greater preservation of activating effects on the EEG, processes of recognition and decision-making in the associative zones of the cortex, and less pronounced activity of stress-implementing mechanisms. Indicators of EEG spectral analysis and characteristics of the P300 cognitive evoked potential are predictors of the cognitive status dynamics.*

Key words: *carotid atherosclerosis, P300 cognitive potential, carotid endarterectomy, cognitive functions, artificial neural networks, cluster analysis*

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ПРЕДИКТОРЫ ДИНАМИКИ ИЗМЕНЕНИЯ КОГНИТИВНЫХ ФУНКЦИЙ У ПАЦИЕНТОВ ЧЕРЕЗ 6 МЕСЯЦЕВ ПОСЛЕ КАРОТИДНОЙ ЭНДАРТЕРЭКТОМИИ

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РЕЗЮМЕ

Актуальность. *Атеросклеротическое поражение сонных артерий является одной из актуальных проблем в связи с высоким риском развития ишемического инсульта и когнитивных нарушений. Динамика клинических нарушений у пациентов с каротидными стенозами определяется комплексом нейрофизиологических, ангиологических, тканевых и молекулярно-биологических реакций, характеристики которых могут выступать в роли предикторов течения патологии.*

Цель работы. *Определение нейрофизиологических параметров и предикторов когнитивной дисфункции у пациентов, перенёсших каротидную эндартерэктомию.*

Материалы и методы. *В исследование было включено 59 человек с атеросклеротическим поражением сонных артерий. Все включённые пациенты подвергались каротидной эндартерэктомии. У пациентов в различные сроки (до операции, через 6 месяцев после вмешательства) производилась оценка степени стеноза внутренней сонной артерии, регистрация электроэнцефалограммы (ЭЭГ), когнитивных вызванных потенциалов P300, вариабельности сердечного ритма, а также оценка когнитивного статуса по шкалам FAB (Frontal Assessment Battery), MoCA (Montreal Cognitive Assessment) Test. Пациенты были разделены на группы на основе динамики когнитивных тестов методом кластерного анализа (k-средних) с идентификацией элементов, входящих в кластеры: кластер 1 – с «сохранным» профилем когнитивного статуса; кластер 2 – с умеренными когнитивными нарушениями.*

Результаты. *В кластере 1 определяется более высокая мощность бета-колебаний в лобных отведениях, амплитуда компонента P3 потенциала P300, а также большая вариабельность R-R интервалов по суммарному показателю и высокочастотной мощности. Нами была предложена модель, позволяющая классифицировать пациентов в группы по динамике балльной оценки когнитивных функций. Согласно полученным данным, наиболее значимыми предикторами динамики когнитивного статуса являлись исходные характеристики ЭЭГ и когнитивного вызванного потенциала P300.*

Выводы. *Выявлены клинико-нейрофизиологические корреляты когнитивной дисфункции: ассоциация с большей сохранностью активирующих влияний на ЭЭГ, процессов опознания и принятия решения в ассоциативных зонах коры, меньшей выраженностью активности стресс-реализующих механизмов. Показатели спектрального анализа ЭЭГ и характеристики когнитивного вызванного потенциала P300 являются предикторами динамики когнитивного статуса.*

Ключевые слова: *атеросклероз сонных артерий, когнитивный потенциал P300, каротидная эндартерэктомия, когнитивные функции, искусственные нейронные сети, кластерный анализ*

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INTRODUCTION

Carotid atherosclerosis is one of the urgent problems of both neurology and vascular surgery due to the high risk of developing ischemic stroke and cognitive impairment in asymptomatic patients [1–3].

The dynamics of clinical disorders in patients with carotid stenosis is determined by a complex of neurophysiological, angiological, tissue and biomolecular pathological and compensatory-adaptive reactions, the characteristics of which can act as predictors of the course of cerebrovascular pathology [4–6].

An important clinical characteristic of patients with hemodynamically significant stenosis of the carotid arteries is cognitive impairment [7, 8] associated with brain perfusion limitations and secondary changes in the trophism of the associative cortical zones [9, 10].

One of the objective correlates of cognitive impairments are endogenous evoked potentials (potentials associated with events) – P300, reflecting the psychophysiological processes of stimulus recognition and decision-making in relation to it [11]. There are also data on the relationship between neurodynamic cognitive impairments and the activity of ergotropic and trophotropic mechanisms of autonomic support of behavior [12]. Ultrasound and clinical laboratory characteristics in this group of patients can equally be considered as variables influencing cognitive status [13, 14].

In this regard, a relevant issue is the study of the role of these indicators as correlates of the cognitive status dynamics in patients with carotid stenosis.

THE AIM OF THE STUDY

To determine the neurophysiological parameters and predictors of cognitive dysfunction in patients who underwent carotid endarterectomy.

MATERIALS AND METHODS

The study included 59 patients between 2021 and 2022. Patients included in the study were grouped as "symptomatic" (group 1; $n = 33$), with acute cerebrovascular accidents in a medical history, and as "asymptomatic" (group 2; $n = 26$), without acute cerebrovascular accidents in a medical history, according to brain magnetic resonance imaging. All included patients underwent carotid endarterectomy according to clinical guidelines [15]. The surgical technique (classical or eversion) was selected based on the preferences of the operating surgeon, the atherosclerotic plaque extent on the internal and common carotid arteries, and the carotid bifurcation level. A Dacron patch was used for the classical technique. The groups were comparable in terms of the surgery type. All surgical interventions were performed using total combined anesthesia. Artificial lung ventilation was performed using a Dräger Primus breathing apparatus (Dräger, Germany) in the IPPV mode under normoventilation conditions ($PaCO_2 = 35–45$ mm Hg) with ventilation parameter control. General data on the patients are presented in Table 1; both groups of patients were comparable in age and the presence of concomitant pathology, as well as in the intervention technique.

TABLE 1

GENERAL CHARACTERISTICS OF THE PATIENTS

Indicators	Group 1 ($n = 33$)	Group 2 (n = 26)	p*
Age (years)	66.7 ± 8.2	68.2 ± 7.1	–
Gender	male – 21 (63.6 %) female – 12 (36.4 %)	male – 17 (65.3 %) female – 9 (34.7 %)	–
Side of the ICA lesion	left – 19 right – 14	left – 17 right – 9	0.288 0.234
Hypertension	30 (90.9 %)	23 (88.4 %)	0.424
Ischemic heart disease	10 (30.3 %)	12 (46.1 %)	0.123
Postinfarction cardiosclerosis	3 (9 %)	4 (15.3 %)	0.302
Type 2 diabetes mellitus	8 (24.2 %)	4 (15.3 %)	0.155
CEA procedure technique	classical – 15 (45.4%) eversion – 18 (54.6%)	classical – 12 (46.1%) eversion – 14 (53.9%)	0.519 0.457
Temporary intraluminal shunting	7 (21.2 %)	5 (19.2 %)	0.378
Intraoperative ICA clamping time (s)	1567 [1390; 1780]	1597 [1335; 1723]	0.759

Note. ICA – internal carotid artery; CEA – carotid endarterectomy.

The inclusion criteria were the presence in patients of: internal carotid artery stenosis according to expert ultrasound examination of the brachiocephalic arteries (US-BCA) of 70–99 % according to NASCET (North American Symptomatic Carotid Endarterectomy Trial) in the absence of transient ischemic attack (TIA) episodes or acute cerebrovascular accident (CVA); internal carotid artery stenosis according to expert BCA ultrasound from 50 to 60 % according to NASCET, considering morphological instability of the atherosclerotic plaque (ulceration, hemorrhage into the plaque, intimal flotation, mural thrombus) and neurological symptoms – TIA or stroke; internal carotid artery stenosis according to expert BCA ultrasound of more than 60 % according to NASCET in the presence of episodes of TIA or CVA [16]. Patients with contralateral internal carotid stenosis were not included in the study. Exclusion criteria were: contralateral internal carotid artery stenosis; hearing impairment that prevents testing; patient refusal to participate in the study; systemic disease (renal, liver, gastrointestinal, hematological, immunological disease, etc.); degenerative diseases of the nervous system (Alzheimer's disease, Huntington's chorea, Parkinson's disease, amyotrophic lateral sclerosis, frontotemporal degeneration).

Exclusion criteria during the observation period were: death; any cardiovascular events; refusal to continue the study.

Patients received standard antiplatelet (monotherapy with aspirin 100 mg or in combination with clopidogel 75 mg) and lipid-lowering (3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors in a dose according to the level of low-density lipoproteins) therapy, and did not take drugs that affect cognitive functions (central acetylcholinesterase inhibitors or acatinol memantine, etc.).

In patients, the degree of internal carotid artery stenosis was assessed using ultrasound data at different times (before surgery (1–5 days before the intervention) – visit 1; 6 months after the intervention – visit 2); in some cases, computed tomography with angiography and/or digital subtraction angiography were used as a confirmation method. Electroencephalogram (EEG), cognitive evoked potentials (P300), heart rate variability (HRV) were recorded, and cognitive status was assessed using the Frontal Assessment Battery (FAB) and the Montreal Cognitive Assessment (MoCA Test).

Ultrasound examination was performed on a GE Vivid S5 device (GE HealthCare, USA) with a linear sensor with a frequency of 12 MHz; magnetic resonance imaging was performed on a MAGNETOM Symphony 1.5 Tesla device (Siemens, Germany).

EEG was recorded using a 19-channel digital electroencephalograph Neurospectr-3 and the corresponding Neuron-spectr software (Neurosoft LLC, Russia) [17]. The electrodes were mounted according to the 10–20 % scheme (Fp1, Fp2, F7, F3, F4, F8, T3, C3, C4, T4, T5, P3, P4, T6, O1, O2, Fz, Cz, Pz) with reference electrodes on the ears A1 and A2. The quantization frequency of the analog-to-digital converter was 200 Hz, the high-pass filter (time constant) was 0.5 Hz (0.32 s), and the low-pass filter was 75 Hz. The average analysis epoch was 20.48 s (4096 samples). EEG was recorded in the background state. Before conducting the EEG mathematical analysis, artifacts were identified and excluded from the analysis, as well as epileptiform activity was identified both visually and by software detection of spikes and sharp waves. Recording fragments without artifacts were used for the analysis. The EEG analysis was performed using spectral analysis methods based on the fast Fourier transform with the allocation of the following frequency ranges: delta (0.5–3.9 Hz), theta (4.0–7.9 Hz), alpha (8–13 Hz), beta-1 (14–19.9 Hz), beta-2 (20–35 Hz), gamma (36–100 Hz). The following parameters were studied during the spectral analysis: total power; average power; average amplitude and average frequency of alpha oscillations, beta1 and beta2 oscillations, theta and delta oscillations. Cognitive evoked potential P300 registration was performed to assess the activity of the sensory systems. The auditory evoked potentials (EP) study was carried out by recording long-latency auditory EPs, reflecting to a greater extent the function of the auditory analyzer thalamocortical section. For stimulation using headphones, binaurally, clicks with a duration of 50 ms, a filling of 1000 Hz, a frequency of 1–1.5 Hz with a random component (to exclude the phenomenon of habituation to periodicity) were used; the gain was 5–20 μV/mm, the analysis epoch was 500 ms, the number of summations (averaging) was 100. The cognitive evoked potential P300 was recorded in the odd-ball paradigm during the presentation of sound stimuli and the patient's active response to a significant (deviant) stimulus (button pressing) using the "Neuron-Spectrum.DVP" hardware and software complex (Neurosoft LLC, Russia) [17]. Electrocardiogram (ECG) was recorded using the "VNS-Micro" device and the "Poli-Spectrum.NET" program (Neurosoft LLC, Russia). The vegetative regulation mechanisms were assessed by recording and analyzing heart rate variability using the "Poli-Spectrum.NET" hardware and software complex (Neurosoft LLC, Russia) [18]. The assessment was performed in a sitting position in the 1st standard ECG lead; then statistical and spectral analyses of the dynamic series of R-R intervals were performed.

Statistical data processing was performed using the Statistica 10.0 software package (StatSoft Inc., USA). Descriptive statistics included the median (Me), lower and upper quartiles (LQ; UQ). Quantitative data comparison was performed using the Mann – Whitney statistical criterion (U; the normalized indicator is Z); for qualitative data, 2 × 2 conjugation tables and the chi-square criterion were used. Differences were considered statistically significant at $p < 0.05$. To isolate subgroups of patients, the cluster analysis method (k-means) was used with identification of elements included in the clusters. The problem of classifying patients into groups with known clinical characteristics was solved based on machine learning technology (artificial neural networks) using the studied neurophysiological parameters as input parameters. The artificial neural network performance was assessed on the training, control, and test samples. The software package Statistica 10.0 (StatSoft Inc., USA) was used.

All patients signed informed consent; the study was approved by the local Ethics Committee of the Ryazan State Medical University named after Academician I.P. Pavlov of the Ministry of Health of the Russian Federation (protocol No. 3 dated October 11, 2021).

RESULTS OF THE STUDY

To solve the problem of identifying factors that predict the trajectory of patients' cognitive functioning, we implemented a formalized procedure for categorizing patients into homogeneous groups based on the dynamics of cognitive tests using the cluster analysis method (clustering by the k-means method).

Table 2 shows the results of cluster analysis based on changes in FAB and MoCA Test indicators at visit 1 (before surgery) and 6 months after surgery.

As follows from Table 2, cluster 1 is characterized by a more preserved cognitive status compared to cluster 2 according to the selected indicators, in connection with which cluster 1 is designated as a group with a preserved profile of cognitive status, cluster 2 – as a group with moderate cognitive impairment (according to the MoCA Test indicator).

A fundamentally important fact is the absence of statistically significant differences in the presence of the previous ischemic stroke factor in the anamnesis in the groups (in cluster 1: 55 % with a history of stroke, 45 % without a history of stroke; in cluster 2 – 52 % and 48 %, respectively; $\chi^2 = 0.06$; $p = 0.83$).

Table 3 presents the neurophysiological indicators in the clusters.

A statistically significantly higher power of beta oscillations in the frontal leads in cluster 1 is determined; as well as higher amplitude of the P300 potential P3 component in this cluster. When studying the heart rate variability in cluster 1, a greater variability of the R-R intervals by the total indicator and high-frequency power are revealed.

Table 4 presents the correlations between neurophysiological indicators and the scoring level of cognitive functions in clusters.

As follows from the table, statistically significant correlations are determined between the P300 potential amplitude and the cognitive function score level at visit 1 (for groups 1 and 2) and at visit 2 (after 6 months).

TABLE 2

DYNAMICS OF INDICES OF COGNITIVE FUNCTIONS

Indicators	Cluster 1 (*n* = 33)	Cluster 2 (n = 26)	Statistical indicators	
	Me (LQ; UQ)	Me (LQ; UQ)	U (Z)	*p*
FAB, visit 1	17 (17; 18)	16 (14; 16)	70 (3.6)	0.001
FAB, visit 2	18 (17; 18)	16 (16; 17)	62 (3.8)	0.001
MoCA Test, visit 1	26 (25; 28)	23 (22; 24)	43 (4.3)	0.001
MoCA test, visit 2	27 (26; 29)	25 (24; 26)	33 (4.6)	0.001

TABLE 3

NEUROPHYSIOLOGICAL PARAMETERS IN SELECTED CLUSTERS

Indicators	Cluster 1	Cluster 2	Statistical indicators	
	Me (LQ; UQ)	Me (LQ; UQ)	U (Z)	*p*
Power of EEG beta oscillations in lead F3, μV × ms	5 (4; 6)	4 (3; 5)	48 (2.01)	0.048
Amplitude of potential P300 in lead P3 after surgery, μV	6.9 (2.9; 7.4)	2.8 (0.9; 6.4)	14.5 (2.25)	0.024
Amplitude of potential P300 in lead Cz after surgery, μV	6.4 (5.8; 8.0)	2.4 (0.8; 5.4)	9 (2.73)	0.007
RMS HRV, ms	29 (16; 44)	17 (10; 25)	45 (2.12)	0.034
HF HRV, ms	177 (36; 328)	26 (17; 120)	48 (1.9)	0.047

Note. RMS HRV – variability of R-R intervals according to the total indicator, HF HRV – high-frequency power.

We proposed a model based on artificial neural network (ANN) technology, which allows classifying patients into groups based on the cognitive function score dynamics based on the above-described neurophysiological indicators. The model with optimal characteristics was a multilayer perceptron with 29 input neurons, 7 intermediate layer neurons, and 2 output neurons (MLP 29-7-2). The model demonstrated 100 % training performance, 78 % test sample performance (BFGS 7 training algorithm).

In the test sample, a smaller number of correct solutions were found in cluster 2 (70 % correct answers; in cluster 1 – 100 %); that is, the more difficult task was to classify subjects into cluster 2. The area under the ROC-curve was 0.950 (good quality of the model) (fig. 1).

Table 5 presents the ranked (in order of decreasing importance) neurophysiological indicators used to solve the classification problem.

As follows from Table 5, the most significant predictors of the cognitive status dynamics in patients who underwent carotid endarterectomy were the EEG characteristics and the cognitive evoked potential P300 characteristics.

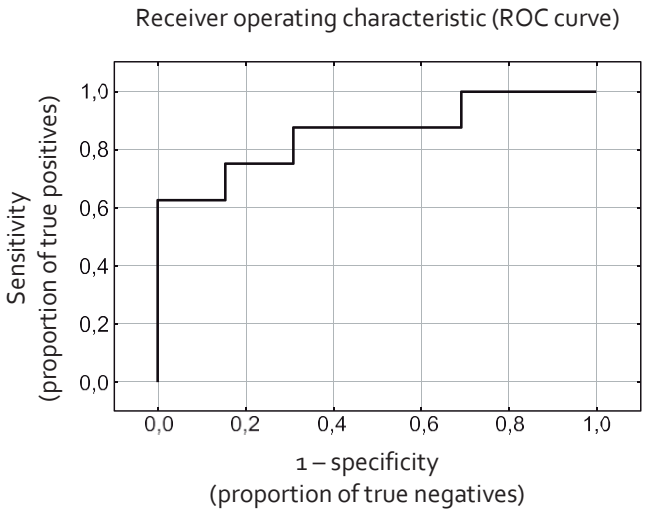

FIG. 1.
ROC-curve of the classification model into the studied clusters

TABLE 4

CORRELATIONS OF NEUROPHYSIOLOGICAL INDICES IN CLUSTERS

Groups of indicators	Cluster 1		Cluster 2	
	correlation	*p*	correlation	*p*
FAB (visit 1), P200 CEP amplitude in lead Fz	0.494	0.044	–	–
MoCA Test (visit 1), P300 CEP amplitude in lead Cz	–	–	0.632	0.029
MoCA Test (visit 2), P300 CEP amplitude in lead Cz	–	–	0.539	0.039

Note. CEP – cognitive evoked potential.

TABLE 5

NEUROPHYSIOLOGICAL INDICATORS USED FOR CLASSIFICATION AND RANKED IN THE DESCENDING ORDER OF SIGNIFICANCE

Groups of indicators	Characteristics	
	Rank*	Sensitivity
EEG alpha oscillation power in lead O1, μV × ms	1	3.0
HF HRV	2	2.5
P2N2 P300 amplitude in lead Fz	3	2.5
P3 P300 latency in lead Fz	4	2.0
EEG beta oscillation frequency in lead F3	5	2.0
EEG alpha oscillation power in lead O2, μV × ms	6	2.0
EEG theta oscillation frequency in lead F3, counts/s	7	2,0

Note. * – the first 7 indicators with the highest level of sensitivity are presented; HF HRV – high-frequency power.

DISCUSSION

The absence of statistically significant neurophysiological differences in the previous stroke factor in patients with carotid stenosis indicates the need to search for other predictors of the cognitive status dynamics, which is consistent with the literature data [19, 20]. This report presents mainly neurophysiological correlates and predictors of the dynamics of cognitive functions; at the same time, the role of factors associated with clinical status [19], biomolecular and neurotransmitter mechanisms [21, 22] is undoubted.

It was revealed that the preserved cognitive status (cluster 1) is associated with indirect signs of greater brain activation (according to beta1 EEG oscillations), a greater cognitive evoked potential P300 amplitude, as well as with a lesser expression of sympathetic influences (less activity of stress-realizing systems). At the same time, statistically significant correlates of a higher score for cognitive functions of the first visit and the visit after surgery were indicators reflecting the preservation of the stimulus recognition and decision-making mechanisms in relation to it, which also corresponds to literature data [11].

Machine learning technologies are widely used to solve problems in both clinical neurology and clinical angiology [23]. The use of neurophysiological indicators as predictors allowed us to create a model for classifying subjects into groups based on ANN technology. The model was more sensitive to identifying a group with less pronounced cognitive impairment. The most significant predictors of the cognitive status dynamics were the characteristics of the EEG spectral analysis and the cognitive evoked potential P300 indicators.

CONCLUSIONS

In patients with atherosclerotic lesions of the carotid arteries who underwent carotid endarterectomy, the presence of clinical and neurophysiological correlates was revealed: an association of preserved cognitive functions with a more pronounced activation of cortical structures, activity of cortex associative zones, and a lesser expression of the activity of stress-realizing mechanisms. The EEG spectral analysis indicators and the cognitive evoked potential P300 characteristics during recognition and decision-making in relation to a sensory stimulus can be used as additional predictors of the cognitive status dynamics in patients with carotid stenosis using machine learning technologies. At the same time, the stroke factor does not have a statistically significant effect on the cognitive status of patients.

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Conflicts of interest

No potential conflict of interest relevant to this article reported.

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DRUG-RESISTANT EPILEPSY: CURRENT CONCEPTS, PATHOGENESIS, RISK FACTORS, OUTCOMES OF SURGICAL TREATMENT

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ABSTRACT

Despite the wide choice of antiepileptic drugs (AEDs), a third of patients remain resistant to the effects of modern AEDs. Drug-resistant epilepsy (DRE) is characterized by the inability to control seizures in a patient when using at least two adequate AED regimens at an effective daily dose as monotherapy or in combination. In this case, the mechanisms responsible for drug resistance are mainly either increased excretion of AEDs by transporters from epileptogenic tissue (the multidrug transporter hypothesis) or a decrease in the sensitivity of drug receptors in epileptogenic brain tissue. It is assumed that there are other mechanisms, but they remain understudied. A number of factors are associated with the risk of DRE developing in patients with diagnosed epilepsy, including genetic, iatrogenic, brain malformations, and others. Patients with DRE have a higher probability of developing psychopathological disorders (depression, anxiety, psychosis), the proportion of which is significantly higher than in the general population. They have a 10-fold increased risk of death due to injury, cognitive decline, and sudden unexpected death in epilepsy (SUDEP). The priority treatment method for DRE is surgery. Early identification of DRE is critical for identifying potential treatment alternatives and determining whether a patient is a surgical candidate. Analysis of data from clinical and instrumental research of operated patients with DRE in the early and late postoperative period will allow us to identify factors of unfavorable outcome and to increase the effectiveness of treatment for this category of patients.
The aim *was to study and to summarize literature data on the pathogenesis and risk factors of drug resistance to antiepileptic drugs in patients with epilepsy, justifying the need for timely identification of drug resistance and referral of patients with drug-resistant epilepsy to specialized centers for possible surgical treatment.*

Key words: *drug-resistant epilepsy, pathogenesis, surgical treatment of drug-resistant epilepsy, outcomes of surgical treatment, risk factors*

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СОВРЕМЕННЫЕ ПРЕДСТАВЛЕНИЯ О ФАРМАКОРЕЗИСТЕНТНОЙ ЭПИЛЕПСИИ, ПАТОГЕНЕЗЕ, ФАКТОРАХ РИСКА И ИСХОДАХ ЕЁ ХИРУРГИЧЕСКОГО ЛЕЧЕНИЯ

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РЕЗЮМЕ

Несмотря на большой выбор противоэпилептических препаратов (ПЭП), треть пациентов остаются устойчивыми к действию современных ПЭП. Фармакорезистентная эпилепсия (ФРЭ) характеризуется невозможностью контроля над приступами у больного при применении по крайней мере двух адекватных схем ПЭП в эффективной суточной дозе в качестве монотерапии или в комбинации. При этом механизмами, ответственными за резистентность к фармакопрепаратам, в основном являются либо повышенное выведение ПЭП переносчиками из эпилептогенной ткани (гипотеза мультилекарственных транспортеров), либо снижение чувствительности рецепторов к лекарству в эпилептогенной ткани головного мозга. Предполагается наличие и других, но недостаточно изученных механизмов. С риском развития ФРЭ у пациентов с диагностированной эпилепсией связан ряд факторов, включая генетические, ятрогенные, пороки развития головного мозга и другие. Больные с ФРЭ имеют более высокую вероятность развития психопатологических расстройств (депрессия, тревога, психозы), доля которых значительно выше, чем в общей популяции. У них в 10 раз повышается риск летального исхода вследствие травм, снижения когнитивных функций и внезапной смерти (SUDEP, sudden unexpected death in epilepsy). Приоритетным методом лечения ФРЭ является хирургическое. Раннее выявление ФРЭ имеет решающее значение для установления потенциальных альтернатив лечения и определения того, является ли пациент кандидатом на хирургическое вмешательство. Анализ данных клинических, инструментальных методов исследования оперированных больных ФРЭ в раннем и отдалённом послеоперационном периоде позволит выявить факторы неблагоприятного исхода и повысить эффективность лечения данной категории больных.

***Целью исследования** явилось изучение и обобщение данных литературы по патогенезу, факторам риска фармакоустойчивости к ПЭП больных эпилепсией с обоснованием необходимости своевременного выявления фармакорезистентности и направления пациентов с ФРЭ в специализированные центры для возможного оперативного лечения.*

***Ключевые слова:** фармакорезистентная эпилепсия, патогенез, хирургическое лечение фармакорезистентной эпилепсии, исходы хирургического лечения, факторы риска*

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MODERN CONCEPTS OF PHARMACORESISTANT EPILEPSY

Epilepsy is one of the most common chronic disabling neurological diseases, affecting more than 70 million people worldwide [1, 2]. Patients with epilepsy have 3.1 times more physical, mental, or social limitations compared to patients without epilepsy due to cognitive, psychopathological, and other comorbid diseases [3]. Despite the availability of more than 20 modern antiepileptic drugs (AEDs) for the symptomatic treatment of epilepsy, about 30–40 % of patients with epilepsy remain resistant to pharmacotherapy [1, 2, 4].

Drug-resistant epilepsy (DRE) is characterized by the inability to achieve seizure cessation with two "adequate" AED regimens as monotherapy or in combination [5]. It is considered a multifactorial phenomenon based on numerous genetic and acquired mechanisms.

Among the DRE genetic causes are the increased rate of metabolism of AEDs in individuals homozygous or heterozygous for the fast allele of genes that are biotransformed in the liver) [6]; decreased or absent sensitivity of cortical neuron receptors to AEDs. One of the acquired mechanisms can be considered the epileptogenesis initiation by seizures with changes in nervous tissue through neuroplasticity [7].

In recent years, the development of neuroscience, neuroimaging and the use of mathematical models based on graph theory in clinical and fundamental neurology have made it possible to consider epilepsy as a disease of neural networks [8]. In patients with epilepsy, disturbances in the structural and functional connectomes, i.e. the set of structural and functional networks in the nervous system, have been identified. The networks are divided into nodes and connections between nodes (edges), in which changes are noted.

The nodes typically correspond to different areas of the temporal lobe and extratemporal structures [9].

The medical and social DRE consequences are significant for the patient's physical and mental health. Socioeconomic and psychological limitations that reduce their quality of life increase the risk of mortality [10]. According to numerous studies, they have higher levels of cognitive deficits, emotional disorders, mental illnesses, and difficulties or inability to perform certain social roles [11].

The quality of life (QoL) of patients with epilepsy is quite low. This is influenced by the presence of comorbid mental and behavioral disorders, cognitive impairment, the inability to receive timely consultation from a specialist, and the high cost of modern AEDs. The presence of epileptic seizures and the personal characteristics of patients cause a wary attitude towards them from others and stigmatization of such patients in society.

In patients with focal onset seizures, taking two or more anticonvulsants for at least 2 years, or in patients with significant side effects from anticonvulsants and in case the seizures affect or limit daily life and its quality, surgical treatment is indicated [1, 12]. The epileptogenic focus removal in patients with DRE allows achieving complete seizure control in an average of 59–80 % of patients [13–15], as well as significantly improving their QoL [16, 17].

Patients with epilepsy who are indicated for surgical treatment need to assess the risks of remote surgical outcomes, as well as to evaluate their neuropsychological status and QoL. However, there are currently insufficient prospective long-term studies on the efficacy and safety of various surgical treatment methods for patients with DRE. To assess the surgical treatment efficacy, it is important to determine the prognostic factors for a favorable outcome. Surgical treatment of patients with DRE generally yields good results, but the scope and methods of surgical intervention, the risks of adverse outcomes, the state of the psychoemotional and cognitive sphere, especially in the remote postoperative period, remain insufficiently studied.

PATHOGENESIS OF DRUG-RESISTANT EPILEPSY

In recent years, several putative mechanisms underlying drug resistance in epilepsy have been identified. Based on experimental and clinical studies, two main neurobiological theories have been proposed: 1) decreased sensitivity to the target drug in epileptogenic brain tissue (target hypothesis); 2) removal of AEDs from epileptogenic tissue due to overexpression of multidrug transporters (multidrug transporter hypothesis). However, none of them fully explains the neurobiological basis of pharmacoresistance [2, 18].

According to **the target hypothesis**, drug resistance is considered to be the result of the absence or loss of sensitivity of ion channel receptors and neurotransmitter receptors to AEDs [2, 19]. It is assumed that in order to provide an antiepileptic effect, a drug must affect target molecules in the brain. These are primarily voltage-dependent ion channels, neurotransmitter receptors, and transporters or metabolic enzymes involved in the release, absorption, and metabolism of neurotransmitters [20].

In the work of T.A. Sazhina et al. (2019), the presence of local changes in the structure in the epileptic focus area and a decrease in the activity of receptors to gamma-aminobutyric acid (GABA) were noted. It was shown that pathological processes affecting the glutamatergic and GABAergic systems in patients with DRE are accompanied by a change in the content of apoptotic proteins. This could be one of the causes of neuronal death [21]. However, the presence of a significant number of patients with resistance to several AEDs with different action mechanisms simultaneously does not exclude other mechanisms of resistance.

Multidrug transporter hypothesis. It is known that lipophilic substances, which include AEDs, are transported across the blood-brain barrier (BBB) with the help of proteins, in particular P-glycoprotein (PGP) and the family

of multidrug resistance-associated proteins (MRP) – proteins located in the capillary endothelium membrane [2, 18, 22]. They are able to transport excess lipophilic substances, including AEDs, back into the bloodstream, which have penetrated beyond the BBB by diffusion. It has also been shown that multidrug transporters can control the movement of AEDs from the extracellular spaces of the brain to endothelial cells with their subsequent release into the blood [2, 23]. A special genetically determined system controls the process of movement of substances across the BBB. This system limits the passage of ionized hydrophilic substances and large molecules through the BBB.

PGP and MRP in the BBB are thought to act as an active defense mechanism limiting the penetration of lipophilic substances into the brain [24]. A wide variety of compounds, including many lipophilic drugs, are substrates for either PGP or MRP, or both. P-glycoprotein is secreted by tissues with secretory activity (small intestine, liver, kidneys) and at the blood-tissue level (BBB, placenta, blood-testis barrier), which determines the concentration of the drug in the body, its excretion, and concentration in susceptible tissues such as the brain [25]. Most AEDs (phenobarbital, oxcarbazepine, lamotrigine, gabapentin, topiramate, etc.) are substrates for P-glycoprotein [19].

Thus, increased expression of such transporters in epileptogenic tissue likely reduces the amount of drug reaching epileptic neurons, which may be a possible explanation for pharmacoresistance.

Recent advances in neuroscience, particularly in the field of connectomics (**neural network hypothesis**); allow detailed assessment of network organization, dynamics, and functions at the individual level. Data can be assessed using fundamental forms of network analysis based on graph theory, which can reveal patterns of organization prone to abnormal dynamics and epileptogenesis [26]. A single pathological focus involves other, distant areas of the brain in epileptogenesis, forming an epileptic system. The connectomics approach allows for the assessment of network organization personalized measures and the variability elucidation in clinical outcomes [26]. The neural network hypothesis requires further research to determine their structural and functional organization in DRE, as well as changes during the course of the disease and against the background of treatment (medication and surgery).

It is necessary to emphasize the importance of acquired drug resistance mechanisms, in particular, epileptic seizures themselves can trigger the kindling mechanism. Kindling is a phenomenon when repeated subconvulsive stimulation of certain brain areas leads to progressive development of seizure activity [27]. Based on this and insufficient information on drug resistance from the point of view of cellular and molecular factors, a hypothesis of **the drug resistance intrinsic severity to AEDs** was formulated. According to it, drug resistance is an integral property of epilepsy associated with the severity of the disease [2, 28]. According to this hypothesis, drug resistance is the result of the impact of neurobiological factors that determine a particular level of disease severity as a whole, that is, the phenotypic variability of this form of epilepsy [8, 29]. Therefore, drug resistance in this situation may be a consequence of the factors underlying epilepsy and its severe course.

In addition, there are undoubtedly other mechanisms of drug resistance that need to be identified and studied in detail.

RISK FACTORS FOR THE DEVELOPMENT OF PHARMACORESISTIENCE IN PATIENTS WITH EPILEPSY

Identification of patients with DRE and their timely referral for specialized treatment is often delayed. Such patients are more susceptible to high risk of morbidity and mortality. Identifying risk factors for DRE and changing the approach to treating a specific patient allows avoiding the use of ineffective AEDs, side effects from drug therapy, and worsening of the disease.

In clinical practice, some therapeutic errors are made that result in failure to control seizures or even worsening of the disease. Therefore, with a high probability, these errors incorrectly indicate the presence of DRE in patients. Such errors most often include incorrect assessment of the type of seizures; the presence in the patient of a condition that imitates epilepsy (psychogenic non-epileptic seizures, fainting, transient ischemic attacks, metabolic disorders, various motor disorders, especially of the extrapyramidal system, sleep disorders) and/or their combination with epilepsy [5].

In the treatment of patients with epilepsy, as in the treatment of other diseases, iatrogenic or nosogenic factors may occur that reduce or neutralize the effect of the therapy. Iatrogenic factors are associated with medical activity (inadequate dose and/or incorrectly selected drug (or drugs), irregular treatment of the patient, drug withdrawal for diagnostic purposes, etc.). Nosogenic factors are associated with the patient's behavior [30]. These may include the patient's failure to comply with the rules for taking the medication (frequency, dosage) or stopping taking it, etc.

There are factors that provoke attacks and, accordingly, increase the risk of developing DRE. In this regard, the patient should be informed about the adverse effects on health of stressful situations, sleep deprivation, alcohol, hyperthermia, etc. Attacks can be provoked by surgical interventions, metabolic and hormonal disorders, menstruation, pregnancy, childbirth, etc. [31]. Such provoking factors can be determined during the initial collection of the patient's anamnesis.

According to foreign researchers, risk factors for the DRE development may include early onset of the disease and its long course, frequent seizures (especially focal type). The anamnesis of these patients often includes indications of febrile seizures, possibly

epileptic status. It is also necessary to take into account the polymorphism of epileptic seizures, neurological deficit or mental retardation at the time of diagnosis. With regard to drug resistance, the lack of response to the first AED (if chosen correctly), abnormal electroencephalogram (EEG) and the presence of interictal epileptiform activity should be alarming [1, 32, 33].

Genetic factors probably play an important role in the development of many epileptic conditions, from classical idiopathic (genetic) generalized epilepsies to epileptic encephalopathies, focal epilepsy, and DRE. In recent years, numerous studies have shown that genetic variability is associated with drug resistance in epilepsy, including genes for voltage-gated sodium and potassium channels, as well as genes for the metabolism of endogenous and xenobiotic substances [34].

Epilepsy is a common manifestation of brain tumors. The type of brain tumor and its location will certainly be determining factors in the risk of developing epilepsy. Brain tumors that are most at risk of seizures are slow-growing primary tumors (low-grade gliomas), tumors with hemorrhage, and multiple metastases. Seizures, which are symptoms of a brain tumor, are difficult to treat. According to S. Dupont (2008), tumor evolution, modifications of tumor and peritumor tissue, and related treatments are usually associated with drug resistance when prescribing AEDs [35].

Cerebral cortex malformations (CCM) are considered to be one of the significant causes of epilepsy and developmental disorders in children. CCM are macroscopic or microscopic abnormalities of the cerebral cortex that occur as a result of disruption of the cortical plate formation stages. In most cases, they are genetically determined (there are abnormalities in genes that participate in neuronal proliferation, migration, and cortex stratification during embryogenesis) [36]. CCM may be caused by intrauterine factors associated with infection, hypoxia, and intoxication [5].

A number of diseases (tuberous sclerosis, focal cortical dysplasia, hemimegalencephaly, lissencephaly, subcortical laminar heterotopia, etc.) are accompanied by the DRE development [5, 37].

As practice shows, in temporal lobe epilepsy, the most frequent histopathological finding during surgical interventions, in cases of drug resistance, is mesial temporal sclerosis with the death of neurons in the hippocampus and adjacent structures. Similar changes are often found in the amygdala, entorhinal cortex, temporopolar areas of the cortex and temporal lobe. In patients with DRE requiring surgical intervention, the most frequent histological diagnosis in adults is hippocampal sclerosis [38]. It has been revealed that cortical dysplasia, atypical febrile seizures, brain tumors, traumatic brain injury, cerebral malformations have a fairly high risk of damage to the hippocampal region [39]. All these factors lead to a decrease in the number of neurons and hyperexcitability of unaffected nervous tissue.

PSYCHOEMOTIONAL STATUS IN PATIENTS WITH PHARMACORESISTANT EPILEPSY

The underlying cause of cognitive, emotional and behavioral disorders that commonly occur in patients with DRE is seizure activity [40].

Among the comorbid disorders accompanying epilepsy, depression occupies a special place, accounting for 4–12 % during remission and 20–55 % or more in DRE. Depression most often develops in patients with structural focal epilepsy with frequent (more than once a month) seizures and taking 2–3 AEDs [41, 42].

Considering the interhemispheric functional brain asymmetry, one can assume the importance of focus lateralization in the development of depressive disorders. In the studies conducted to study the dependence of the risk of developing depression on the epileptogenic focus lateralization, ambiguous results were obtained. However, most scientists believe that depression is more typical for patients with left-hemispheric focal epilepsy [43]. This can be explained by the fact that patients with a left-sided focus are more critical of their condition, and patients with right-sided hemispheric damage are characterized by understatement or denial of the negative aspects of their behavior. A. Grabowska-Grzyb et al. (2006) found that depression was observed in 49.5 % of 203 patients with DRE. It was also shown that depression and epilepsy can be caused by the same reasons [44].

A two-way relationship between epilepsy and depression, as well as epilepsy and suicidality, has been confirmed [45]. Depression may act as an independent risk factor for the development of the first unprovoked epileptic seizure [46]. It should be noted that depressive disorders in epilepsy have their own characteristics, differing from depression in other neurological diseases and from primary depression [47]. Depressive disorders are usually classified by the temporal relationship with epileptic seizures as: 1) preictal depression; 2) ictal depression; 3) postictal depression. Preictal depression occurs several hours, less often – days, before the onset of the seizure and is characterized by dysphoria, irritability and anxiety. Ictal depression is usually observed against the background of simple focal seizures. They are short-lived, stereotypical, associated with the emergence of guilt, anhedonia and suicidal thoughts. Postictal depression lasts for several hours or days after the attack and is characterized by increased sensitivity to frustrating factors, anhedonia, feelings of helplessness, guilt, irritability, and a sense of failure. Crying attacks, sometimes suicidal thoughts and suicidal tendencies are possible. These patients may have a medical history of major depression or bipolar disorder [47].

Anxiety disorders in patients with epilepsy are detected in 10–25 % of cases, according to other authors – in 50 % or more [48, 49].

There may be cause-and-effect and temporal relationships between clinical manifestations of anxiety disorder and epileptic seizures. Thus, anxiety and the emergence

of fears may precede the onset of a seizure, and these symptoms are often part of the structure of simple focal (most often temporal) and complex focal seizures with automatisms [50].

Behavioral disorders in patients with epilepsy are also more common than in the general population. In DRE, they can be diagnosed as independent disorders or associated with affective disorders (recurrent and bipolar depressions, dysthymia), such as hyperkinetic disorder [50].

Cause-and-effect relationships have not been sufficiently studied, since it can be difficult to obtain objective information about the presence and typology of behavioral disorders throughout the life of a patient with epilepsy.

Thus, the study of psychoemotional status disorders in DRE remains a pressing issue in modern neurology and psychiatry. The occurrence of psychoemotional disorders is an integral part of the DRE course and is reflected in the general condition of the patient and his QoL at different stages of the disease.

Cognitive impairments are quite common in patients with epilepsy, among which memory and attention impairments and bradyphrenia in the interictal period predominate. Organic damage to brain structures, neuronal dysfunction, interictal epileptic activity, repeated seizures, and the use of certain AEDs play an important role in the pathogenesis of cognitive impairments [51].

Seizures cause progressive cellular and metabolic changes that correlate with hippocampal neuronal loss, neoneurogenesis, and synaptic reorganization, as well as increased susceptibility to induced and spontaneous seizures. Behavioral and cognitive impairments occur and worsen with the cumulative number of seizures [52]. Memory problems are more pronounced in focal epilepsies than in generalized forms of epilepsy, with short-term memory being particularly affected. Memory impairments correlate with the long-term course of uncontrolled epilepsy [53].

OUTCOMES OF SURGICAL TREATMENT OF PHARMACORESISTANT EPILEPSY

The priority method of DRE treating is surgical treatment. Currently, data on the long-term results of surgical treatment of patients with DRE after various types of surgical interventions are accumulating [54]. However, there is no single point of view on the effectiveness of different treatment methods, risk factors for favorable and unfavorable outcomes of surgical treatment. Surgical treatment of epilepsy is usually performed on young people who need an assessment of the risks and long-term results of the operation. Prognostic factors for a good outcome of epilepsy surgical treatment include the presence of structural changes in the brain according to neuroimaging data (mesial temporal sclerosis, space-occupying process), the absence of focal cortical dysplasia and other cortical congenital malformations. In addition, it is necessary to consider the "consistency" of the results of neuroimaging and electroencephalographic monitoring, and there should be a sufficient volume of epileptogenic focus surgical resection [12, 13, 55].

The traditional surgical approach is considered to be anterior temporal lobectomy. Anterior temporal lobectomy with amygdalohippocamectomy (AGHP) includes resection of the medial complex, which consists of the amygdala, hippocampus, and parahippocampal gyrus. Additionally, the neocortex, which is not involved in the pathological process, is resected. Many studies have shown that AGHP is superior to long-term drug therapy in terms of seizure control in patients with DRE [56, 57]. Other surgical techniques are also currently used. Such techniques include stereotactic radiosurgery, MRI-guided laser interstitial thermal therapy (MgLiTT), and stereoelectroencephalography-guided radiofrequency thermocoagulation (SEEG-guided RFTC) [58]. In some cases, DRE surgical treatment is impossible. Limitations are associated with the presence of multiple epileptogenic foci, the impossibility of localizing the foci, or the location of the pathological substrate that is dangerous for any surgical intervention (proximity to functionally significant zones). For such patients, neurostimulation techniques are used, including vagus nerve stimulation, deep brain stimulation, and responsive neurostimularion [58, 59].

The central epileptogenic role of mesial temporal structures in temporal lobe epilepsy has been demonstrated in animal models of temporal lobe epilepsy as well as in structural cerebral pathology under the control of electrophysiological and neuroimaging studies. Thus, more targeted mesial temporal resections that spare the temporal neocortex, selective amygdalohippocampectomy (SAH), have been considered as a possible means of providing equivalent seizure control with fewer neuropsychological consequences [60, 61].

According to a large study (prospective and retrospective), including 745 and 766 people, respectively, who underwent SAH and AGHP, the proportion of the IA outcome according to J. Engel in the overall group was 68 %. For SAH this figure was 66 %, for AGHP – 71 %. A meta-analysis demonstrated a statistically significant decrease in the chances of being seizure-free in patients who underwent SAH compared to patients who underwent AGHP [62]. According to another study, seizure control in SAH was achieved in 78.2 %, and in 85.7 % of cases with AGHP [63]. Another study indicates good results of seizure control and IA outcome according to J. Engel in patients with DRE: 72 % with AGHP, 71 % with SAH [64]. Another meta-analysis found no statistically significant differences in outcomes between AGHP and SAH [65].

Our study showed that patients who underwent AGHP had a favorable outcome in terms of seizure control. The outcomes of surgical treatment were studied in 31 patients 6 months after surgery, in 21 patients – 1 year after surgery, and in 2 patients – 2 years after surgery. The proportion of patients with significant improvement (I and II

outcome classes according to J. Engel) was 87.1 %, 76.2 %, and 50 %, respectively, during the observation period [66]. As a rule, after surgery, patients remain on a reduced dose of AED, which reduces the expected effect of the surgery and requires further monitoring of the patient's somatic and mental functions with correction or discontinuation of pharmacotherapy [59].

In the middle of the last century, the effectiveness of epilepsy surgical treatment was assessed mainly by such indicators as complete or partial remission, reduction in seizure frequency, and the degree of changes in instrumental research methods. In recent years, the results of epilepsy surgical treatment have been assessed not only by reducing the frequency and/or cessation of epileptic seizures, but also by improving the quality of life, neuropsychological status, and cognitive sphere of operated patients.

Neuropsychological assessment of the functions of specific brain regions subject to resection and the patient's mental reserve capabilities allows predicting postoperative cognitive impairment. Successful surgery can halt the decline in mental abilities due to resistant epilepsy and reverse this negative trend by "releasing" functions that were secondarily affected before surgery [67]. However, surgery carries a risk of additional impairments that, together with comorbid disorders, can accelerate the decline in cognitive functions, especially in old age. From a neuropsychological point of view, early detection of drug resistance is of great importance, along with early and complete seizure control with maximum preservation of functional tissues during surgical treatment [67]. Many studies demonstrate the superiority of SAH in preserving neurocognitive functions [68, 69].

The study by W. Chengxiong et al. (2018) reported equal results in J. Engel's outcomes for SAH and AGHP, but worse results in neurocognitive impairment were observed with AGHP [70]. A large study by H. Clusmann reported better results after SAH in terms of attention, verbal memory, and overall neuropsychological performance [71]. U. Gleissner et al. (2002) reported the first results after 3 months and then after a year in 140 patients who underwent SAH. They noted that a more selective procedure may have important cognitive consequences. After 3 months, almost half of the patients with left-sided SAH had significant verbal memory loss; functional impairment was less pronounced in right-sided surgeries. Of 115 individuals who were followed for one year, no significant recovery in verbal memory was observed compared to an earlier time period [72].

The problem of QoL of patients with DRE is associated not only with the clinical manifestations of the disease, but also with the need for constant medication, with a personal reaction to it, with difficulties in integrating into society and their stigmatization. In the medical literature, there are more than 80 questionnaires for assessing QoL in epilepsy. At the same time, many of them assess the impact of epilepsy in general or its individual symptoms on the patient's life. An example of the most common special questionnaire for patients with epilepsy is the Quality Of Life In Epilepsy Patients (QOLIE) questionnaire, presented in different length versions for adults (QOLIE-89, QOLIE-31 and QOLIE-10) [73–75].

According to the results of a systematic review by A. Saadi et al. (2016), including data from more than 7,000 patients with epilepsy, the average QoL score on the QOLIE-31 questionnaire was 59.8 points with a maximum score of 100 points. Moreover, in high-income countries, this indicator was significantly higher [75]. Other studies using various QoL questionnaires in patients with epilepsy have shown a positive effect of surgical treatment of epilepsy on this indicator [76, 77]. According to the study by V. Ives-Deliperi, J.T. Butler (2017), there is also a significant improvement in QoL scores on the QOLIE-31 scale in patients with DRE after surgical treatment at 6 and 12 months compared to patients on drug therapy [77].

CONCLUSIONS

Understanding the mechanisms underlying resistance to AEDs may help develop more effective therapeutic options for patients with DRE. Development of a P-glycoprotein inhibitor is an important goal in the pharmacotherapy of resistant epilepsies. Identification of genes that influence the risk of developing DRE is of great importance for both research and clinical purposes. The discovery of new genes and their effects may expand our knowledge of the processes underlying susceptibility to DRE, potentially leading to the discovery of new treatments.

Identification of patients with DRE and timely provision of specialized care to them is often delayed. These patients are more susceptible to a high risk of comorbid diseases and mortality. However, identifying risk factors for DRE and changing the approach to treating a specific patient allows avoiding the use of ineffective AEDs and their side effects, worsening the course of the disease, etc.

Surgical treatment of this category of patients shows good results, however, the volume and methods of surgical intervention, the risks of adverse outcomes, the state of the psychoemotional and cognitive sphere, especially in the late postoperative period, remain insufficiently studied.

Evaluation of QoL in DRE is necessary, like other indicators, to determine the effectiveness of the treatment and rehabilitation of patients.

Conflicts of interest

No potential conflict of interest relevant to this article reported.

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OPHTHALMOLOGY

CHOOSING THE OPTIMAL METHOD FOR SURGICAL TREATMENT OF RHEGMATOGENOUS RETINAL DETACHMENT

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ABSTRACT

The problem of the structural and functional effectiveness of episcleral and endovitreal treatment methods of rhegmatogenous retinal detachment remains open to this day.
The aim of the study. *To assess the clinical effectiveness of surgical treatment of rhegmatogenous retinal detachment using episcleral and endovitreal methods.*
Material and methods. *An analysis of the electronic database and a detailed assessment of the treatment of 285 patients with rhegmatogenous retinal detachment for 2005–2022 were carried out. A comparative analysis was made in two groups: group 1 – patients after episcleral surgery (n = 155); group 2 – patients after endovitreal surgery (n = 130). The initial condition and the extent of surgery were comparable.*
Results. *From 2005 to 2009 in 65.9 % of cases, episcleral buckling predominated; from 2009 to 2021 – posterior closed vitrectomy (in 64.8–88.7 % of cases). The incidence of primary retinal reattachment was 74.2 % and 71.5 %. The number of relapses after vitreoretinal surgery slightly exceeded the values in the group 1 – 28.4 % versus 25.7 %, and in 20 % of cases the first relapse occurred before silicone aspiration as a result of subsilicone proliferation. The total number of surgical interventions per person, taking into account mandatory silicone aspiration, in the group 1 was 1.3, in the group 2 – 2.25 for the entire observation period. The visual acuity of patients in group 1 was 2 times higher than that of the comparison group – 0.21 ± 0.02 and 0.1 ± 0.03, respectively ($p < 0.05$).*
Conclusion. *Episcleral treatment methods of rhegmatogenous retinal detachment are characterized by better anatomical, reconstructive and functional effects with fewer re-operations.*

Key words: *rhegmatogenous retinal detachment, circular episcleral buckling, posterior closed vitrectomy, silicone oil tamponade*

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ВЫБОР ОПТИМАЛЬНОГО МЕТОДА ХИРУРГИЧЕСКОГО ЛЕЧЕНИЯ РЕГМАТОГЕННОЙ ОТСЛОЙКИ СЕТЧАТКИ

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РЕЗЮМЕ

Вопрос о структурной и функциональной эффективности эписклеральных и эндовитреальных методов лечения регматогенной отслойки сетчатки остаётся открытым до сих пор.

Цель исследования. *Оценить клиническую эффективность хирургического лечения регматогенной отслойки сетчатки с помощью эписклеральных и эндовитреальных методов.*

Материал и методы. *Проведены анализ электронной базы данных и детальная оценка лечения 285 пациентов с регматогенной отслойкой сетчатки за 2005–2022 гг. Сравнительный анализ проведён в двух группах: первая – после эписклеральной хирургии (n = 155); вторая – после эндовитреальных вмешательств (n = 130). Исходное состояние, объём хирургического вмешательства были сопоставимы.*

Результаты. *С 2005 по 2009 гг. в 65,9 % случаев преобладало эписклеральное пломбирование, с 2009 по 2021 г. – задняя закрытая витрэктомия (в 64,8–88,7 %). Частота первичного прилегания сетчатки составила 74,2 % и 71,5 %. Число рецидивов после витреоретинальной хирургии несколько превышало значения первой группы — 28,4 % против 25,7 %, и в 20 % случаев первый рецидив возник ещё до аспирации силикона в результате субсиликоновой пролиферации. Общее количество хирургических вмешательств на человека с учётом обязательной аспирации силикона в первой группе составило 1,3, во второй – 2,25 за весь период наблюдения. Острота зрения больных 1-й группы превышала значения группы сравнения в 2 раза – 0,21 ± 0,02 и 0,1 ± 0,03 соответственно (p < 0,05).*

Заключение. *Эписклеральные методики лечения регматогенной отслойки сетчатки характеризуются лучшим анатомо-реконструктивным и функциональным эффектом при меньшем количестве повторных операций.*

Ключевые слова: *регматогенная отслойка сетчатки, круговое эписклеральное пломбировние, задняя закрытая витрэктомия, тампонада силиконовым маслом*

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Surgical treatment of retinal detachment dates back to 1929, when J. Gonin first proposed blocking the retinal defect by draining the subretinal fluid and coagulating the sclera with a thermal cautery in the projection of the break [1, 2]. The method proposed by E. Custodis in 1953 involved local occlusion of the retinal break with a polyviol buckle and application of a series of coagulates to the depression ridge. The author assumed that after the retinal break was blocked, the pigment epithelium would adsorb the subretinal fluid and the retina would adhere. C. Schepens in 1953 and H. Arruga in 1958 increased the scleroretinal barrier to a circular buckle, on which extensive diathermocoagulation was performed [1, 3, 4].

The evolution of retinal detachment surgery is associated with the use of operations accompanied by tamponade of the gap by introducing air into the eye after diathermocoagulation and drainage of subretinal fluid. This method was proposed in 1938 by B. Rosengren. In 1972, R. Machemer developed a safe method for removing the vitreous body through small incisions in the pars plana of the ciliary body; this method was called "vitrectomy" and opened a new era in the treatment of diseases of the retina and vitreous body [5, 6].

Since then, both episcleral and endovitreal methods of treatment have been actively used in the surgical treatment of retinal detachment. The debate about their effectiveness continues to this day. If episcleral methods were used more often in the second half of the last century, then currently preference is given to endovitreal surgery [5]. The main reasons for the change in the ratio of these treatment methods, when the number of endovitreal interventions significantly exceeds the number of episcleral operations, are: technical complexity and conditional "traumatism" of episcleral surgery, difficulties in mastering and insufficient visualization of retinal changes using binocular ophthalmoscopy and the use of neutralizing lenses. In turn, the improvement of technology and the wide availability of microinvasive vitrectomy have led to a significant reduction in the time of this operation.

Despite the fact that many authors show a similar percentage of primary retinal reattachment using episcleral and endovitreal approaches, the question of functional effectiveness, duration of rehabilitation measures and economic costs in surgical treatment of rhegmatogenous retinal detachment using various methods remains open to this day [7–13]. These facts determined the relevance of this study.

THE AIM OF THE STUDY

To assess the clinical effectiveness of surgical treatment of rhegmatogenous retinal detachment using episcleral and endovitreal methods.

MATERIALS AND METHODS

The study included two stages. At the first stage, an analysis of the electronic database of the Irkutsk Branch of the S. Fyodorov Eye Microsurgery Federal State Institution was conducted in order to assess the number of episcleral and vitreoretinal interventions performed on patients with rhegmatogenous retinal detachment from 2005 to 2022. The search was performed using the following key words: "retinal detachment", "circular episcleral buckling", "vitrectomy", "silicone oil tamponade".

At the second stage, the treatment results of 285 patients were analyzed based on medical records. The main inclusion criteria were: the presence of rhegmatogenous retinal detachment involving the macular zone; proliferative vitreoretinopathy (PVR) at stages B to C1; the duration of retinal detachment from 2 weeks to 2 months. Axial length of the eye from 22 to 25 mm, extremely high and low anterior-posterior axis of the eye were exclusion criteria.

Depending on the method of surgical treatment, two groups were formed. The first group included 155 patients after episcleral surgery, and 130 of them underwent circular episcleral buckling using a silicone sponge, and in 25 cases, circular buckling was supplemented with a radial buckle. The second group consisted of 130 patients after endovitreal intervention. All patients in this group underwent standard three-port posterior closed vitrectomy (PCV) 25G with endolaser coagulation of the retinal break and silicone endotamponade with subsequent silicone aspiration for a period of 3 to 6 months. The study included patients aged 23 to 67 years; the proportion of women was 41.4 %, men – 58.6 %. The duration of observation was from 5 to 7 years. The clinical characteristics of the patients are presented in Table 1.

The criteria for assessing the effectiveness of treatment were: primary reattachment of the retina; the number and timing of relapses of retinal detachment; the dynamics of changes in visual acuity after surgical treatment.

RESULTS

It was found that in 2005, episcleral buckling was predominantly used, in 2008, the number of episcleral and endovitreal interventions was equal. Starting from 2009, the obtained trend indicated the predominant use of PCV with silicone oil tamponade of the vitreous cavity in the treatment of retinal detachment. In 2021, this type of surgery was already used in the overwhelming majority of cases – 88.7 %, episcleral buckling was performed only in 11.3 % of patients (fig. 1).

In our opinion, the data on the timing and frequency of relapses with different methods of treating retinal detachment are important. A detailed analysis of the treatment results of 285 patients based on medical records showed that primary retinal reattachment in patients of group 1 was achieved in 74.2 % of cases and was comparable with the number of successfully treated patients in group 2 – 71.5 %. Consequently, the first relapse of retinal detachment occurred in almost every fourth patient in both groups – 25.8 % and 28.5 % of cases, respectively. In most cases, relapses occurred in the first 6 months after surgery, and their number in both groups was

TABLE 1

CLINICAL CHARACTERISTICS OF PATIENTS AND TREATMENT METHODS

	Indicators	Group 1 (*n* = 155)	Group 2 (*n* = 130)
Episcleral method	Circular scleral buckling	130	–
	Circular scleral buckling with a radial buckle	25	–
PCV with endolaser coagulation	Oxane 1300 silicone oil tamponade	–	68
	Oxane 1300 + Densiron silicone oil tamponade	–	62
Age		45.3 ± 0.07	44.2 ± 0.07
Sex	Female	64	54
	Male	91	76
PVR	B	134	117
	C1	21	13
APA		23.7 ± 0.04	23.5 ± 0.04
Duration of existence of retinal detachment	2 weeks	67	65
	4 weeks	51	39
	6 weeks	26	18
	8 weeks	11	8

Note. APA – anterior-posterior axis of the eye.

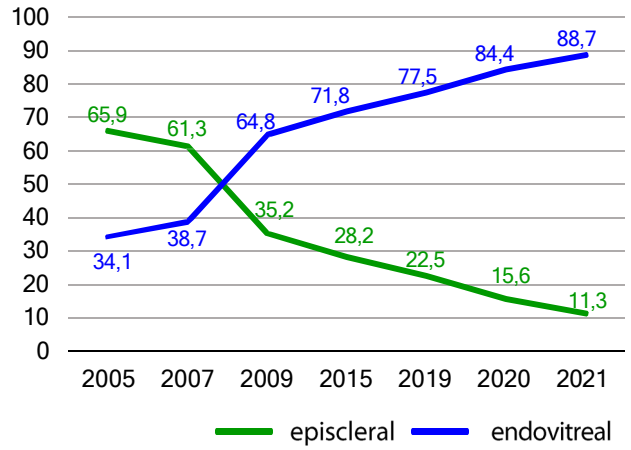

FIG. 1.
Dynamics of change in the number of episcleral and endovitreal interventions in retinal detachment surgery (%)

comparable – 17.4 % and 20 %, respectively. In the period from 6 to 12 months, the first relapse occurred in 2.5 % and 6.9 % of cases, in the late postoperative period of more than 12 months – in 5.8 % and 1.5 % of cases after episcleral and vitreoretinal surgery, respectively. It should be noted that in patients with endovitreal interventions, the first relapse in 20 % of cases occurred before silicone aspiration and was due to the development of subsilicone proliferation.

The frequency of the second and third relapses was calculated relative to the total number of operated patients in each group. Comparative analysis of repeated relapses of retinal detachment showed that after endovitreal interventions they occurred in 11.5 % of cases, and after episcleral surgery – only in 5.8 % of patients. Such distribution of the frequency of retinal detachment relapses can most likely be explained by the slower progression of proliferative vitreoretinopathy after episcleral interventions. The frequency of the third relapse of retinal detachment was almost the same in both groups and did not exceed 3 % of the total number of operated patients (Table 2).

In order to treat recurrent retinal detachment, radial buckles were additionally installed in the patients of the 1st group, and vitreous cavity revision with silicone oil retamponade was performed in the patients of the 2nd group. An important criterion for the effectiveness of surgical treatment of retinal detachment is not only the anatomical and reconstructive effect, but also the ability to improve the visual functions of patients. The best corrected visual acuity of patients in both groups before

TABLE 2

THE NUMBER AND TIMING OF RECURRENT RETINAL DETACHMENT IN PATIENTS OF TWO GROUPS

Indicators	Time of occurrence of recurrent retinal detachment	Group 1 (*n* = 155)		Group 2 (*n* = 130)	
% of primary retinal attachment		115 (74.2 %)		93 (71.5%)	
1st relapse	Up to 6 months	27 (17.4%)	25.8%	26 (20%)	28.5%
	6-12 months	4 (2.5%)		9 (6.9%)	
	Over 12 months	9 (5.8%)		2 (1.5%)	
2nd relapse	Up to 6 months	4 (2.6%)	5.8%	4 (3.1%)	11.5%
	6-12 months	3 (1.9%)		5 (3.8%)	
	Over 12 months	2 (1.3%)		6 (4.6%)	
3rd relapse	Up to 6 months	0 (0%)		0 (0%)	
	6-12 months	0 (0%)		0 (0%)	
	Over 12 months	4 (2.6%)		4 (3%)	

surgery varied from 0.01 to 0.09. After surgery, the visual acuity of patients in the 1st group exceeded the values of patients in the comparison group by 2 times, averaging 0.21 ± 0.02 compared to 0.1 ± 0.03 in the 2nd group ($p < 0.05$) (fig. 2).

Distribution of patients depending on the obtained best corrected visual acuity demonstrated that after episcleral surgery in 59.4 % of cases the visual acuity was from 0.05 to 0.3, in 16.1 % – 0.3 and higher. After endovitreal intervention, patients with the corresponding visual acuity were 46.2 % and 12.3 %, respectively (fig. 1).

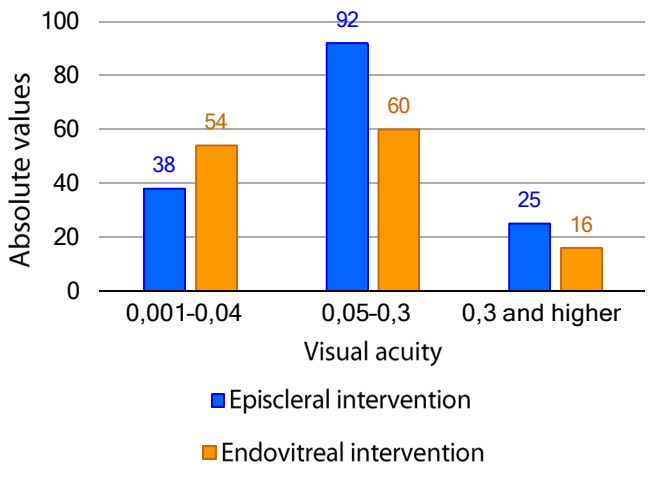

FIG. 2.
Distribution of patients depending on the maximum corrected visual acuity in the early postoperative period

DISCUSSION

Currently, various methods of treating retinal detachment are available. The choice of method is individual and should be made considering the patient's initial condition and the risk of recurrent detachment. Current data provide some information about the advantages and disadvantages of various methods.

W. Park et al. in 2023 published a work devoted to the development of criteria for predicting the recovery of visual acuity and the development of recurrent retinal detachment after removal of silicone oil. A retrospective analysis of the treatment of 1017 eyes of patients with retinal detachment who underwent vitrectomy with silicone endotamponade was conducted [14]. In this study, recurrent retinal detachment after silicone removal occurred in 8.2 % of patients. Several studies show that the cause of recurrence after silicone removal is proliferative vitreoretinopathy. G.W. Abrams et al. in their study reported that 19 % of patients experienced recurrent retinal detachment, 14 % of whom had PVR [15]. According to studies, most often (69 %) PVR occurs within the first 6 months after vitreectomy and silicone oil tamponade. A retrospective analysis showed that the risk of developing recurrent PVR is higher in patients with PVR stage C and above [15].

In 2021, M. Bunajem et al. compared the anatomical and functional results in patients after treatment of rhegmatogenous retinal detachment using two different methods. 68 eyes were examined after episcleral buckling and 64 eyes in the group after vitrectomy with silicone endotamponade. In eyes after vitrectomy,

the likelihood of developing recurrent retinal detachment requiring reoperation was higher than in patients after episcleral surgery. The functional results of the two groups were comparable [16].

In 2022, A.S. Dhoot et al. conducted a comprehensive meta-analysis of the treatment of 15,947 eyes with rhegmatogenous retinal detachment to compare the effectiveness of posterior closed vitrectomy with silicone oil tamponade with the episcleral method of treatment [5]. According to the study, the rates of primary and reattachment of the retina were similar. Primary reattachment with episcleral surgery was achieved in 86.5 % of cases, with vitrectomy – in 84.8 % (p = 0.13). Reattachment was achieved in 96.7 % and 97.7 % of cases, respectively (p = 0.12). However, the final best corrected visual acuity was statistically significantly better in patients after episcleral buckling (0.38 ± 0.53 vs. 0.33 ± 0.53 (20/48 vs. 20/43 on the Snellen chart). This is consistent with the data obtained in our study: when using episcleral treatment techniques, patients received a more stable anatomical result over many years of follow-up and visual acuity that was 2 times higher than in patients after endovitreal treatment of retinal detachment.

CONCLUSION

The vector of development of surgical interventions for rhegmatogenous retinal detachment is directed towards improving primary vitrectomy and substances used for tamponade of the vitreous cavity. However, episcleral techniques, despite their apparent invasiveness, are characterized by a better anatomical-reconstructive (percentage of primary adhesion) and functional effect (increased visual acuity) with a smaller number of repeated operations. Therefore, the choice of the optimal method of retinal detachment surgical treatment should not exclude episcleral approaches, which will minimize the number of postoperative relapses and achieve a good functional effect.

Conflicts of interest

No potential conflict of interest relevant to this article reported.

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METABOLIC CHANGES IN THE EYE LENS IN THE PROGRESSION OF CATARACT

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ABSTRACT

Background. *Cataract is one of the main causes of decreased visual acuity in the world, and therefore scientists are continuing researches on the mechanisms of development of this ophthalmic pathology.*
The aim. *To study metabolic changes in a cloudy lens using an experimental model.*
Materials and methods. *The study was carried out on adult male Wistar rats (n = 60), which were divided into control (n = 30) and experimental (n = 30) groups. Experimental cataract were simulated by daily ultraviolet irradiation (λ = 300–350 nm) during 6 months for 20 minutes. At the months 2, 4 and 6 of the study, we carried out a biomicroscopic examination of the anterior eye of animals using a slit lamp to monitor the development of cataract. Lenses were collected to determine the content of stearoyl-coenzyme-A desaturases and melatonin using enzyme immunoassay.*
Results. *At the stage of initial cataract, the content of the stearoyl-coenzyme A desaturase was statistically significantly lower than the control values by 38 %; at the stage of immature cataract – by 30 %; at the stage of mature cataract – by 15.4 %. It was revealed that at the month 6 of the study, the concentration of melatonin in lens homogenates was 17 % lower when compared with the control. A statistically significant correlation was established between stearoyl-coenzyme A desaturase and melatonin (r = 0.32).*
Conclusion. *Melatonin and stearoyl-coenzyme A desaturase play an important role in a number of biochemical processes that ensure the proper functioning of the visual analyzer. Changes in the concentration of these biological molecules can play a key role in the pathogenesis of cataract and a number of other ophthalmic diseases.*

Key words: *stearoyl-coenzyme A desaturase, melatonin, cataract, fatty acids, eye lens*

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МЕТАБОЛИЧЕСКИЕ ИЗМЕНЕНИЯ В ХРУСТАЛИКАХ ГЛАЗА ПРИ ПРОГРЕССИРОВАНИИ КАТАРАКТЫ

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РЕЗЮМЕ

Обоснование. *Катаракта является одной из основных причин снижения зрения в мире, в связи с чем, учёными активно продолжаются исследования по изучению механизмов развития данной офтальмопатологии.*
Цель исследования. *Изучить метаболические изменения в мутнеющем хрусталике на экспериментальной модели.*
Материалы и методы. *Работа была проведена на взрослых крысах-самцах линии Wistar (n = 60), которые были разделены на контрольную (n = 30) и опытную (n = 30) группы. Экспериментальная катаракта моделировалась путём ежедневного ультрафиолетового облучения (λ = 300–350 нм) в течение 6 месяцев по 20 минут. На 2-й, 4-й и 6-й месяцы исследования проводилось биомикроскопическое обследование переднего отдела глаза животных с помощью щелевой лампы для наблюдения за развитием катаракты; осуществлялся забор хрусталиков для определения содержания стеарил-коэнзим-А-десатураз и мелатонина методом иммуноферментного анализа.*
Результаты. *На стадии начальной катаракты содержание фермента стеарил-коэнзим-А-десатуразы было статистически значимо ниже контрольных значений на 38 %; на стадии незрелой катаракты – на 30 %; на стадии зрелой катаракты – на 15,4 %. Выявлено, что на 6-й месяц исследования концентрация мелатонина в гомогенатах хрусталиков была ниже на 17 % при сравнении с контролем. Установлено наличие статистически значимой корреляционной зависимости между стеарил-коэнзим-А-десатуразой и мелатонином (r = 0,32).*
Заключение. *Мелатонин и стеарил-коэнзим-А-десатуразы играют важную роль в ряде биохимических процессов, обеспечивающих правильное функционирование зрительного анализатора. Изменение концентрации данных биологических молекул может играть ключевую роль в патогенезе катаракты и ряда других офтальмологических заболеваний.*

Ключевые слова: *стеарил-коэнзим-А-десатуразы, мелатонин, катаракта, жирные кислоты, хрусталик глаза*

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INTRODUCTION

Cataract is one of the most common ophthalmological diseases and one of the leading causes of blindness along with glaucoma and age-related macular degeneration [1]. According to the World Health Organization, cataract accounts for 47 % of all eye diseases [2]. That is why understanding the underlying mechanisms of age-related changes in the structure and function of the ocular apparatus has become critically important for identifying new therapeutic targets and developing multimodal health strategies that meet the needs of the population.

A number of numerous studies devoted to the causes of cataracts have shown that cataracts are a multifactorial disease [3]. The main causes of this pathology include exposure to ultraviolet (UV) radiation. This is evidenced by epidemiological studies that have established a relationship between the medium-wave range of UV radiation (UVB, wavelength 290–315 nm), present in sunlight, and the formation of lens opacities [4]. There is an assumption that lens lipids of the eye are involved in the development of the disease, despite their small content (1 %). In particular, it is known that prolonged exposure to sunlight increases the risk of cataracts due to peroxidation of fatty acids that are part of the cell membrane of lens cells [5].

One of the actively studied enzymes involved in the regulation of fatty acid metabolism is membrane-bound stearyl-coenzyme A desaturase-1 (SCD1; EC 1.14.19.1). This enzyme catalyzes the reaction during which saturated fatty acids are converted into monounsaturated ones. As a rule, stearic and palmitic acids undergo desaturation reactions with the formation of oleic and palmitoleic acids, respectively [6]. It is known that the ratio of fatty acids, in particular stearic acid, to oleic acid affects the viscosity of the lipid bilayer of cell membranes. For this reason, SCD1 is vital for maintaining the structural integrity and fluidity of membranes. Foreign scientists have found that changes in the level of desaturases in humans and animals are associated with the development of ophthalmological pathologies [7, 8].

In addition, according to modern literature, the role of melatonin in the normal and pathological functioning of the visual system is actively discussed in ophthalmology [9, 10]. Melatonin is a hormone secreted by the pineal gland in accordance with the circadian rhythm and regulates chronobiological processes in the body, including endocrine and non-endocrine functions [11]. Other functions of melatonin are associated with the oxidation-reduction status of cells and tissues and include its antioxidant and anti-inflammatory properties [12]. It should be noted that melatonin is produced not only in the pineal gland – its synthesis has been found in many organs and tissues. Using specific antibodies to melatonin, its presence was found in almost all biological fluids, including cerebrospinal fluid, saliva, bile, amniotic fluid, breast milk and tear fluid [11]. Melatonin is also found in the structures of the eyeball – the ciliary body, lens and retina [13].

The working hypothesis of the study is based on the assumption that desaturases and melatonin participate in cataractogenesis. In this regard, the assessment of the levels of these enzymes in lens homogenates is a necessary step in the study of the visual analyzer functional state.

THE AIM OF THE STUDY

To study metabolic changes in a cloudy lens using an experimental model.

MATERIALS AND METHODS

The studies were conducted in the experimental biological clinic (vivarium) of the Federal Research Centre of Biological Systems and Agrotechnologies of the Russian Academy of Sciences and S. Fyodorov Eye Microsurgery Federal State Institution of the Ministry of Health of the Russian Federation. The experiment was performed on the Wistar rat model in accordance with the protocols of the Geneva Convention and the principles of good laboratory practice (National Standard of the Russian Federation GOST R 53434-2009), as well as according to the recommendations of The Guide for the Care and Use of Laboratory Animals (National Academy Press Washington, D.C. 1996). The design of the experiment was approved by the local Ethics Committee of the Federal Research Centre of Biological Systems and Agrotechnologies of the Russian Academy of Sciences (protocol No. 4 dated February 05, 2019).

For the experiment, 60 male rats weighing 180–200 g and aged 10 months were selected. Two groups were formed from the selected animals using the pair-analogue method: control ($n = 30$) and experimental ($n = 30$). Age-related cataracts were modeled in the animals of the experimental group by daily ultraviolet irradiation using a mercury gas discharge lamp DRL ($\lambda = 300–350$ nm). The exposure time was 20 minutes for 6 months.

The animals were kept on a standard diet (according to GOST R 50258-92), with free access to water and food, at a temperature of 22 ± 1 °C and 12-hour lighting.

At the 2nd, 4th and 6th months of the study, a biomicroscopic examination of the anterior segment of the animal's eye was performed using a BQ 900 slit lamp (Haag-Streit, Switzerland) to monitor the development of cataracts; the lenses were isolated and then homogenized on ice in sterile saline (in a ratio of 1:20 – 20 mg of tissue sample was added to 1 ml of the solution) to determine the content of stearyl-coenzyme-A desaturase and melatonin using an enzyme immunoassay and the Rat SCD (Stearoyl Coenzyme A Desaturase) Elisa Kit and Rat MT (Melatonin) Elisa Kit according to the instructions.

The obtained data were processed using the methods of variation statistics using the statistical package Statistica 10 (StatSoft Inc., USA). The compliance of the obtained data with the normal distribution law was checked using the Kolmogorov goodness-of-fit test. The hypothesis of the data belonging to the normal distribution was

rejected in all cases with a probability of 95%, which justified the use of nonparametric procedures for processing statistical populations (Mann – Whitney U-test). The obtained data are presented as the median (Me) and the 25th and 75th centiles (Q25–Q75). The relationships between the parameters were assessed using the Spearman's rank correlation method.

RESULTS

According to biomicroscopic examination of the eyeball, no clinical signs of cataract development were detected in laboratory animals during the first month of ultraviolet irradiation. Only by the end of the 2nd month of ultraviolet exposure did the rats develop initial subcapsular cataract, characterized by heterogeneity of the lens fibers. In the 4th month of exposure, according to slit lamp analysis of the eye, signs of subcapsular cataract became more pronounced, and by the 6th month of irradiation, signs of mature cataract were observed in the animals – lens clouding was noted at the equator and in the center in all layers (fig. 1).

Analysis of the enzyme immunoassay results showed that in animals of the experimental group, the content of SCD1 in lens homogenates throughout the experiment was statistically significantly lower than in the control (fig. 2).

When comparing centile values, it was found that Q75 of the experimental group was lower than Q25 of the control group by 38 % ($p \leq 0.05$) at the initial cataract stage (2nd month of observation), by 30 % ($p \leq 0.001$) at the immature cataract stage (4th month), and by 15.4 % ($p \leq 0.05$) at the mature cataract stage (6th month). It should be noted that in the 4th month of the experiment, the range of SCD1 enzyme concentrations was the widest, while the 25th centile value was more than 2 times lower than the same indicator in the 2nd and 6th months of observation.

In the experimental group animals, the melatonin content in lens homogenates was stable throughout the experiment, but somewhat lower than in the control. However, against the background of the development of mature cataract (6th month), the melatonin concentration in the experimental group statistically significantly decreased relative to the control values: the median value was 17 % lower; Q75 of the experimental group was 5 % lower than Q25 of the control group ($p \leq 0.05$) (fig. 3).

The conducted correlation analysis allowed us to establish the presence of a positive moderate association between melatonin and SCD1 ($r = 0.32$; $p \leq 0.05$) in lens

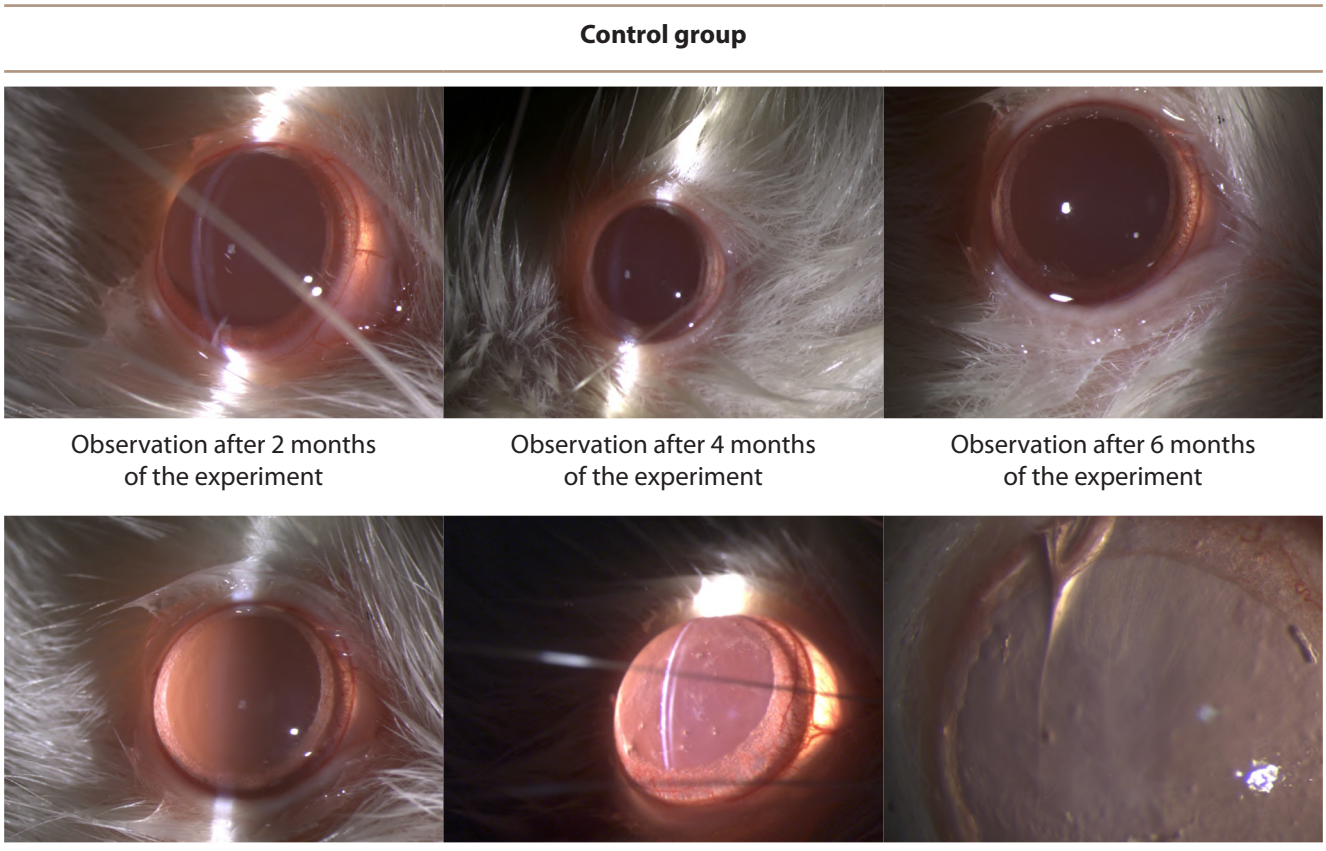

FIG. 1.
Biomicroscopy of the lens at the different stages of the experiment

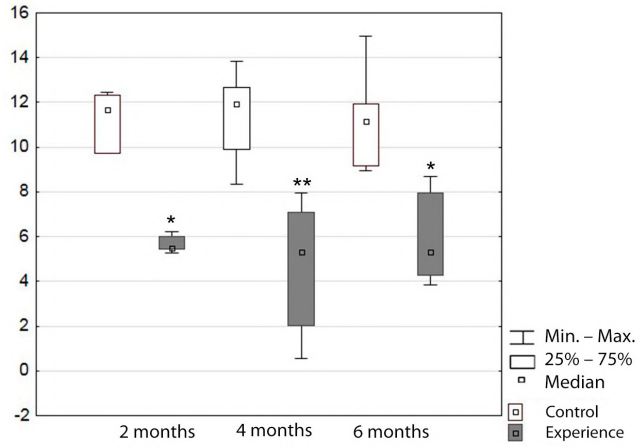

FIG. 2.
*Content of stearoyl-coenzyme A desaturase in eye lens homogenates: statistically significant difference between the experimental group and the control * – at p ≤ 0.05, ** – at p ≤ 0.01; data are presented as Me (Q25–Q75)*

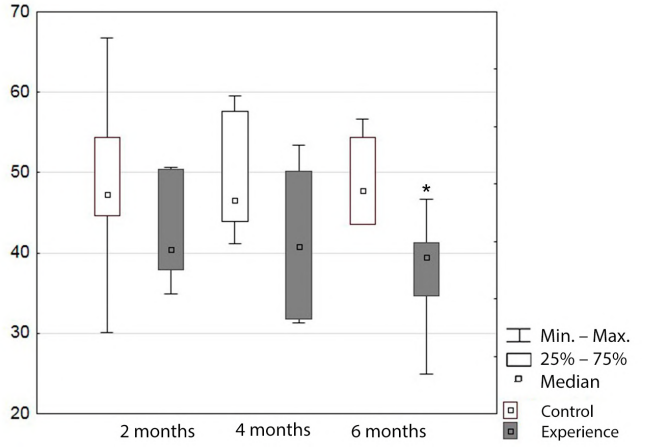

FIG. 3.
*Melatonin content in eye lens homogenates: * – statistically significant difference between the experimental group and the control at p ≤ 0.05; data are presented as Me (Q25–Q75)*

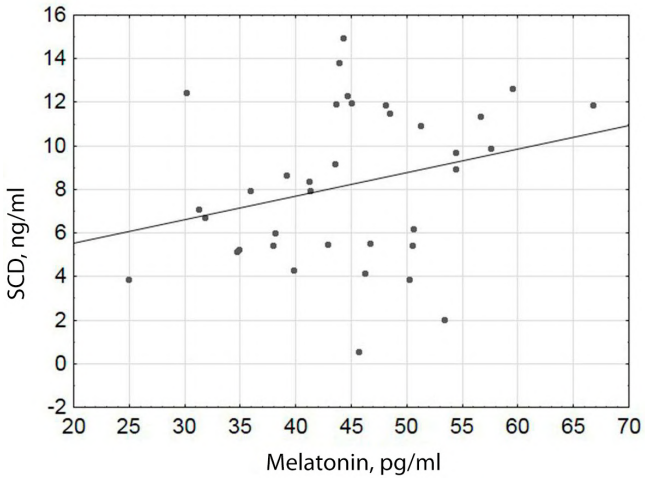

FIG. 4.
Correlation between melatonin and stearyl-coenzyme A desaturase in eye lens homogenates

homogenates (fig. 4). As can be seen from the presented graph, the higher the melatonin level, the higher the desaturase level.

DISCUSSION

The study found that after 2 months of ultraviolet irradiation, there was a statistically significant decrease in the SCD1 level in animal lens homogenates by more than 30 %. This fact may be due to the protective and compensatory body reaction aimed at ensuring the correct functioning of the system elements under conditions exceeding the adaptation norm, due to the reaction of the structures of the system itself [14]. As is known, the body maintains physiologically favorable membrane fluidity by changing the ratio of saturated and unsaturated fatty acids. Ultraviolet radiation promotes the activation of oxidation processes and the accumulation of photolysis products. Lipids containing unsaturated fatty acids are primarily subject to peroxidation. This is due to the fact that they contain a π-bond, which is less strong than a σ-bond and is more easily broken during chemical reactions [15]. As a result, there is a loss of phospholipids (phosphatidylcholines and phosphatidylethanolamines), which mainly include unsaturated fatty acids. In order to provide resistance to oxidation and maintain the transparency of the lens membranes, the acyl chains of phospholipids are saturated, and the content of lipids with residues of saturated fatty acids increases [16, 17]. Such processes provide relative physical and chemical stability of membranes, rigidity and resistance to peroxidation. This is the basis for the physiological compensatory decrease in the concentration of the SCD1 enzyme, which catalyzes the biosynthesis of unsaturated fatty acids. It should be noted that the established increase in the SCD1 content in the 6th month of the experiment compared to the 2nd and 4th months may indicate adaptation processes in response to the stress factor, as well as the high metabolic flexibility of this enzyme. This is probably due to the fact that in the process of peroxidation, the fluidity of cell membranes decreases, but remains within a certain range due to the action of desaturases. On the other hand, it can be assumed that the activity of desaturase in lens cells is subject to cyclic changes, allowing to maintain a constant ionic composition in the cells and membrane potential, thereby preventing the development of cataracts [18, 19]. However, the results of studies by Chinese scientists show that SCD1 inhibition may be involved in cataractogenesis by changing the lipid composition of the lens [20]. In our previous studies, it was shown that against the background of cataract development, the content

of saturated palmitic acid increases and the level of unsaturated linoleic acid decreases [21]. Such changes provide an increase in the "rigidity" of the membranes, which helps protect cells from oxidative stress. However, along with this, the ratio of saturated and unsaturated fatty acids changes, which leads to a violation of the fluidity and structural integrity of the lens cell membranes and, as a consequence, to the development of cataracts. Thus, chronic exposure to unfavorable environmental factors on the body, as a rule, causes stress in the regulatory mechanisms, which may result in a breakdown of compensatory reactions, leading to the so-called state of distress, against the background of which there is an imbalance in the regulatory systems of the body and the development of the disease. It is assumed that the violation of lipid metabolism and damage to cell membranes is the leading link in the process of changing the optical and physical properties of the lens.

The decrease in the melatonin level in the studied biosamples revealed during the experiment is entirely justified. As is known, in physiological concentrations, this hormone protects cells from oxidative stress by binding hydroxyl radicals that are formed during lipid peroxidation, as well as stimulating the expression of antioxidant genes and inhibiting the genes of prooxidant enzymes [22]. A number of experimental studies have shown that melatonin is able to suppress the development of cataracts [23–25]. For example, a study by Turkish scientists found that intraperitoneal administration of melatonin reduced the manifestations of oxidative stress and contributed to the restoration of the lens optical properties after UV irradiation [26]. Thus, a decrease in the concentration of this hormone in lens homogenates is due to the high production of active oxygen forms. The results of the study also indicate that the antioxidant system cannot cope with the progression of cataracts.

Of great interest is the fact that there is a direct correlation between the content of melatonin and SCD1 in lens cells. Perhaps this association is due to the fact that melatonin in this case acts, as described earlier, as a strong antioxidant with direct and indirect antioxidant properties [27]. Due to these properties of melatonin, the level of active oxygen species in lens cells decreases, due to which the SCD1 enzyme concentration increases and the ratio of saturated and unsaturated fatty acids is normalized, thereby restoring the functional activity of membranes. Thus, we assume that the higher the level of melatonin, the higher the degree of cell protection from oxidative stress, and, consequently, the higher the level of unsaturated fatty acids and desaturases. It is interesting to note that in 1981, D.F. Horrobin in the journal "Medical Hypotheses" identified melatonin as one of the factors regulating desaturases [28]. Thus, in the 2000s. Japanese scientists have shown that the introduction of melatonin to rats with type 2 diabetes mellitus contributed to the activity restoration of one of the enzyme isoforms – liver Δ-5-desaturase, which in turn led to the normalization of the ratio of fatty acids in the blood plasma and liver [29]. In addition, there is evidence that the activity of the enzymes of the isoenzymes Δ-5- and Δ-6-desaturases plays an important role in the expression and regulation of melatonin itself [30].

CONCLUSION

To summarize the study, it should be concluded that the enzyme stearyl-coenzyme-A desaturase and the hormone melatonin play an important role in a number of biochemical processes that ensure the proper functioning of the visual analyzer. The study noted that at the stage of initial cataract, the content of stearyl-coenzyme-A desaturase is statistically significantly lower than the control values by 38 %, at the stage of immature cataract – by 30 %, at the stage of mature cataract – by 15.4 %. It was found that during the mature cataract formation, the melatonin concentration in lens homogenates statistically decreases by 17 % when compared with the control. The presence of a statistically significant correlation between stearyl-coenzyme-A desaturase and melatonin (r = 0.32) was established. Changes in the concentration of these biological molecules may play a key role in the pathogenesis of cataracts and a number of other ophthalmological diseases.

Conflicts of interest

No potential conflict of interest relevant to this article reported.

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PEDIATRICS

CAUSES OF INTRAVENTRICULAR HEMORRHAGES IN EXTREMELY PREMATURE NEWBORNS AND FEATURES OF THEIR EARLY OUTCOMES

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ABSTRACT

The aim. *To study the predisposing factors for the development and timing of development of intraventricular hemorrhage (IVH) in extremely premature newborns.*
Materials and methods. *We carried out retrospective analysis of 32 case histories of children born at a gestational age of less than 32 weeks. The children were divided into three groups: group 1 (n = 13) – children death was caused by non-traumatic IVH; group 2 (n = 12) – surviving infants with IVH; group 3 (comparison group; n = 7) – premature infants without IVH. We assessed risk factors for the development of IVH, their severity, and main indicators predisposing to death in newborns of these groups.*
Results. *Children of the group 1 had statistically significantly low values of body weight – 670 [640–860] g (p_{1-2} = 0.007; p_{1-3} = 0.012), head circumference – 23 [22–24] cm (p_{1-2} = 0.008; p_{1-3} = 0.049), gestational age – 24.5 [23.5–25.5] weeks (p_{1-2} = 0.002; p_{1-3} = 0.007). Gender differences were revealed: in the group 1, there were 92.3 % of boys, in the group 2 – 33.3 % (p_{1-2} = 0.008). Maternal smoking increased the risk of fatal IVH by 3.5 ± 0.15 times, polyhydramnios – by 3.3 ± 0.37 times, chorioamnionitis – by 12.8 ± 0.47 times, placenta previa – by 3.2 ± 0.15 times. In newborns of the group 1, seizures developed on the day 1 of life in 84.6 % (more often than in group 2; p = 0.00001), and shock in the first 3 hours of life was recorded in 46.1 % of cases (p_{1-2} = 0.034), which increased the risk of death by 4.3 ± 0.47 times. In newborns of group 1, compared with newborns of groups 2 and 3, pulmonary hypertension was more often detected (60.8 [50.1–69.2] mm Hg; p_{1-2} = 0.028; p1–3 = 0.047).*
Conclusion. *Confirmed infectious diseases in the mother, clinical manifestation of convulsions, pulmonary hypertension, development of multiple organ failure and shock in extremely premature newborns increase the risk of intraventricular hemorrhage and the frequency of deaths.*

Key words: *prematurity, intraventricular hemorrhage, risk factors*

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ПРИЧИНЫ РАЗВИТИЯ ВНУТРИЖЕЛУДОЧКОВЫХ КРОВОИЗЛИЯНИЙ У ГЛУБОКО НЕДОНОШЕННЫХ НОВОРОЖДЁННЫХ И ОСОБЕННОСТИ ИХ РАННИХ ИСХОДОВ

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РЕЗЮМЕ

Цель исследования. *Изучить предрасполагающие факторы развития и сроки реализации внутрижелудочковых кровоизлияний (ВЖК) у глубоко недоношенных новорождённых.*

Материалы и методы. *Ретроспективно проанализированы 32 истории болезни детей, рождённых на сроке гестации менее 32 недель. Дети были разделены на три группы: 1-я группа (n = 13) – дети, причиной смерти которых стало нетравматическое ВЖК; 2-я группа (n = 12) – выжившие младенцы с ВЖК; 3-я группа (сравнения; n = 7) – недоношенные дети, у которых не развились ВЖК. Оценивали факторы риска развития ВЖК, их тяжесть, основные показатели, предрасполагающие к летальному исходу у новорождённых этих групп.*

Результаты. *У детей 1-й группы получены статистически значимо низкие показатели массы тела – 670 [640–860] г (p_{1-2} = 0,007; p1–3 = 0,012), окружности головы – 23 [22–24] см (p_{1-2} = 0,008; p_{1-3} = 0,049), срока гестации – 24,5 [23,5–25,5] недели (p_{1-2} = 0,002; p_{1-3} = 0,007). Выявлены гендерные различия: в 1-й группе преобладали мальчики (92,3 %), во 2-й группе их доля составила 33,3 % (p_{1-2} = 0,008). Увеличивали риск ВЖК с летальным исходом курение матери – в 3,5 ± 0,15 раза, многоводие – в 3,3 ± 0,37 раза, хориоамнионит – в 12,8 ± 0,47 раза, предлежание плаценты – в 3,2 ± 0,15 раза. У детей 1-й группы судороги развились в 1-е сутки жизни у 84,6 % (чаще, чем во 2-й группе; p = 0,00001), а шок в первые 3 часа жизни регистрировался у 46,1 % (p_{1-2} = 0,034), повышая риск летального исхода в 4,3 ± 0,47 раза. У детей 1-й группы по сравнению с детьми 2-й и 3-й групп была чаще выявлена лёгочная гипертензия (60,8 [50,1–69,2] мм рт. ст.; p_{1-2} = 0,028; p_{1-3} = 0,047).*

Заключение. *Наличие инфекционных заболеваний у матери, клиническое проявление судорог, лёгочной гипертензии, развитие полиорганной недостаточности и шока у глубоко недоношенных новорождённых увеличивают риск реализации ВЖК и частоту летальных исходов.*

Ключевые слова: *недоношенность, внутрижелудочковые кровоизлияния, факторы риска*

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Premature birth remains an important medical problem. A special group of newborns requiring the use of modern medical technologies for nursing are infants with a gestational age (GA) of less than 32 weeks. Premature babies with very and extremely low body weight most often develop life-threatening diseases, one of which is intraventricular hemorrhage (IVH) of hypoxic and hemorrhagic genesis [1–3].

According to European researchers, the highest incidence of non-traumatic IVH is associated with the very fact of prematurity, that is, with the anatomical, physiological, and morphological features of the vascular wall structure of the brain germinal matrix, imperfect autoregulation of cerebral blood flow, and the state of the hemostasis system of premature infants. The likelihood of non-traumatic IVH is aggravated by conditions accompanied by chronic intrauterine hypoxia, birth asphyxia, and the development of hypocoagulation, including primary coagulopathy, generalized purulent-septic and viral diseases [4, 5]. Coagulation mechanisms in premature infants are characterized by low hemostatic potential, as reflected in the studies of P. Monagle et al. [6]. As gestational age (GA) increases, the probability of IVH decreases, which is certainly associated with neuronal and migratory regression of the germinal matrix. This is also associated with the tectonics of IVH localizations of different GAs. The most important independent risk factor for IVH is GA. At 28–32 weeks of GA, the germinal matrix is preserved for the longest time on the surface of the caudate nucleus head and in the caudate sulcus, and it is in this area that hemorrhages form in children with such GAs [7].

The above factors explain the negative relationship between the frequency of IVH and its severity in very premature infants. IVH is diagnosed in 20–30 % of very premature infants with a GA of less than 29 weeks and increases the frequency of fatal outcomes [8–11]. In the USA, the share of IVH among the causes of neonatal mortality is 1.7 % [12, 13].

THE AIM OF THE STUDY

To study the predisposing factors for the development and timing of development of intraventricular hemorrhage in extremely premature newborns.

MATERIALS AND METHODS

The study was conducted in the neonatal intensive care unit of the Trans-Baikal Regional Perinatal Center (Chita). Retrospectively, for the period 2020–2021, risk factors on the part of the mother and the child were assessed in 32 premature newborns with a GA of less than 32 weeks, using the following registration forms: No. 096/u-20 "Medical record of a pregnant woman, a woman in labor, and a postpartum woman receiving medical care in stationary conditions", and form No. 097u "Medical history of the development of a newborn". The mothers were examined for their somatic health indicators, obstetric and gynecological history, and the characteristics of the current pregnancy and childbirth.

Gestational, anthropometric, and gender characteristics of the newborns under study were assessed, the Apgar score, respiratory disorders, cardiovascular failure, hemostasis (coagulogram in the first hours after birth), metabolic, and electrolyte abnormalities (venous and capillary blood) were studied, general clinical laboratory diagnostic methods (blood, urine), as well as methods of therapeutic measures to stop the identified abnormalities were used. The venous blood coagulogram was studied using the STA Compact Max device (Stago, France); the results were compared with the reference values given in the instructions for this device. In order to identify the causes of hypocoagulation, infant diseases that affect hemostasis and the development of IVH were analyzed.

X-ray examinations were performed to verify the causes of respiratory disorders, were included in the standard examination of newborns with congenital pneumonia or respiratory distress syndrome. The study was conducted on equipment included in the perinatal center. Echocardiography was performed on all newborns to assess hemodynamic changes, including pulmonary hypertension, determine the volume of drug therapy, exclude heart defects and infectious myocardial damage.

The diagnosis of IVH was made after a neurosonographic (NSG) examination using the ALOKA SSD 1700 (Hitachi, Japan) device [14]. The severity of hemorrhage was determined according to the classification of M. Levene et al. [15, 16] and K.V. Vatoli et al. [17, 18]. The cause of death was confirmed by the results of autopsies according to D.A. Duskaliev et al. [19].

The inclusion criteria were: prematurity at less than 32 weeks; development of non-traumatic IVH of varying severity in children.

The children were divided into groups:

Group 1 – premature newborns with a fatal outcome, the cause of death was non-traumatic IVH and posthemorrhagic hydrocephalus ($n = 13$).

Group 2 – premature infants with the development of IVH who survived ($n = 12$).

Inclusion criteria for the comparison group (3rd group): prematurity; GA less than 32 weeks; absence of IVH ($n = 7$).

Exclusion criteria: prematurity after 32 weeks of gestation; presence of congenital malformations; fatal cases caused by generalized infectious diseases, anoxia ($n = 6$).

Statistical processing was performed using Statistica 6.0 (StatSoft Inc., USA), MS Excel 2010 (Microsoft Corp., USA). Groups of children were compared for normal distribution using the Kolmogorov – Smirnov method. Due to the non-normal distribution in the groups, the description is given as a median (Me) and 25-75th quantiles. Comparison of non-parametric indicators was performed using the Fisher's criterion. Risk factors were assessed by calculating the odds ratio (OR) ± standard error (SE, standard error) [95% confidence interval (95% CI)]. Differences were considered statistically significant at $p < 0.05$.

To predict an unfavorable outcome, a logistic regression model (logit regression) was calculated; indicators with a statistically significant resulting feature were included in the equation [20].

The work complied with ethical standards; the participants signed voluntary informed consent; the work was approved by the Ethics Committee of the Chita State Medical Academy of the Ministry of Health of the Russian Federation (Protocol No. 128 dated November 14, 2023).

RESULTS

The characteristics of the study groups are presented in Table 1. When assessing the anthropometric parameters, infants with a fatal outcome and diagnosed IVH had the lowest body weight and length, head circumference, chest circumference and gestational features compared to newborns in groups 2 and 3. Weight fluctuations in group 1 were 490–990 g, in group 2 – 540–1400 g, in the group without IVH – 800–1300 g with a statistically significant difference (p_{1-2} = 0.007; p_{1-3} = 0.012). The body length of infants had a range from 28 to 37 cm and corresponded to the average percentile indicators for gender and GA, but had no statistically significant differences in the comparison groups.

The infants' height ranged from 28 to 37 cm and corresponded to the average percentile values for gender and GA, but had no statistically significant differences in the comparison groups. The head circumference values were statistically significantly lower in the 1st group and ranged from 21 to 27 cm, in the 2nd group Me was 24.2 cm (from 22 to 28 cm; p_{1-2} = 0.008), in the 3rd group Me = 26 cm (from 23 to 29 cm; p_{1-3} = 0.049). Smaller chest circumference was also found in the infants of the 1st group – from 18 to 24 cm (p_{1-2} = 0.041), in the 2nd group – from 15 to 24 cm, in the 3rd group – from 18 to 27 cm. Small anthropometric values corresponded to the GA of premature infants.

In the group of infants who developed IVH with an unfavorable outcome, GA was statistically significantly lower – 24 [22; 27] weeks, that is, the infants were extremely immature; in the 2nd group, infants were born with a GA of 26 [26; 28] weeks, although they were extremely premature, but were more mature than the children in the 1st group (p = 0.002). Extreme immaturity of newborns (GA – 22-26 weeks) increased the risk of developing IVH and a fatal outcome from it by 27.5 ± 0.5 times [95% CI: 10.21-74.01]. Infants who did not develop IVH were born at an average gestational age of 27 weeks (minimum 25 weeks 4 days, maximum 29 weeks), which also statistically significantly differed from children in group 1 (p = 0.007).

TABLE 1

ANTHROPOMETRIC, GESTATIONAL AND GENDER INDICATORS OF THE STUDIED GROUPS

Indicators	Comparison groups, Me [Q25–Q75]			
	Group 1 (n = 13)	Group 2 (n = 12)	Group 3 (n = 7)	p
Weight, g	670 [640–860]	960 [845–985]	940 [870–1260]	**p_{1-2} = 0.007*** p_{2-3} = 0.703* **p_{1-3} = 0.012***
Height, cm	31 [30–33]	33 [30–35.5]	34 [33–38]	p_{1-2} = 0.513* p_{2-3} = 0.204* p_{1-3}=0.079*
Head circumference, cm	23 [22–24]	24.25 [24–27]	26 [23–28]	**p_{1-2} = 0.008*** p_{2-3} = 0.703* **p_{1-3} = 0.049***
Chest circumference, cm	20 [19–22]	22.5 [21–24]	22 [20–23]	**p_{1-2} = 0.041*** p_{2-3} = 0.767* p_{1-3}=0.292*
Gestation age, weeks	24.5 [23.5–25.5]	26.6 [26.4–28]	27 [25.6–28.1]	**p_{1-2} = 0.002*** p_{2-3} = 0.582* **p_{1-3} = 0.007***
Gender, n (%)				
Boys	12 (92.3)	4 (33.3)	6 (85.7)	**7.034; p_{1-2} = 0.008#** 2.99; p_{2-3} = 0.064#
Girls	1 (7.7)	8 (66.7)	1 (14.3)	0.096; p_{1-3} = 0.755#

Note. p_{1-2} – statistically significant differences between groups 1 and 2 (p < 0.05); p_{2-3} – statistically significant differences between groups 2 and 3 (p < 0.05); p_{1-3} – statistically significant differences between groups 1 and 3 (p < 0.05); * – Mann – Whitney test; # – Fisher's test.

Among infants with a fatal outcome associated with IVH, boys predominated (12 out of 13 children), which was statistically significantly different from children in group 2, in which girls predominated **(p_{1-2} = 0.008).** Male gender increased the odds of an adverse outcome with IVH by **24.0 ± 0.48 times [95% CI: 9.18–62.6].**

To assess the impact of maternal diseases and the perinatal period course on the IVH development and its lethal outcome in the comparison groups, the socio-biological and obstetric-gynecological anamnesis were assessed. When assessing a possible lethal outcome, the risk assessment of their development was calculated against the background of the IVH development (OR ± SE; 95% CI). In all observation groups, a favorable age for childbirth was noted for mothers – from 20 to 35 years; the proportion of mothers over 35 years of age did not have statistically significant differences in the groups (from 15 to 28 %).

Nicotine dependence of mothers in the 1st group was recorded in more than half of the observations (53.8 %), in the 2nd group – in a quarter, in the 3rd group – in every 6th case. No statistically significant differences were found. However, maternal smoking increased the risk of IVH and its unfavorable outcome by **3.5 ± 0.15 times [95% CI: 3.01–4.06].**

When analyzing the somatic history of mothers in all study groups, no statistically significant differences were obtained. Burdened obstetric history in the study groups was diagnosed in 2/3 of women, which affected the likelihood of premature delivery, but statistically significant differences between the groups were not established. Preeclampsia was detected in mothers of the 2nd and 3rd groups (8.3 % and 28.6 %, respectively), and fetoplacental insufficiency (FPI) was diagnosed in all observation groups. At the same time, the subcompensated form was registered in 8 out of 13 patients (61.5 %) in the 1st group and in 2/3 of observations in the 2nd group; decompensated – in 15 % in the 1st group and in 16.7 % in the 2nd. In the group of children without IVH, decompensated FPI was not detected; subcompensated FPI was detected in 85.7 %, compensated – in 14.3 %. No statistically significant differences were found between the groups. Complications of pregnancy with the threat of termination in all observation groups were noted in almost half of the cases, the development of isthmic-cervical insufficiency – in every 4th case, which is associated with premature birth. Oligohydramnion was diagnosed in the 1st and 2nd groups with a frequency of 23 % and 8 %, respectively, which increased the probability of developing IVH with a fatal outcome by **3.3 ± 0.37 times [95% CI: 1.6–6.77].** In the 3rd observation group, polyhydramnios was detected in 14.3 %. Emergency operative delivery was required in all observation groups in more than half of the cases (53.8 %, 58.3 % and 57.1 %, respectively).

Pregnancy was complicated by infectious factors. Cervicitis was registered in almost every 3rd case (30.8 % in group 1; 25.0 % in group 2; 28.6 % in group 3). Chorioamnionitis during labor in group 1 occurred in 7 of 13 cases (53.8 %), in group 2 – in 1 of 12 (8.3 %), in group 3 – in 1 of 7 (14.3 %) and statistically significantly prevailed in the group of newborns with IVH that ended in death **(p_{1-2} = 0.03).** This pathology increased the probability of death from IVH by **12.8 ± 0.47 times [95% CI: 5.05–32.44].** Endometritis was also registered statistically significantly more often in the 1st group – in 10 of 13 observations (76.9 %), against 33.3 % in the 2nd group **(p_{1-2} = 0.047)**, and 28.6 % in the 3rd group. Bacterial inflammation of the endometrium increased the chance of IVH with a fatal outcome by **6.7 ± 0.5 times [95% CI: 2.48–18.03].** Placenta previa was detected only in the group with IVH and led to a fatal outcome in 5 of 13 cases. In the 2nd group, transverse fetal position was diagnosed in 1 patient, the risk of IVH development was increased by **3.2 ± 0.5 times [95% CI: 1.2–8.5].**

An increase in the placental-fetal coefficient (PFC) as a marker of the placental infectious process by more than 0.23 was diagnosed in all observations (100 %) of the 1st group, in 75 % of the 2nd group, and in 85.7 % of the 3rd group. The PFC Me in the 1st group was 0.36 [0.32–0.42], the minimum value was 0.28, and the maximum was 0.48, which is statistically significantly higher than the level of this indicator in the 2nd group: Me = 0.295 [0.225–0.34], the minimum value was 0.11, and the maximum was 0.46 **(p_{1-2} = 0.005)**; in the 3rd group Me = 0.34 [0.25–0.4], the minimum value is 0.23, the maximum is 0.52 (p_{2-3} = 0.404; p_{1-3} = 0.579). A prolonged anhydrous period (more than a day) in premature infants of the comparison groups was detected with a frequency of 46 % in the 1st group, 33.3 % in the 2nd group, and 43 % in the 3rd group.

Among the pathological conditions in newborns leading to the IVH development, convulsions are the most common, in the development of which, in addition to hypoxia, the implementation of infection plays a special role. In infants of the 1st group, convulsions were recorded in the first day in 11 of 13 cases (84.6 %), on average, the development time was 3 hours from the moment of birth [1 hour 10 minutes – 6 hours], minimum – 30 minutes, maximum – the 4th day of life. At the age of over 1 day, convulsions developed in 2 children (15.4 %). In the 2nd group, convulsions developed in 3 newborns (25.0 %) at the age of over 24 hours (Me = 2 days 7 hours). Convulsions were not recorded in 9 of 12 newborns (75.0 %) of the 2nd group. In the 3rd observation group, convulsions were diagnosed in 3 of 7 children (42.8 %), Me age – 8 days 8 hours. Statistically significant differences were revealed when comparing the 1st group with the 2nd and 3rd **(χ^2 = 26.692; p = 0.0001).**

Clinical and functional manifestations of various types of shock in premature infants in the 1st group were diagnosed in the first 3 hours of life in 6 of 13 cases (46.1 %) compared to the 2nd group (2 of 12 children (16.7 %)), increasing the risk of developing IVH with a fatal outcome by **4.3 ± 0.47 times [95% CI: 1.69–10.89].** Shock was diagnosed at the age of 3–6 hours in 3 (23.1 %) children in the 1st group, and after 6 hours of life – in 4 (30.7 %) newborns; in the 2nd group, the clinical picture of shock developed at the age of 3–6 hours in 3 (25.0 %) children, and after 6 hours – also in a quarter. In the group of children without IVH, shock was diagnosed less frequently

– in the first 3 hours of life in 2 (28.6 %) children, at the age of over 6 hours – in 1 (14.3 %) infant. When comparing the timing of shock, statistically significant differences were obtained in the comparison groups **(χ^2 = 10.420; p = 0.034).**

Persistent pulmonary hypertension (PPH) in infants of group 1 was detected in 12 of 13 cases (92.3 %), Me = 60.8 [50.1–69.2] mmHg, from 31.8 to 89.6 mmHg. In group 2, the frequency of PPH was recorded in 9 of 12 children (75 %) at a level of 44.1 [34.1–52.1] mmHg, from 23 to 78.7 mmHg, statistically significantly differing in the groups **(p_{1-2} = 0.028)**. PPH in group 3 was detected in 4 of 7 children (57.1 %), Me = 44 [28.6–57] mmHg, from 23 to 64.8 mmHg, in comparison with the 1st group, the differences are statistically significant **(p_{1-3} = 0.047)**.

Neurosonographic (NSG) criteria of IVH in the 1st group were detected in the first day of life in 6 (46.2 %) infants; bilateral IVH of grade III was detected in 53.8 % of cases from the 2nd to the 5th day of life. In the 2nd group, IVH was detected in 5 (41.6 %) children on the 1st day of life, in 1 child – of grades II-III, with the development of posthemorrhagic hydrocephalus. At the age of 3 to 6 days, IVH was realized in 50 % in the form of subependymal hemorrhage.

According to the acid-base balance (ABB) assessment, all infants had uncompensated mixed acidosis and increased lactate levels after birth: in group 1 Me = 4.35 [3.3–5.95] mmol/l, minimum – 2.2 mmol/l, maximum – 9.7 mmol/l; in group 2 Me = 4.4 [2.3–5.6] mmol/l, minimum – 1.4 mmol/l, maximum – 11.3 mmol/l; in group 3 Me = 5.1 [3.4–7.9] mmol/l, minimum – 2.5 mmol/l, maximum – 12.5 mmol/l; no statistically significant differences were found.

The base deficit in infants of group 1 was –9.1 [–7.05 ÷ –11.6] mmol/l, minimum –0.6 mmol/l, maximum –14.6 mmol/l; in group 2 Me = –9.35 [–5.9 ÷ –11.25] mmol/l, minimum –1.7 mmol/l, maximum –15 mmol/l; in group 3 Me = –6.4 [–4.8 ÷ –11.7] mmol/l, minimum –4.8 mmol/l, maximum –15 mmol/l; no statistically significant differences were found. These changes were nonspecific and were associated not with the fact of IVH, but with prematurity, the implementation of an infectious disease, and the presence of respiratory failure.

In order to identify violations of vascular-platelet hemostasis parameters and insufficiency of the blood coagulation system, laboratory criteria (platelet level), coagulogram parameters (activated partial thromboplastin time (APTT), fibrinogen level, international normalized ratio (INR)) were studied. The results are presented in Table 2.

In all study groups, thrombocytopenia was diagnosed in the first hours after birth. In the 1st group, the minimum platelet level was 92 × 109/l, the maximum was 252 × 109/l; in the 2nd group, the range of values was from 93 to 325 × 10 9/l; in the 3rd group – from 107 to 251 × 109/l.

In the coagulogram, the fibrinogen level in all groups corresponded to the average gestational values. In the 1st group, the minimum fibrinogen value was

TABLE 2

LABORATORY PARAMETERS OF HEMOSTASIS IN THE STUDIED GROUPS AT BIRTH, ME [Q25–Q75]

	Comparison groups			
Hemostasis parameters at birth	**Group 1 (n = 13)**	**Group 2 (n = 12)**	**Group 3 (n = 7)**	**p**
Platelets, thousand	163.5 [141.5–232.5]	198 [163–239.5]	183 [121–247]	p_{1-2} = 0.270 p_{2-3} = 0.735 p_{1-3} = 0.799
Fibrinogen, g/l	1.38 [1.14–1.9]	2.2 [1.3–2.4]	1.3 [0.81–3.6]	p_{1-2} = 0.426 p_{2-3} = 1.0 p_{1-3} = 0.853
APTT, s	52 [42–57]	47.35 [29.7–60.8]	43.8 [41.1–46.6]	p_{1-2} = 0.624 p_{2-3} = 0.865 p_{1-3} = 0.267
INR	1.87 [1.44–2.28]	1.42 [1.2–2.45]	1.72 [1.59–5.7]	p_{1-2} = 0.602 p_{2-3} = 0.361 p_{1-3} = 0.609

Note. p_{1-2} – statistically significant differences between groups 1 and 2 ($p < 0.05$; Mann – Whitney test); p_{2-3} – statistically significant differences between groups 2 and 3 ($p < 0.05$; Mann – Whitney test); p_{1-3} – statistically significant differences between groups 1 and 3 ($p < 0.05$; Mann – Whitney test).

0.75 g/l, the maximum – 4.1 g/l; in the 2nd group, its level was from 0.8 to 4.2 g/l; in the 3rd group – from 0.81 to 3.6 g/l; statistically significant differences were not revealed. In the group of children with a fatal outcome, an increase in APTT was determined from 41.4 to 118.9 s, in the group of surviving children with IVH – from 25.7 to 109 s. In the group of children who did not have developing IVH, APTT corresponded to the standard values – from 41.1 to 46.6 s. In all study groups, INR indicated hypocoagulation; statistically significant differences were not revealed. The indicators in the 1st group ranged from 1.26 to 2.8, in the 2nd – from 1.14 to 3.24, in the 3rd – from 1.59 to 5.7; statistically significant differences were not identified.

Based on the obtained statistical data, a logit regression equation was calculated, which made it possible to predict the implementation of an unfavorable outcome of IVH in the early neonatal period [21, 22].

Y = exp (0.1 – 8.05 chorioamnionitis + 10.89 endometritis + 9.86 PFC + 12 gender – 6.43 maternal smoking – 1.17 placenta previa – 1.05 PPH – 5.6 shock in the first 3 hours – 45.8 convulsions in the first 3 hours) / 1 + exp (0.1 – 8.05 chorioamnionitis + 10.89 endometritis + 9.86 PFC + 12 gender – 6.43 maternal smoking – 1.17 placenta previa – 1.05 PPH – 5.6 shock in the first 3 hours – 45.8 convulsions in the first 3 hours); χ^2 = 34.29641; df = 9; *p* = 0.0000797.

DISCUSSION

IVH in premature infants has a multifactorial genesis. There are three groups of factors: antenatal, intranatal and postnatal. According to the results of our study, antenatal factors include: maternal smoking; infectious processes causing oligohydramnios; chorioamnionitis; endometritis. Intranatal factors include: complicated delivery; abnormal placenta previa. Postnatal factors are: fluctuations in systemic arterial pressure, which is manifested by shock; PPH; coagulopathy; oxidative stress, confirmed by the data of the acid-base balance study. As is known, germinal matrix cells are rich in mitochondria and are very sensitive to oxygen deficiency, which explains the importance of germinal matrix hypoxia in the pathogenesis of IVH development [23].

CONCLUSION

Thus, our observations established that extreme prematurity, infectious diseases of the mother, bad habits, and placental pathology exert their adverse effects on the fetus through pathophysiological processes. IVH is realized as a result of a violation of hemodynamic autoregulation and structural immaturity at the level of the germinal matrix. The clinical picture of shock, persistent pulmonary hypertension, and convulsions in the first hours of life can aggravate the severity of IVH and lead to death.

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Conflicts of interest

No potential conflict of interest relevant to this article reported.

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Popova N.G. – developed the concept; worked with literature sources; wrote the manuscript (33 %).

Shcherbak V.A. – worked with literature sources; wrote the manuscript; edited the manuscript (33 %).

PREVENTIVE MEDICINE

QUALITY ASSESSMENT OF THE SCREENING TEST FOR PREDICTORS OF CORONARY HEART DISEASE

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ABSTRACT

Background. *Coronary heart disease (CHD) ranks first among the causes of death, morbidity, and disablement. The development of innovative methods for predicting CHD will reduce these burden.*
The aim of the work. *To assess the quality of the screening test for predictors of coronary heart disease using statistical quality control of a verifiable diagnostic test (VDT) (with binary outcomes).*
Materials and methods. *In 2008–2013, 70 cases of CHD were registered in a group of 7959 initially healthy men aged 18–66 years old who were the members of locomotive crews. Statistical analysis identified CHD predictors: arterial hypertension; psychosocial stress; hyperglycemia; dyslipidemia; excessive alcohol consumption; I-III degree of obesity; age 34–66 years; microalbuminuria; thickening of the intima-media complex/atherosclerotic plaque (IMC/ASP); pulse wave velocity (PWV) > 12 m/s; left ventricular hypertrophy; grade I-II grade of retinopathy; atherosclerosis of aorta. DiagStat software (Russian Federation) determined their predictive ability when used in screening tests to predict CHD. We demonstrated the use of this method to assess the predictive ability of risk factors for any disease.*
Results. *CHD predictors have high to moderate specificity for the absence of CHD in individuals who test negative for the above-listed factors. IMC/ASP, microalbuminuria, PWV > 12 m/s, grade III obesity moderately increase the posterior odds of developing CHD versus its absence in comparison with the prior odds after receiving a positive result of the verifiable diagnostic test for these factors. Age 34–66 years moderately increases the posterior odds in favor of the absence of CHD versus its occurrence compared with the prior odds after receiving a negative result of the verifiable diagnostic test.*
Conclusion. *When assessing the result of the verifiable diagnostic test, we should focus on both the probability of occurrence and the absence of CHD in the presence or absence of a predictor in the patient. Since the determination of PWV > 12 m/s, atherosclerosis of aorta, microalbuminuria, stress, and excessive alcohol consumption among workers of locomotive crews is not mandatory, it is necessary to conduct a targeted search for them.*

Key words: *coronary heart disease, risk factors, prognosis, screening study, verifiable diagnostic test, prevention*

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ОЦЕНКА КАЧЕСТВА СКРИНИНГ-ТЕСТА ПРЕДИКТОРОВ ИШЕМИЧЕСКОЙ БОЛЕЗНИ СЕРДЦА

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РЕЗЮМЕ

Введение. *Ишемическая болезнь сердца (ИБС) занимает первые позиции среди причин смерти, заболеваемости, профессиональной непригодности. Разработка инновационных методов прогнозирования ИБС позволит сократить эти потери.*

Цель работы. *Оценить качество скрининг-теста предикторов ишемической болезни сердца методом статистического контроля качества проверяемого диагностического теста (ПДТ) (с бинарными исходами).*

Материалы и методы. *В 2008–2013 гг. в группе изначально здоровых 7959 мужчин 18–66 лет – работников локомотивных бригад – зарегистрировали 70 случаев ИБС. С помощью статистического анализа идентифицировали предикторы ИБС: артериальная гипертензия; психосоциальный стресс; гипергликемия; дислипидемия; чрезмерное потребление алкоголя (ЧПА); ожирение I–III степени; возраст 34–66 лет; микроальбуминурия (МАУ); утолщение комплекса интима-медиа/атеросклеротическая бляшка (ТИМ/АСБ); скорость распространения пульсовой волны (СРПВ) > 12 м/с; гипертрофия левого желудочка; ретинопатия I–II степени; атеросклероз аорты (Ат.АО). В программе DiagStat (Россия) выяснили их предсказательную способность при использовании в скрининг-тестах для прогнозирования ИБС. Показано применение этого метода для оценки предсказательной способности факторов риска любого заболевания.*

Результаты. *Предикторы ИБС обладают высокой и умеренной специфичностью в отношении отсутствия возникновения ИБС у лиц, имеющих отрицательный результат на наличие этих факторов. ТИМ/АСБ, МАУ, СРПВ > 12 м/с, ожирение III степени умеренно повышают апостериорные шансы возникновения ИБС против её отсутствия в сравнении с априорными шансами после получения положительного результата ПДТ этих факторов. Возраст 34–66 лет умеренно повышает апостериорные шансы в пользу отсутствия ИБС против её возникновения в сравнении с априорными шансами после получения отрицательного результата ПДТ.*

Заключение. *При оценке результата ПДТ следует ориентироваться как на вероятность возникновения, так и на отсутствие ИБС при наличии или отсутствии у пациента предиктора. Так как определение СРПВ > 12 м/с, Ат.АО, МАУ, стресса, ЧПА у работников локомотивных бригад в обязательном порядке не предусмотрено, необходимо проводить их целенаправленный поиск.*

Ключевые слова: *ишемическая болезнь сердца, факторы риска, прогнозирование, скрининг-исследование, проверяемый диагностический тест, профилактика*

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INTRODUCTION

Statistical data indicate that cardiovascular disease (CVD) morbidity and mortality remain leading causes worldwide and in the Russian Federation [1, 2]. The largest share in the mortality structure from diseases of the circulatory system falls on coronary heart disease (CHD) [3], with acute forms predominating [4]. The widespread use of modifiable risk factors (RF) for CVD has placed cardiovascular causes and CHD in the first positions in the ranking of ten leading causes of death in the world [5, 6]. Despite the fact that chronic CHD is characterized by a stable long-term course, CHD occupies the first positions among the causes of death, temporary and permanent loss of working capacity of the population [7-9]. These problems are also relevant for railway medicine, since cases of this disease are regularly registered among locomotive crew workers (LCW). CHD is included in the list of diseases that do not allow LCWs to work in the profession, and is the leading cause of professional unsuitability. The share of professional unsuitability due to CHD reaches 40–50 %. Admission by the medical expert commission to train work does not guarantee the absence of this latent CVD in LCW and the safety of railway traffic, does not prevent early retirement from the profession and economic losses associated with the health of workers [10-14]. Therefore, it is important for such professional groups of clinically asymptomatic individuals to undergo primary examination – screening testing to prevent CVD. Screening examination helps to detect the disease at an early stage and completely cure or prevent it. The disease should be understood as any clinical outcome under study that has a latent period and forms long before the appearance of clinical manifestations [13]. Despite their obvious usefulness, screening methods have varying effectiveness, since they do not exclude diagnostic error or the formation of a false opinion about the absence of the disease. Therefore, a screening test should meet an acceptable level of prediction [15] and comply with the World Health Organization (WHO) principles for conducting a screening study [16]: the course and treatment of the actual disease should be known; case finding should be carried out continuously, and the diagnostic costs should be justified. An ideal screening test should detect the disease before its manifestation, not give false-positive or false-negative results, and reduce disability and mortality. The party performing the screening study should have an idea of how effective a particular diagnostic test is in predictive performance in order to compare its capabilities and the expected benefit of conducting the screening study. In this study, the predictive performance of previously established predictors of coronary heart disease [17] is assessed, their exposure (concentration, dose) capable of causing coronary heart disease in the observation group and the range of probability of the coronary heart disease in the presence or absence of the predictor in the patient are shown. A method for assessing the quality of coronary heart disease predictors is proposed, and its application is shown, since no other scientific publications on this topic could be found.

THE AIM OF THE STUDY

To determine the predictive performance of coronary heart disease predictors using statistical quality control of a verifiable diagnostic test (with binary outcomes).

MATERIALS AND METHODS

According to the criteria of the recommendations on arterial hypertension (AH) of the Russian Medical Society on Arterial Hypertension and the All-Russian Scientific Society of Cardiologists (ARSC) for 2008 and 2011 and in accordance with the regulatory order [10, 18, 19], from 2008 to 2013, all LCWs of the Trans-Baikal Railway (TBR) annually for 6 years during medical expert commissions underwent underwent annual screening for CVD, target organ damage (TOD) and CVD. The following risk factors were determined: age; hypertension; dyslipidemia – total cholesterol > 5.0 mmol/l, and/or low-density lipoprotein cholesterol (LDL) > 3.0 mmol/l, and/or high-density lipoprotein HDL < 1.0 mmol/l, and/or triglycerides > 1.7 mmol/l; hyperglycemia > 5.5 mmol/l; family history of early CVD; excessive alcohol consumption (EAC); psychosocial stress; smoking; overweight – body mass index (BMI) ≥ 25.0 kg/m^2. According to BMI gradations, obesity stages I–III were distinguished: BMI = 30.0–34.9, 35.0–39.9, and ≥ 40.0 kg/m^2, respectively. The following were identified from the TOD: left ventricular hypertrophy (LVH) by electrocardiography (Sokolov–Lyon sign > 38 mm, Cornell product > 2440 mm×ms) and echocardiography (left ventricular mass index ≥ 125 g/m^2); aortic atherosclerosis (AA) by radiography and/or ultrasound diagnostics (USD); thickening of the intima-media complex (IMC) > 0.9 mm or atherosclerotic plaques (ASP) by USD of the brachiocephalic arteries [18]; reduced glomerular filtration rate; microalbuminuria (MAU); hypercreatininemia; retinopathy stages I–II; pulse wave velocity (PWV) > 12 m/s; ankle-brachial index < 0.9; type 2 diabetes mellitus (T2DM) [10, 12]. Lipid and carbohydrate metabolism parameters were determined and assessed according to the above criteria [18] and were included in the sample as qualitative variables – "yes" or "no". According to the order [10], the LCWs had no CVD at baseline except for hypertension stage I, 1–2 stages. They dropped out of the study in case of death, dismissal, or if their health did not meet the criteria of the order [10]. The observation was approved by the local Ethics Committee of the Chita State Medical Academy of the Ministry of Health of the Russian Federation (protocol No. 30 dated November 09, 2011) and was conducted using official laboratory and instrumental diagnostic methods, on licensed equipment by certified specialists. The study design, collected material on 22 items at the beginning and end of the observation are shown in Figure 1 and Table 1. Comparison of the collected data

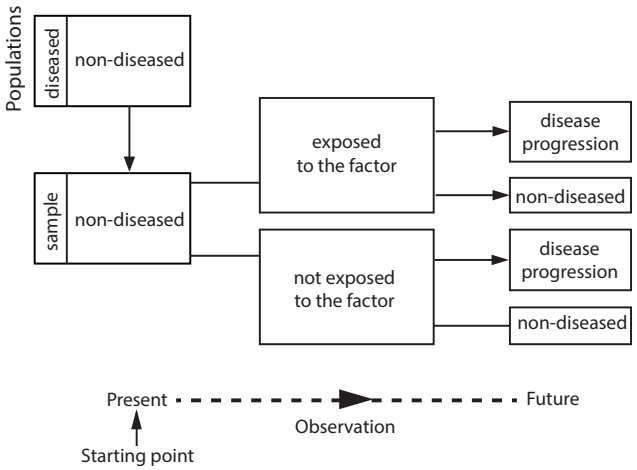

FIG. 1.
Prospective cohort design of locomotive crews members of the Trans-Baikal Railway [20]

at the beginning and end of the observation showed that CVD risk factors are cumulative, increasing over time both in the population and within individuals. [12].

The age of the LCWs at the beginning of the observation was 35.7 ± 10.6 years, at the end of the observation – 38.6 ± 10.3 years. 53.4 % (n = 4251) of the LCWs in the observation group were under 40 years of age (fig. 2).

The dynamics of cardiovascular risk according to the SCORE scale by observation years in the LCW of the Trans-Baikal Railway is presented in Figure 3.

Predicted probability of 10-year prediction of fatal cases of atherosclerosis-associated diseases according to the SCORE scale in the year of their prediction and the same year of death occurring among LCWs of the Trans-Baikal Railway is shown in Figure 4.

The structure of the identified SVDs in 2008–2013 is shown in Figure 5. In 2008, 7959 LCWs of the Trans-Baikal Railway were observed, in 2009 – 7851, in 2010 – 7141, in 2011 – 6817, in 2012 – 6016, in 2013 – 5722.

TABLE 1

OCCURRENCE OF RISK FACTORS, TARGET ORGAN LESIONS IN LOCOMOTIVE CREW MEMBERS OF THE TRANS-BAIKAL RAILWAY AT THE BEGINNING AND AT THE END OF THE OBSERVATION [12]

Risk factors, target organs	Start of observation		End of observation		%#/%*	McNemar's test	
	n*	%*	n*	%*		χ^2	p
Arterial hypertension	1401	17.6	2033	25.5	1.4	2381.6	0.00
Overweight (BMI = 25.0–29.9 kg/m^2)	2602	32.7	3135	39.4	1.2	580.4	0.00
Obesity stage I (BMI = 30.0–34.9 kg/m^2)	923	11.6	1215	15.3	1.3	4104.4	0.00
Obesity stage II (BMI = 35.0–39.9 kg/m^2)	167	2.1	234	2.9	1.4	7114.4	0.00
Obesity stage III (BMI ≥ 40.0 kg/m^2)	16	0.2	24	0.3	1.5	7868.3	0.00
Smoking	4600	57.8	4918	61.8	1.1	293.7	0.00
Dyslipidemia	700	8.8	2534	31.8	3.6	2278.1	0.00
Left ventricular hypertrophy	446	5.6	597	7.5	1.3	5895.1	0.00
Psychosocial stress	1249	15.7	1635	20.5	1.3	3084.3	0.00
Family history of early CVD	597	7.5	906	11.4	1.5	5038.6	0.00
Retinopathy stages I–II	533	6.7	337	4.2	0.6	6470.7	0.00
Hyperglycemia	80	1.0	445	5.6	5.6	6636.4	0.00
Aortic atherosclerosis	8	0.1	458	5.8	58.0	6674.0	0.00
Excessive alcohol consumption	48	0.6	71	0.9	1.5	7697.6	0.00
IMC/ASP	8	0.1	24	0.3	3.0	7876.3	0.00
PWV > 12 m/s	0	0	19	0.2	-	7899.2	0.00
Creatininemia	24	0.3	116	1.5	5.0	7590.7	0.00
Microalbuminuria	3	0.04	8	0.1	2.5	7929.0	0.00
Decreased glomerular filtration rate	0	0	6	0.1	-	7938.0	0.00
Ankle-brachial index < 0.9	0	0	5	0.1	-	7941.0	0.00
Diabetes mellitus type 2	24	0.3	45	0.6	2.0	7798.0	0.00

Note. * – start of observation; # – end of observation.

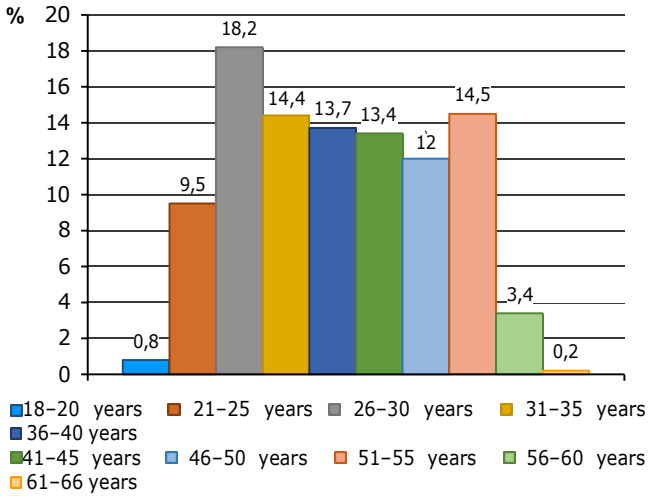

FIG. 2.
Age structure of respondents

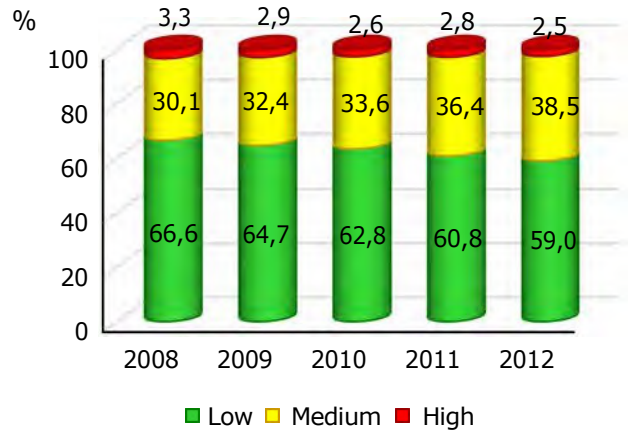

FIG. 3.
Cardiovascular risk assessment using the SCORE scale in the observation group

In 2008–2013, medical expert commissions of 14 non-governmental healthcare institutions of the Trans-Baikal Railway diagnosed 70 chronic cases of coronary heart disease, which were confirmed during inpatient examination at the Railway Clinical Hospital at Chita station, as well as by the Central Medical Expert Commission of Russian Railways (Moscow) in the case of their complex resolution. The diagnostics were carried out in accordance with the clinical recommendations of the All-Russian Society of Cardiology (ARSC) in 2009, the Ministry of Health and Social Development of Russia in 2013, and the ESC (European Society of Cardiology) in 2013 [12, 21–23].

Considering the identified risk factors, the individual cardiovascular risk was calculated annually using the SCORE scale as the most adapted to the Russian population and recommended by the State Research Center for Preventive Medicine for individuals aged 40 and older. LCWs aged under 40 were classified as having a low cardiovascular risk. In the SCORE scale, the overall cardiovascular risk is expressed as the absolute risk of cardiovascular mortality in the next 10 years. Since the absolute overall cardiovascular risk is highly dependent on age, it can be low in young patients even with a combination of high blood pressure (BP) and other RF [24]. Individuals aged under 40, regardless of the RF presence (except for very high levels of individual factors), have a low absolute risk of fatal cardiovascular complications in the next 10 years of life. For young people (under 40), not the absolute, but the relative total cardiovascular risk is determined. A person under 40 years of age without RF (non-smoker, with normal BP and total cholesterol levels in the blood) has a 12-fold lower relative total cardiovascular risk compared to a person with the specified RF [12, 25]. We did not analyze further or identify special age groups in the assessment and stopped studying the SCORE indicators in order to save our resources when this scale showed low predictive abilities in this category of workers (fig. 4).

At the end of the LCW observation, the above-listed CHD predictors were determined in a 2 × 2 contingency table, in a multivariate stepwise analysis, and in a survival analysis (in the Cox proportional hazards regression model and in the Kaplan – Meier models), and their relative risk was established [17]. As a result of the performed multivariate analysis, all CHD predictors were divided into three categories, since they showed statistical heterogeneity in the analytical models. The first category included IMC/ASP, hypertension, and retinopathy, which showed a statistically significant assessment in all

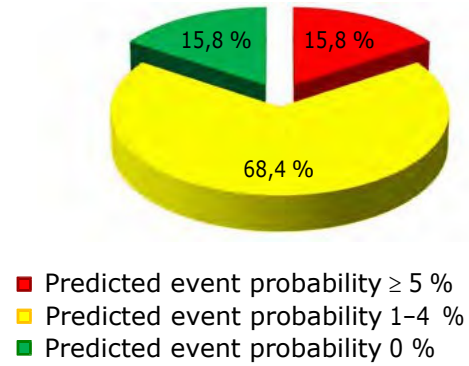

FIG. 4.
Predicted probability of 10-year prediction of fatal cases of atherosclerosis-associated diseases according to the SCORE scale in the year of their prediction and the same year of death coming among locomotive crew members of the Trans-Baikal Railway: sudden cardiac death – 15 cases, cerebral stroke – 4 cases; mean age of the deceased – 46.3 ± 9.2 years

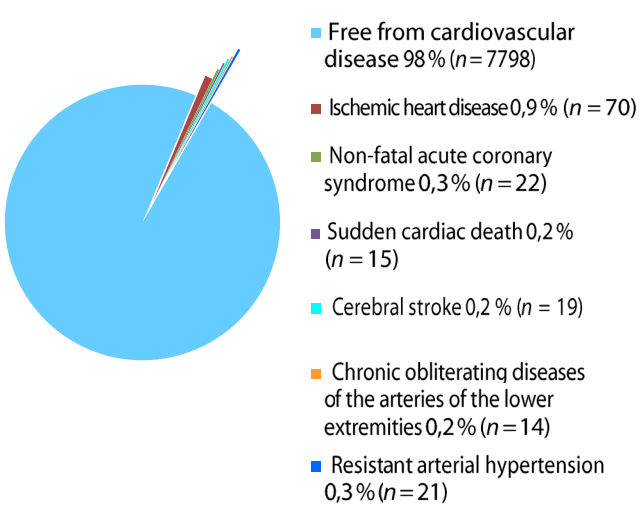

FIG. 5.
Structure of diagnosed cardiovascular diseases in 2008–2013

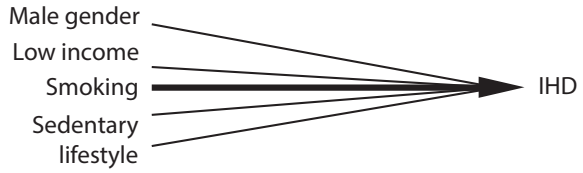

FIG. 6.
Assessment of the influence of various potential risk factors on coronary heart disease [26]

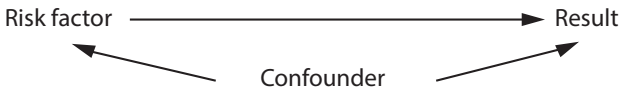

FIG. 6.
Confounder interaction scheme [26]

five models used. These factors were assessed as the main independent predictors capable of being realized in CHD without the participation of other predictors. Such predictors of coronary heart disease as age 34–66 years, AA, obesity stage III, which had a statistically significant result in 4 statistical models, were hypothetically assessed as interacting RFs, realized in coronary heart disease with the participation of other factors as part of a complex variable consisting of 2 or more similar independent factors. The remaining predictors of coronary heart disease, which had a statistically significant result in less than 4 models used, were assessed as confounders, factors influencing the final outcome and the main influencing variable [17, 26] (fig. 6, 7; Table 3). The results of this part of the work were published [12, 17]. Since the predictors of coronary heart disease differ in their statistical characteristics and, therefore, can have different effects on the disease development, we decided to determine their predictive ability using the method of statistical quality control of the verified diagnostic test (VDT) [27] in the DiagStat software (Russian Federation) [28] to clarify the issue of the informativeness and appropriateness of their use in screening examinations. We also decided to show how this method can be used to assess the predictive ability of predictors of any disease.

The main requirement for disease diagnostic methods is that they should have sufficient sensitivity and high specificity. However, when disease factors are tested for a screening test, it is necessary to remember and understand that they are individual, differ from each other in their statistical characteristics and have the ability to interact with each other [17, 26, 29]. In medical and biological issues, three types of factor interaction are described: additivity – summation; synergism – mutual enhancement of the effect; antagonism – mutual weakening of the effects of predictors [30]. Patient examination methods that are usually used as screening tests do not have these qualities. Therefore, these features of environmental factors during their assessment of the VDT quality can manifest themselves in the analysis, and the results interpretation of the study of disease predictors using this technique may differ from the assessment of the VDT quality of diagnostic methods for detecting the disease.

The VDT predictive value is assessed by the accuracy and predictive value indicators, comparing its result with the gold standard – a diagnostic reference test that almost accurately determines the presence or absence of a disease in the patient being examined. The gold standard may be one or several tests for determining the disease. A diagnostic test (T) should be understood as a factor being examined for diagnostic ability (as in this case) or a method for identifying an important disease, which can have two values for the patient being examined: T^+ – a positive result or T^- – a negative result. The disease can be realized in two binary outcomes – "yes" or "no" (CHD^+ and CHD^-). To evaluate the VDT, it is necessary to compare the exposed and unexposed VDT groups by the frequency of disease occurrence in them – in this case, coronary heart disease. For this purpose, a 2 × 2 contingency table is formed. The frequencies of opposite values of the studied binary outcome of both groups are entered in absolute figures. The exposed and unexposed VDT groups are marked in the rows, and the possible outcomes are indicated in the columns. Each study object is included in only one of the groups and has only one of the possible outcomes. Positive results and negative results of the VDT form 4 combinations of the disease outcome: $T+CHD^+$ – true "positive"; $T+CHD^-$ – false "positive"; T^-CHD^+ – false "negative"; T^-CHD^- – true "negative". Their number in the 2 × 2 cross-classification is designated as a, b, c and d, respectively. The methodology for assessing the VDT is shown in Table 2 [27]. All designations in the text, tables and figures

are given in the original terminology of the methodology and the DiagStat program [27, 28].

The indicators of the accuracy and predictive value of the VDT reflecting its ability to determine and predict the outcome under study form two pairs of indicators and counter-indicators, by which the VDT quality is assessed when comparing them. Four conditional probabilities of the indicator of the VDT accuracy form two pairs of opposites – sensitivity (Se) and counter-sensitivity (coSe), specificity (Sp) and counter-specificity (coSp). Their statistical estimates are measured by proportions (f), which can be shown as a percentage, or as the ratio of a part to a whole or the ratio of the constituent parts of a set to its total volume [31, 32]. Four indicators of the VDT predictive value form two pairs of opposites: the positive predictive value (PPV) and its counter-positive predictive value (coPPV); the negative predictive value (NPV) and its counter-negative predictive value (coNPV). Their qualities and capabilities are indicated in the note to table 3 [27].

When checking the VDT quality, it is necessary to have data on the prevalence of the disease *(Prev)* in the tested population. These data are automatically generated in the DiagStat software [28] when entering the study parameters as the proportion of people with the disease (CHD) among all those examined in the group: $f(CHD^+) = (a + c) / n$.

There is an interdependence between the indicators of VDT accuracy and VDT predictive value, which is determined by the likelihood ratios that form two opposite pairs: the likelihood ratio for the CHD "positives" (LR[+]), the likelihood ratio for the CHD "negatives" (LR[–]), and their antipodes. Likelihood ratios and their inversion results are estimated in terms of probabilities and odds – "for" or "against" [27].

The quality of screening tests for CHD predictors was assessed using the DiagStat software (Russia). If the 100 (1 – α)% confidence interval (CI) for the studied CHD predictor θ did not include the uninformative value θ_{ni}, then the estimated unknown value in this CI of the predictor θ_{uk} had a statistically significant difference from its uninformative value θ_{ni} at the significance level α. This means that $\theta_{uk} \neq \theta_{ni}$ and is statistically significant at the level α. When the 100 (1 – α)% CI of the θ indicator included the uninformative value θ_{ni}, the unknown value θ_{uk} of the CHD predictor estimated by this CI did not statistically significantly differ from θ_{ni} at the level α, and a conclusion was made about the insignificance of the result, $\theta_{uk} = \theta_{ni}$, according to the VDT assessment methodology [27].

The value $Se_{ni} = coSe_{ni} = 0.5$ is uninformative for the sensitivity Se and counter-sensitivity coSe. If they are equal, then the VDT cannot be considered accurate in identifying "positives" in individuals with a disease, in this case, coronary heart disease. By analogy, the value $Sp_{ni} = coSp_{ni} = 0.5$ is considered uninformative for the specificity Sp and counter-specificity coSp [27].

TABLE 2

TABLE OF CROSSED CLASSIFICATION MATRIX 2 × 2 FOR CORONARY HEART DISEASE

Object groups	Gold standard (infallible test for disease detection)		Total objects in the group
	Result		
	CHD [+]	CHD [–]	
Exposed Test [+]	True "positive" T^+CHD^+ a	False "positive" T^+CHD^- b	T^+ $P(T^+)$ a + b
	Prevalence of "positives" in the outcome		
	$P(T^+CHD^+)$	$P(T^+CHD^-)$	
Unexposed Test [–]	False "negative" T^-CHD^+ c	True "negative" T^-CHD^- d	T^- $P(T^-)$ c + d
	Prevalence of "negatives" in the outcome		
	$P(T^-CHD^+)$	$P(T^-CHD^-)$	
Total objects in the outcome	Prevalence of outcome (disease) in the observation group		1 *n*
	CHD^+ $Prev = P(CHD^+)$ a + c	CHD^- $(1 – Prev = P(CHD^-))$ b + d	

Note. (here and in Table 3). [+] – positive result of the gold standard (GS) or the VDT compared with it; [–] – negative result of the gold standard or the VDT compared with it; CHD^+ – presence of CHD in the patient according to the GS, CHD– – absence of CHD in the patient according to the GS; T+ – "positive", positive result of the VDT; T– – "negative", negative result of the VDT; Prev, (P) – prevalence of the disease.

If the PPV CI overlaps "overlaps" the CHD Prev, then such a "positive" is considered an uninformative value: PPV_{ni} = Prev [27].

According to the method used, if the "negative" NPV does not increase the probability of the probability of absence of CHD (coPrev), then such a value is also considered uninformative: NPVni = coPrev [27]. The PPV and NPV graphs of the test should differ from Prev, located in the center of the scale grid, that is, the effect of the test on the population prevalence of the disease should be noticeable, and the stronger it is, the more significant the deviation of the test curve from Prev.

According to the methodology for the likelihood ratio for the CHD LR[+] "positives" and the CHD LR[–] "negatives", the values $LR[+]_{ni}$ = 1 and $LR[-]_{ni}$ = 1 are considered uninformative [27]. The practical usefulness of the VDT quality indicators is determined by verbal scales that make it possible to qualitatively evaluate the quantitative result in the proportion values for Se and Sp from 0 to 1.0 [27]. Evaluation in values: 0–0.5 – useless; 0.5–0.7 – low; 0.7–0.9 – moderate; 0.9–1.0 – high. LR[+] and LR[–] in verbal scales are evaluated in the following ranges: 1–3 – insignificant assessment; 3–10 – mediocre; 10–33 – moderate; 33–100 – high; 100–1000 – very high; > 1000 – perfect score.

RESULTS AND DISCUSSION

The results of the quality assessment of the VDT predictors of CHD are shown in Table 3 and Figures 8–23. All CHD predictors had low VDT sensitivity (Se), except for the predictors of hypertension, BMI ≥ 25, and age 34–66 years. According to the method used [27], Se has the ability to "sense" the presence of the disease (CHD) and shows the frequency of "positives" among individuals with CHD. This interpretation of the indicator is used when assessing the VDT of the patient examination method. When assessing environmental factors using this method, it should be understood that Se shows the prevalence of the factor that caused the disease among individuals who have experienced this outcome (Tables 2, 3). That is, Se, when working with predictors, determines and shows the exposure (accumulation, dose) of the factor [29], capable of causing the expected disease in a specific population. This information is important for determining the scope of preventive measures and medical care in a specific society. The range of probability of a disease event is shown by the PPV and NPV indicators. The prognostic CI of the «positives» of the PPV predictors: hypertension, PWV > 12 m/s, age 34–66 years, LVH, MAU, AA, IMC/ASP, obesity stage III, retinopathy, "do not overlap" the central zone of the population prevalence of CHD Prev, which indicates the informativeness of their values. Figures 9–14, 16, 21, 22 confirm this conclusion visually. The CI predictive value of the PPV "positives" predictors: EAC, hyperglycemia, BMI ≥ 25.0, obesity stages I–II, dyslipidemia – cover the zone of population prevalence of CHD Prev and have no informative value (figs. 8, 15, 18–20, 23). In this regard, when assessing these predictors, one should focus on the CI predictive value of the NPV "negatives", showing the ranges of the probability of the CHD absence in individuals who do not have these predictors, as well as the proportion of individuals in the population (Sp) without these factors, guaranteeing the CHD absence in this probability range at the level of the controlled society. The CI of "negatives" and "positives" of the predictor psychosocial stress overlap the zone of prevalence of CHD Prev and have no informative value. This factor (confounder) is associated with and contributes to the CHD formation, but does not have an independent prognostic effect on CHD (fig. 7, 17) [17]. The quantitative assessment of the increase in the odds of a disease event or absence of a disease in the group of "positives" LR[+] and in the group of "negatives" LR[–] is shown by the VDT likelihood ratios. The predictors IMC/ASP, MAU, PWV > 12 m/s, BMI > 40.0, in contrast to other CHD predictors, statistically significantly moderately increase the posterior odds in favor of the CHD development after a positive VDT result is obtained in a patient. The predictor age in the range of 34–66 years statistically significantly moderately increases the posterior odds in favor of the absence of coronary heart disease versus the development of coronary heart disease after a negative VDT result is obtained in a patient. In our study, most of the CHD predictors did not reach the level of statistical significance Se, and, therefore, this indicator does not accurately determine the prevalence (exposure) of risk factors in the group of individuals who developed CHD, which indicates a higher real statistically significant concentration of RF required for the development of this disease in the group of LCWs of the Trans-Baikal Railway. To determine the exact value of the concentration of these RF causing CHD in the RLB population, clarifying population studies are needed on a larger sample or a sample of similar size, but with a longer observation period.

We did not compare the obtained VDT results of CHD predictors with the data of VDT coronary angiography or CT coronary angiography, which are considered the gold standard for CHD diagnosing due to the lack of publications on the assessment of the latter using the VDT method. At the same time, it is not possible to compare predictors of the disease and methods of diagnosing the disease, since they belong to different categories and have different qualities. Predictors form the preclinical course and progress the disease until clinical manifestations appear; methods of diagnosing the disease do not have this quality, as well as a number of other properties of RF.

Graphical assessment of the VDT statistical quality control method of the CHD predictors as screening tests is the final stage of the methodology [27, 28] and a useful tool for visualizing the obtained estimates and the influence of predictors on the population prevalence of the disease. The extent to which the predictor under study is invasive and dangerous is visible on the graphs.

TABLE 2

TABLE OF CROSSED CLASSIFICATION MATRIX 2 × 2 FOR CORONARY HEART DISEASE

CHD predictors	*Se*	*Sp*	The probability of CHD developing				Likelihood ratio	
			PPV (+), %		*NPV (–), %*		*LR[+]*	*LR[–]*
			PPV	*coPPV*	*NPV*	*coNPV*		
EAC	~~**0.03**~~	**0.99**	$_{0.1}$ 2.7 $_{9.9}$	$_{90.1}$ 97.3 $_{99.9}$	$_{98.8}$ **99.1** $_{99.3}$	$_{0.7}$ **0.9** $_{1.2}$	3.0	1.0
Arterial hypertension	**0.80**	**0.75**	$_{2.0}$ **2.9** $_{3.9}$	$_{96.1}$ **97.1** $_{98.0}$	$_{99.5}$ **99.7** $_{99.9}$	$_{0.1}$ **0.3** $_{0.5}$	~~**3.2**~~	~~**3.6**~~
Retinopathy stages I–II	~~**0.23**~~	**0.96**	$_{2.5}$ **5.0** $_{8.5}$	$_{91.5}$ **95.0** $_{97.5}$	$_{99.0}$ **99.3** $_{99.5}$	$_{0.5}$ **0.7** $_{1.0}$	~~**5.7**~~	1.3
LVH	~~**0.29**~~	**0.93**	$_{1.9}$ **3.5** $_{5.7}$	$_{94.3}$ **96.5** $_{98.1}$	$_{99.0}$ **99.3** $_{99.5}$	$_{0.5}$ **0.7** $_{1.0}$	~~**3.9**~~	1.3
Stress	~~**0.19**~~	**0.79**	$_{0.4}$ 0.9 $_{1.6}$	$_{98.4}$ 99.1 $_{99.6}$	$_{98.7}$ 99.1 $_{99.3}$	$_{0.7}$ 9.3 $_{1.3}$	0.9	1.0
IMC/ASP	~~**0.05**~~	**0.99**	$_{2.8}$ **15.4** $_{37.4}$	$_{62.6}$ **84.6** $_{97.2}$	$_{98.8}$ **99.1** $_{99.4}$	$_{0.6}$ **0.9** $_{1.2}$	**19.6**	1.1
Microalbuminuria	~~**0.03**~~	**0.99**	$_{1.2}$ **20.0** $_{58.5}$	$_{41.5}$ **80.0** $_{98.8}$	$_{98.8}$ **99.1** $_{99.4}$	$_{0.6}$ **0.9** $_{1.2}$	**27.0**	1.0
Aortic atherosclerosis	~~**0.21**~~	**0.94**	$_{1.5}$ **3.3** $_{5.8}$	$_{94.2}$ **96.7** $_{98.5}$	$_{98.9}$ **99.2** $_{99.5}$	$_{0.5}$ **0.8** $_{1.1}$	~~**3.6**~~	1.2
PWV > 12 m/s	~~**0.04**~~	**0.99**	$_{1.8}$ **14.3** $_{38.7}$	$_{61.3}$ **85.7** $_{98.2}$	$_{98.8}$ **99.1** $_{99.4}$	$_{0.6}$ **0.9** $_{1.2}$	**18.6**	1.0
Dyslipidemia	0.45	~~**0.68**~~	$_{0.8}$ 1.3 $_{2.0}$	$_{98.0}$ 98.7 $_{99.2}$	$_{98.9}$ **99.3** $_{99.5}$	$_{0.5}$ **0.7** $_{1.1}$	1.4	1.2
Hyperglycemia	~~**0.10**~~	**0.94**	$_{0.5}$ 1.6 $_{3.5}$	$_{96.5}$ 98.4 $_{99.5}$	$_{98.8}$ **99.1** $_{99.4}$	$_{0.6}$ **0.9** $_{1.2}$	1.7	1.0
BMI ≥ 25,0	**0.74**	~~**0.42**~~	$_{0.8}$ 1.1 $_{1.6}$	$_{98.4}$ 99.4 $_{99.2}$	$_{99.0}$ **99.4** $_{99.7}$	$_{0.3}$ **0.6** $_{1.0}$	1.3	1.6
BMI = 30,0–34,9	~~**0.26**~~	**0.85**	$_{0.8}$ 1.6 $_{2.6}$	$_{97.4}$ 98.4 $_{99.2}$	$_{98.9}$ **99.2** $_{99.5}$	$_{0.5}$ **0.8** $_{1.1}$	1.7	1.1
BMI = 35,0–39,9	~~**0.08**~~	**0.97**	$_{0.7}$ 2.5 $_{5.9}$	$_{94.1}$ 97.5 $_{99.3}$	$_{98.8}$ **99.1** $_{99.4}$	$_{0.6}$ **0.9** $_{1.2}$	2.8	1.1
BMI ≥ 40,0	~~**0.04**~~	**0.99**	$_{1.4}$ **11.5** $_{32.1}$	$_{67.9}$ **88.5** $_{98.6}$	$_{98.8}$ **99.1** $_{99.4}$	$_{0.6}$ **0.9** $_{1.2}$	**14.1**	1.0
Age 34-66 years	**0.99**	~~**0.38**~~	$_{1.0}$ 1.4 $_{2.0}$	$_{98.0}$ 98.6 $_{99}$	$_{99.0}$ **99.9** $_{100.0}$	$_{0.0}$ **0.03** $_{1.0}$	~~**1.6**~~	**27.5**

Note. Informative values are shown in bold, with the results that did not stand up to verbal assessment crossed out. The assessment results in tables and figures are shown as 99% CI. The Se (sensitivity – how often "positives" are observed in individuals with coronary heart disease, i.e. to what extent the test "feels" the presence of coronary heart disease) and Sp (specificity – how often "negatives" are observed in individuals without coronary heart disease, i.e. to what extent the test "feels" the absence of coronary heart disease) indicators are presented as shares, since they show the specific value of occurrence (accumulation) of RF among the population of individuals who have developed the outcome under study (coronary heart disease) (Se), and the specific value of the absence of this factor among individuals who have not developed coronary heart disease (Sp). The PPV (positive predictive value – the VDT ability to correctly predict coronary heart disease in a person with a "positive") and NPV (negative predictive value – the VDT ability to correctly predict the absence of coronary heart disease in a person with a "negative") indicators and their inversions coPPV (counter-positive predictive value – the VDT ability to erroneously predict the absence of coronary heart disease in a person with a "positive") and coNPV (counter-negative predictive value – the VDT ability to erroneously predict coronary heart disease in a person with a "negative") are presented in %, since they show the range of the probability of the occurrence or absence of a coronary heart disease event in the representatives of the sample. LR[+] – the ratio of the proportion of "positives" among persons with coronary heart disease to the proportion of "positives" among persons without coronary heart disease, shows an increase in the posterior odds in favor of the coronary heart disease presence versus its absence in the respondent in comparison with the a priori odds after receiving a positive VDT result; LR[–] – the ratio of the proportion of "negatives" among persons without coronary heart disease to the proportion of "negatives" among persons with coronary heart disease, shows an increase in the posterior odds in favor of the coronary heart disease absence versus its presence in the respondent in comparison with the a priori odds after receiving a negative VDT result; – independent effect of the factor; – interaction of the factor; – confounding effect.

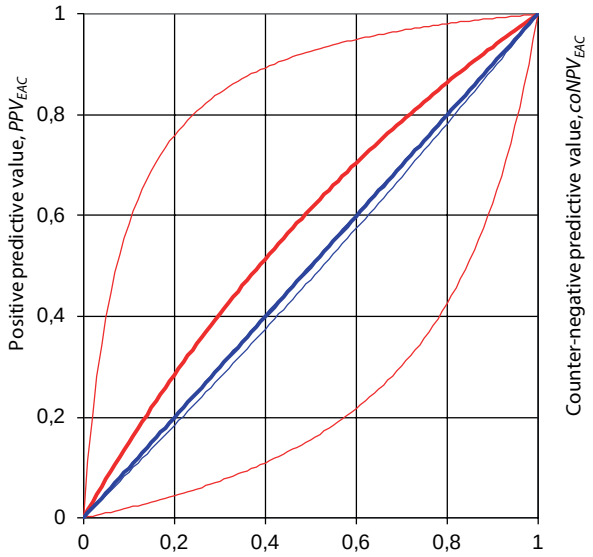

FIG. 8.
PPV_{EAC} and $coNPV_{EAC}$ versus $Prev_{CHD}$ diagram: EAC – excessive alcohol consumption; CHD – coronary heart disease

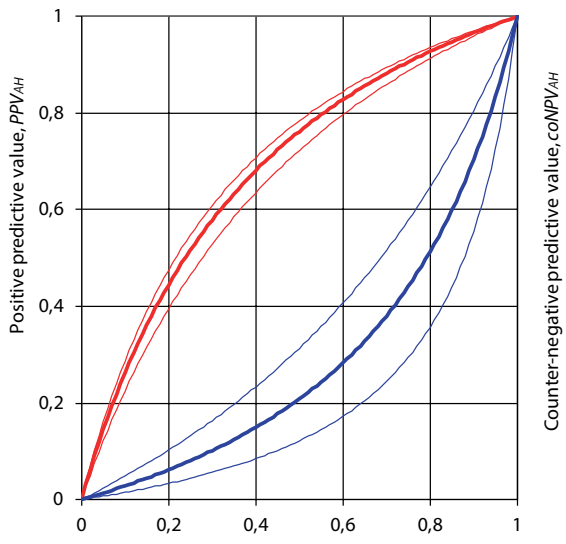

FIG. 9.
PPV_{AH} and $coNPV_{AH}$ versus PrevCHD diagram: AH – arterial hypertension; CHD – coronary heart disease

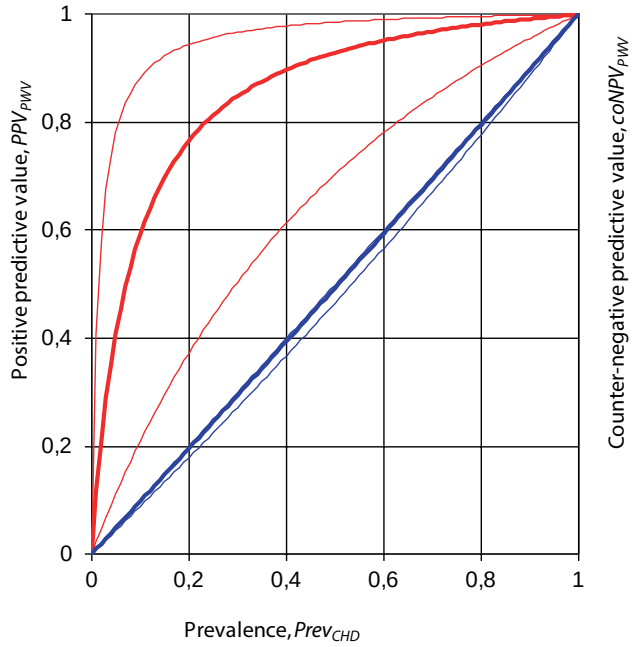

FIG. 10.
PPV_{PWV} and coNPVPWV versus $Prev_{CHD}$ diagram: PWV – pulse wave velocity; CHD – coronary heart disease

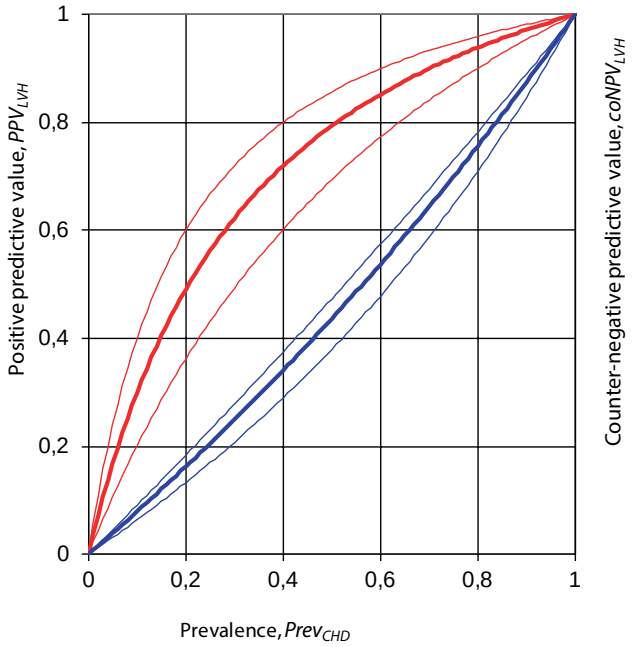

FIG. 12.
PPV_{LVH} and coNPV$_{LVH}$ versus $Prev_{CHD}$ diagram: LVH – left ventricular hypertrophy; CHD – coronary heart disease

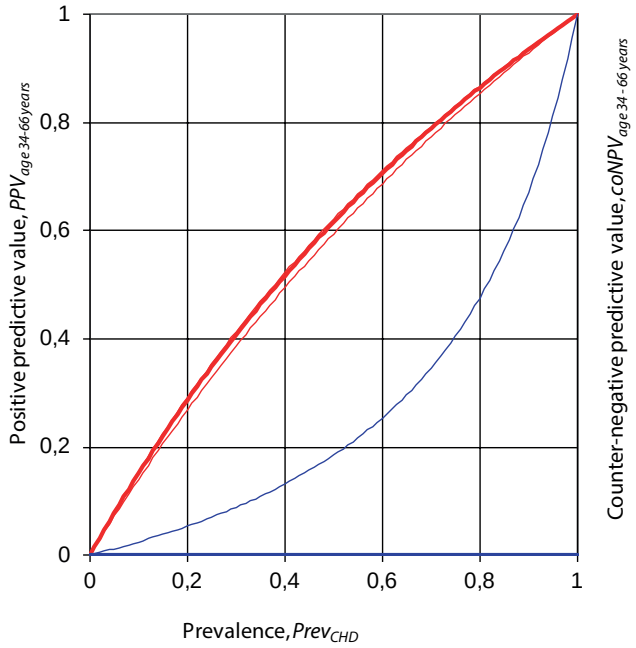

FIG. 11.
$PPV_{age\ 34–66}$ and coNPV$_{age\ 34–66}$ versus $Prev_{CHD}$ diagram: CHD – coronary heart disease

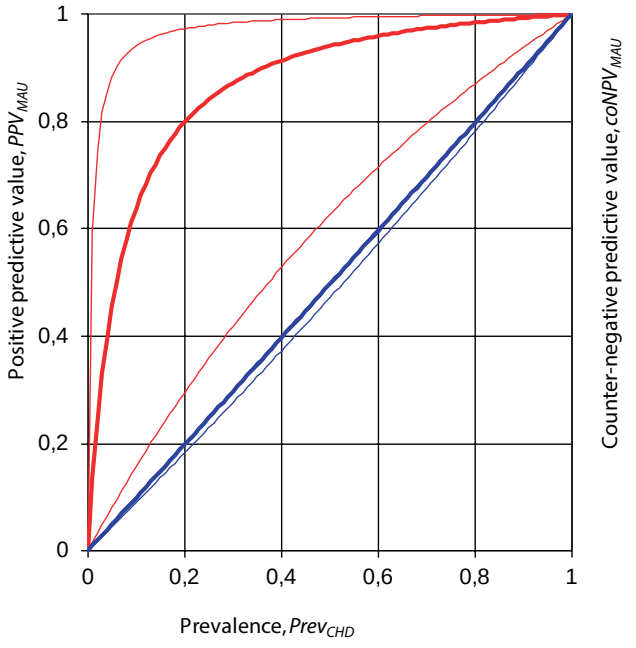

FIG. 13.
PPV_{MAU} and coNPV$_{MAU}$ versus $Prev_{CHD}$ diagram: MAU –microalbuminuria; CHD – coronary heart disease

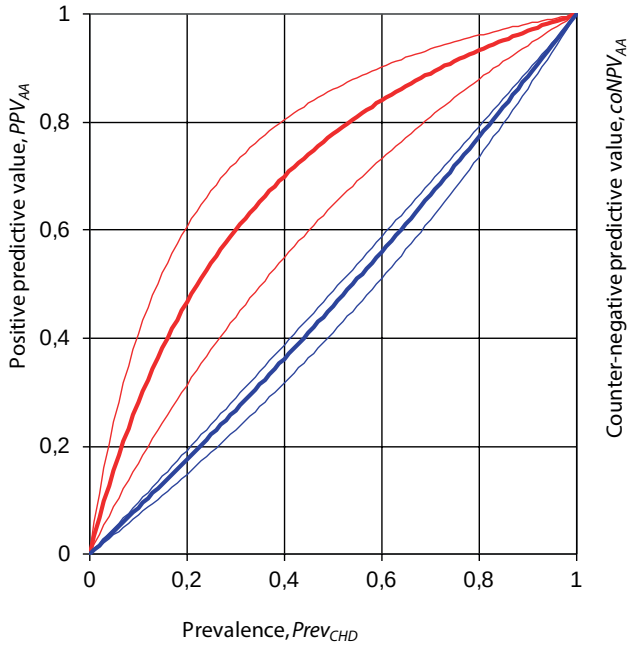

FIG. 14.
PPV_{AA} and $coNPV_{AA}$ versus $Prev_{CHD}$ diagram: AA – atherosclerosis of aorta; CHD – coronary heart disease

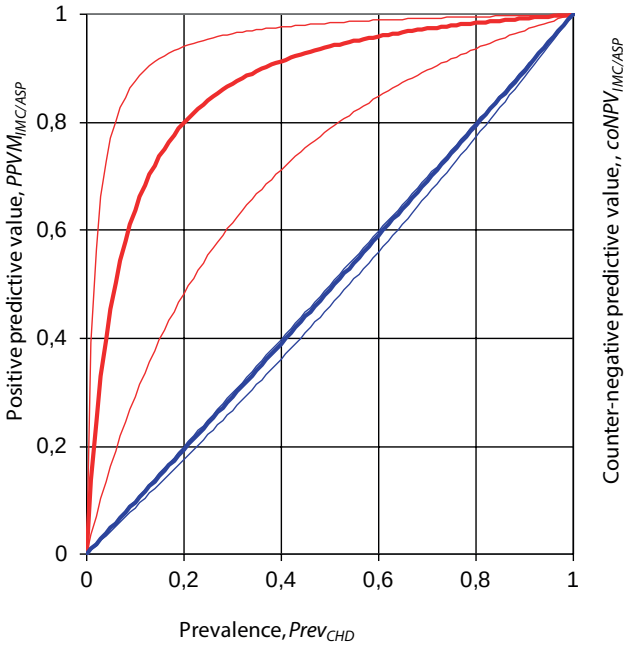

FIG. 16.
$PPV_{IMC/ASP}$ and $coNPV_{IMC/ASP}$ versus $Prev_{CHD}$ diagram: IMC – intima-media thickness; ASP – atherosclerotic plaque; CHD – coronary heart disease

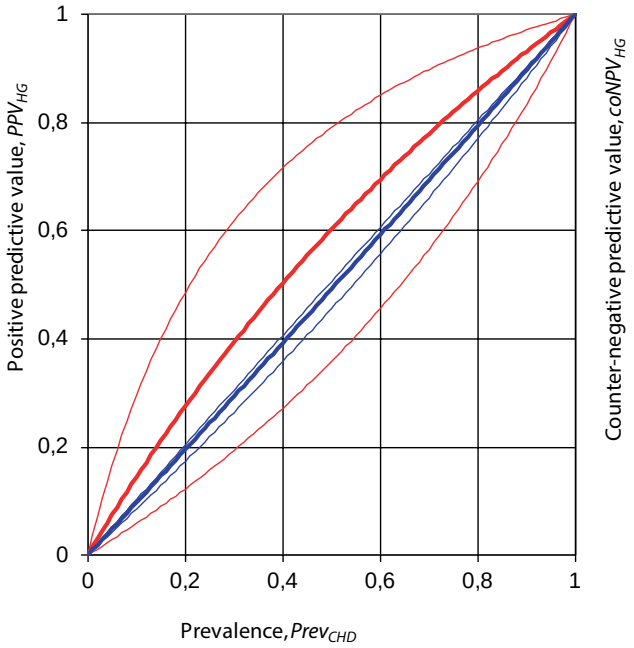

FIG. 15.
PPV_{HG} and $coNPV_{HG}$ versus $Prev_{CHD}$ diagram: HG – hyperglycemia; CHD – coronary heart disease

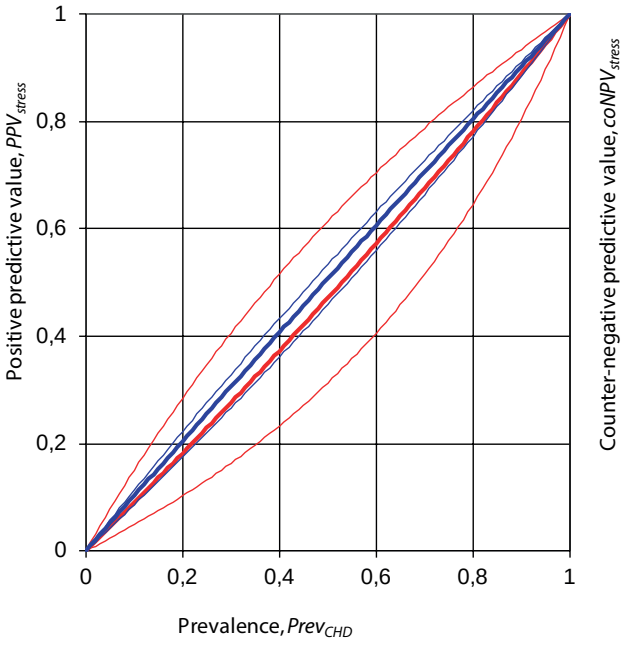

FIG. 17.
PPV_{stress} and $coNPV_{stress}$ versus $Prev_{CHD}$ prevalence: CHD – coronary heart disease

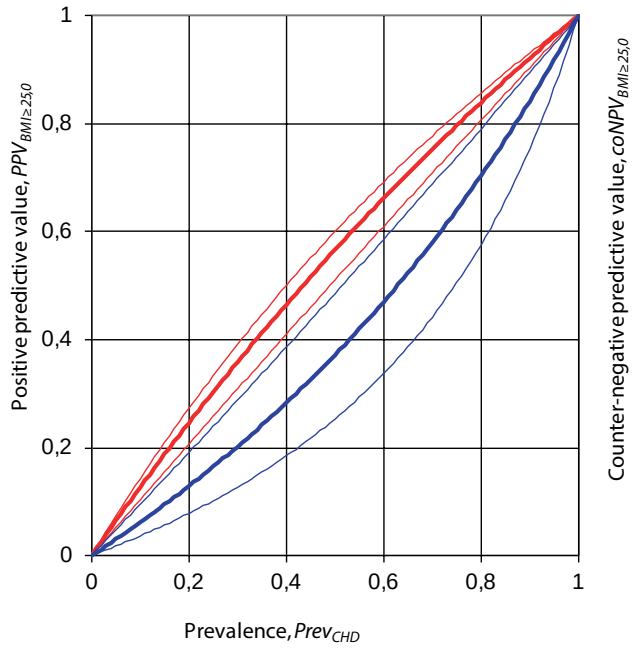

FIG. 18.
$PPV_{BMI \geq 25,0}$ and $coNPV_{BMI \geq 25,0}$ versus $Prev_{CHD}$ diagram: BMI – body mass index; CHD – coronary heart disease

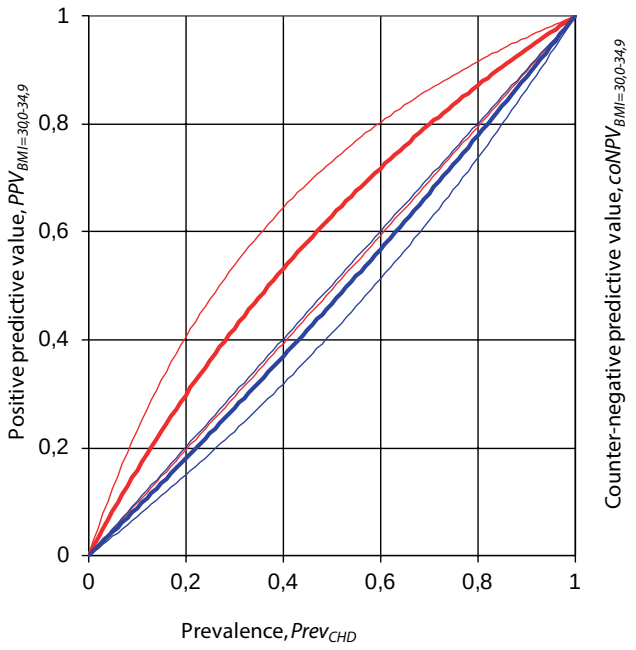

FIG. 20.
$PPV_{BMI = 30,0–34,9}$ and $coNPV_{BMI = 30,0–34,9}$ versus $Prev_{CHD}$ diagram: BMI – body mass index; CHD – coronary heart disease

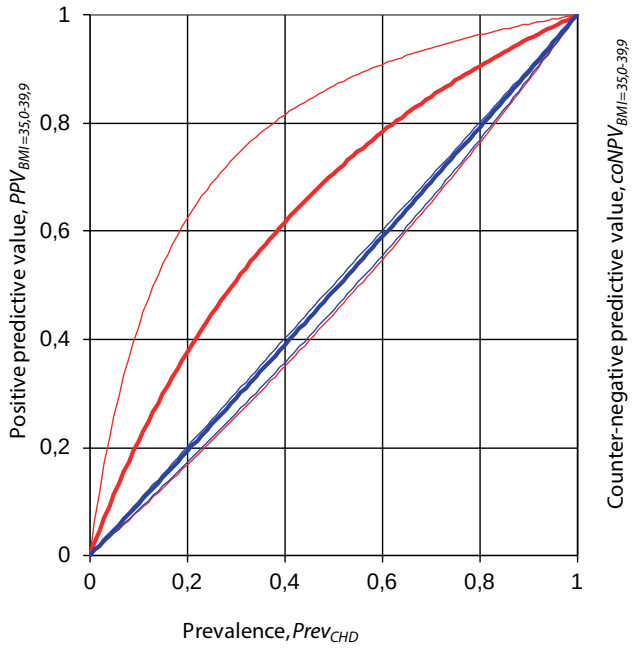

FIG. 19.
$PPV_{BMI = 35,0–39,9}$ and $coNPV_{BMI = 35,0–39,9}$ versus PrevCHD diagram: BMI – body mass index; CHD – coronary heart disease

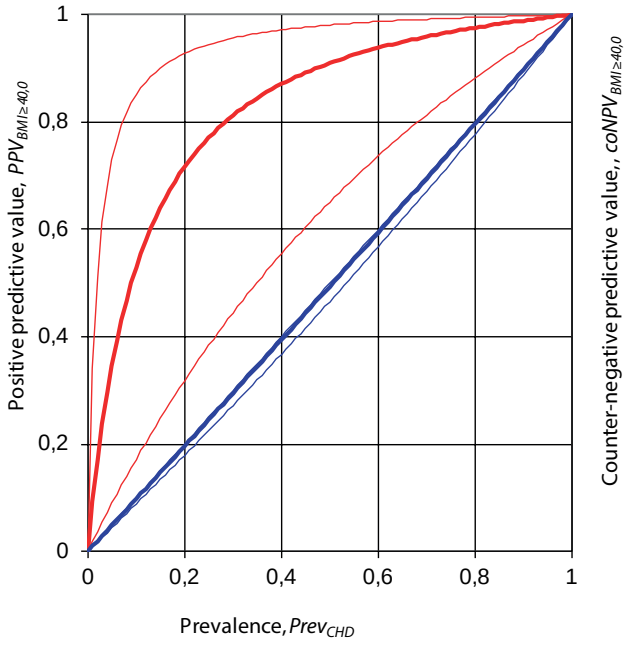

FIG. 21.
$PPV_{BMI \geq 40,0}$ and $coNPV_{BMI \geq 40,0}$ versus PrevCHD diagram: BMI – body mass index; CHD – coronary heart disease

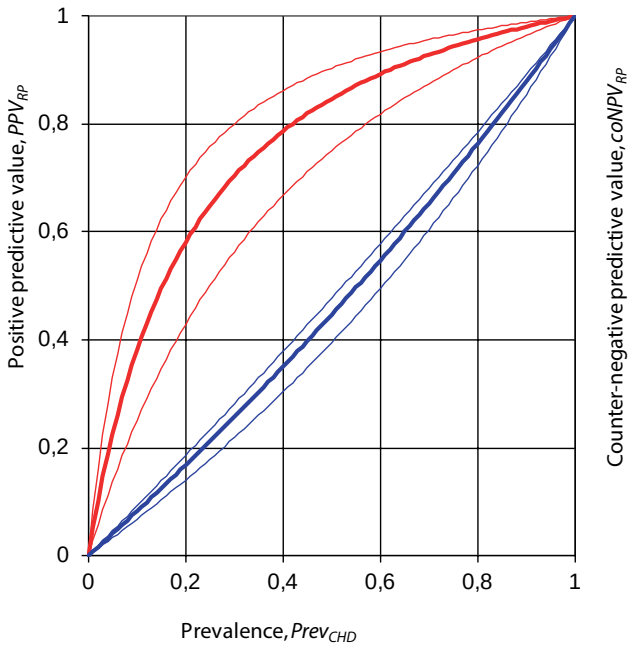

FIG. 22.
PPV_{RP} and $coNPV_{RP}$ versus $Prev_{CHD}$ diagram: RP – retinopathy; CHD – coronary heart disease

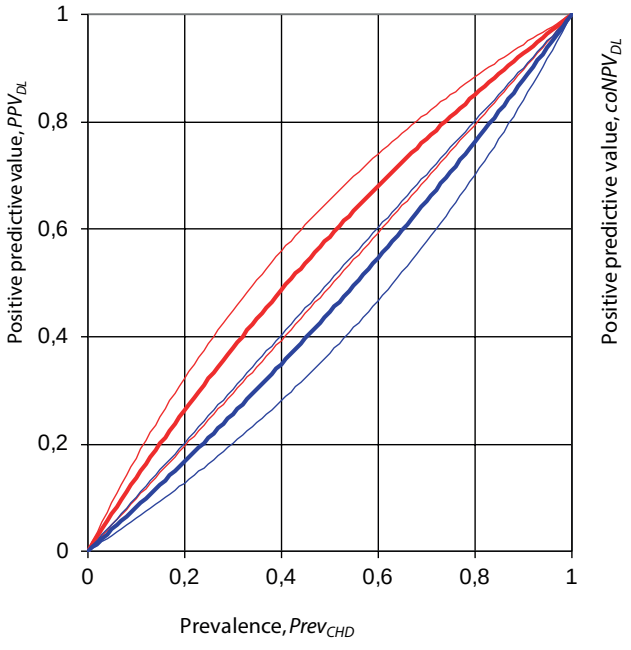

FIG. 23.
PPV_{DL} and $coNPV_{DL}$ versus $Prev_{CHD}$ diagram: DL – dyslipidemia; CHD – coronary heart disease

CONCLUSION

CHD predictors have high and moderate specificity in relation to the absence of CHD occurrence in individuals with a negative result for the presence of factors of this disease. When assessing the CHD probability, one should focus on the probability of both occurrence and absence of the outcome in the presence or absence of a factor of this disease in a patient and consider only estimates that are statistically significant. The obtained data can be used in the formation of the selection (medical screening) of safety-critical operator occupations with increased medical requirements for candidates, for example, LCW, driving a locomotive "alone" without an assistant driver. Modifiable risk factors of the disease should be modified in order to improve the medical indicators of the employee. When recruiting employees, all factors of the disease should be taken into account. Predictors of IMC/ASP, MAU, PWV > 12 m/s, BMI > 40.0, unlike other CHD predictors, statistically significantly moderately increase the a posteriori odds in favor of the CHD occurrence against its absence in comparison with the a priori chances after receiving a positive VDT result in a patient. The predictor age in the range of 34–66 years statistically significantly moderately increases the posterior odds in favor of the CHD absence versus its occurrence in comparison with the prior odds after the patient receives a negative VDT result.

Since the definition of predictors PWV > 12 m/s, AA, MAU, EAC, stress in LCWs is not mandatory provided for by regulatory documents, it is necessary to conduct a targeted search for them, especially if EAC screening is implemented among LCWs in order to prevent other CVDs, a predictor of which is this factor [12, 33, 34]. EAC test: latent daily single alcohol consumption above the WHO safe limit, i.e. more than 2 standard doses of alcohol per day with 1 dose of 13.7 g (18 ml of ethanol), is detected by performing the diagnostic algorithm for chronic alcohol intoxication [12, 35]. Other CHD predictors in LCWs should also be identified in order to carry out therapeutic and preventive measures for workers exposed to these factors in view of the available diagnostic methods and in order to prevent their cumulation in the population and in a specific worker.

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Conflicts of interest
No potential conflict of interest relevant to this article reported.

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TRAUMATOLOGY

MICROBIOLOGICAL PROFILE OF PATIENTS WITH ORTHOPEDIC IMPLANT-ASSOCIATED INFECTION IN THE POST-COVID PERIOD

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ABSTRACT

Background. *The etiological structure of implant-associated infection and antibiotic resistance of pathogens are important when choosing empirical antibiotic therapy. COVID-19 pandemic and increased consumption of antibiotics by the population could provoke an increase in antibiotic resistance.*
The aim of the work. *To compare the spectrum of leading pathogens of implant-associated infection in the pre- and post-Covid period and to assess antibiotic resistance.*
Materials and methods. *A continuous retrospective study of biomaterial samples from traumatology and orthopedic patients with implant-associated infection was carried out for 2018–2019 and 2021–2022. The sample consisted of 548 microorganism strains (n = 237 and n = 317, respectively) in 442 cases of infectious complications. The antibiotic resistance of all isolated microorganisms, including those from microbial associations, was assessed.*
Results. *The leading pathogen of monomicrobial implant-associated infection in both study periods was Staphylococcus epidermidis (33–37 %). In 2021–2022, the proportion of microbial associations increased (from 12.5 to 17.5 %; p = 0.147) with the appearance of fungi in the microbial landscape. In the post-Covid period, the increase in Staphylococcus aureus resistance to tetracycline and doxycycline was revealed; the isolation of methicillin-resistant strains among Staphylococcus aureus decreased from 4 cases (out of 187) to 3 (out of 232); 100 % sensitivity to rifampicin and co-trimoxazole was maintained. An increase in Staphylococcus epidermidis resistance to all tested antibiotics was detected (statistically significant increase in resistance to fluoroquinolones; p = 0.002–0.003) with the isolation of methicillin-resistant strains in 80.5 % and 80.9 % of cases, respectively. All staphylococcal isolates were susceptible to vancomycin and linezolid. Enterobacteriaceae representatives showed a decrease in resistance to carbapenems and an increase in resistance to co-trimoxazole; in Pseudomonas aeruginosa and Acinetobacter baumannii, there is an increase in resistance to carbapenems and fluoroquinolones. All gram-negative microorganisms were sensitive to colistin.*
Conclusion. *The high frequency of isolation of methicillin-resistant staphylococci determines the choice of vancomycin for empirical therapy. Increasing resistance of staphylococci to fluoroquinolones may limit their use. Increasing resistance of gram-negative bacteria and a narrow spectrum of antibiotics acting on carbapenemase producers may reduce the effectiveness of therapy.*

Key words: *implant-associated infection, periprosthetic infection, antibiotic resistance, COVID-19, Staphylococcus epidermidis, microbial associations, carbapenemase producers, methicillin-resistant staphylococci*

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МИКРОБИОЛОГИЧЕСКИЙ ПРОФИЛЬ ПАЦИЕНТОВ С ОРТОПЕДИЧЕСКОЙ ИМПЛАНТАТ-АССОЦИИРОВАННОЙ ИНФЕКЦИЕЙ В ПОСТКОВИДНОМ ПЕРИОДЕ

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РЕЗЮМЕ

Введение. *Этиологическая структура имплантат-ассоциированной инфекции и антибиотикорезистентность патогенов важны при выборе эмпирической антибиотикотерапии. Пандемия COVID-19, увеличение потребления населением антибиотиков могли провоцировать рост антибиотикорезистентности.*

Цель работы. *Сравнить спектр ведущих возбудителей имплантат-ассоциированной инфекции в до- и постковидном периоде с оценкой антибиотикорезистентности.*

Материалы и методы. *Проведено сплошное ретроспективное исследование образцов биоматериала пациентов травматолого-ортопедического профиля с имплантат-ассоциированной инфекцией за 2018–2019 и 2021–2022 гг. Выборка составила 548 штаммов микроорганизмов (n = 237 и n = 317 соответственно) в 442 случаях инфекционных осложнений. Проводилась оценка антибиотикорезистентности всех выделенных микроорганизмов, в том числе из микробных ассоциаций.*

Результаты. *Ведущим возбудителем мономикробной имплантат-ассоциированной инфекции в оба периода исследования был Staphylococcus epidermidis (33–37 %). В 2021–2022 гг. увеличилась доля микробных ассоциаций (с 12,5 до 17,5 %; p = 0,147) с появлением в микробном пейзаже грибов. В постковидном периоде отмечен рост резистентности Staphylococcus aureus к тетрациклину и доксициклину; выделение метициллин-резистентных штаммов среди Staphylococcus aureus снизилось с 4 случаев (из 187) до 3 (из 232); сохранялась 100%-я чувствительность к рифампицину и ко-тримоксазолу. Выявлен рост резистентности Staphylococcus epidermidis ко всем тестируемым антибиотикам (статистически значимый – к фторхинолонам; p = 0,002–0,003) с выделением метициллин-резистентных штаммов в 80,5 % и 80,9 % случаев соответственно. Все выделенные изоляты стафилококков были чувствительны к ванкомицину и линезолиду. У представителей семейства Enterobacteriaceae выявлено снижение резистентности к карбапенемам и её рост к ко-тримоксазолу; у Pseudomonas aeruginosa и Acinetobacter baumannii – рост резистентности к карбапенемам и фторхинолонам. Все грамотрицательные микроорганизмы были чувствительны к колистину.*

Заключение. *Высокая частота выделения метициллин-резистентных стафилококков определяет выбор ванкомицина для эмпирической терапии. Рост резистентности стафилококков к фторхинолонам может способствовать ограничению их использования. Рост резистентности грамотрицательных бактерий, узкий спектр антибиотиков, действующих на карбапенемазопродуцентов, могут снижать эффективность терапии.*

Ключевые слова: *имплантат-ассоциированная инфекция, перипротезная инфекция, антибиотикорезистентность, COVID-19, Staphylococcus epidermidis, микробные ассоциации, карбапенемазопродуценты, метициллин-резистентные стафилококки*

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INTRODUCTION

In the last decade, medicine has seen a trend towards increasing the use of various implants during surgical interventions. Despite the constant improvement of the biomechanical properties of structures, the development of implant-associated infections (IAI) remains a pressing issue.

This term implies a broad concept, including the development of infection in the area of installation of any implants, including orthopedic ones. Their presence in the body leads to the emergence of a lifelong risk of infection. The frequency of infectious complications varies depending on the type of surgical intervention: after primary endoprosthetics of the hip or knee joint, it is low and amounts to 0.3–2 %; after revision endoprosthetics, it increases to 20 % [1–4]; after osteosynthesis of fractures, it ranges from 1.8 to 27 % [5–9]; after external transpedicular fixation of the spine, it occurs in 0.7–20 % of cases [10].

According to a study conducted by the R.R. Vreden National Medical Research Center of Traumatology and Orthopedics of the Ministry of Health of the Russian Federation (St. Petersburg) in 2018, a decrease in the frequency of *Staphylococcus aureus* isolation was noted in the structure of the leading gram-positive pathogens of IAI – from 34.5 % in 2012-2013 to 28.6 % in 2016-2017 – with its leading position maintained and a parallel increase in the share of *Staphylococcus epidermidis* (from 18.4 % to 22.5 %), however, the increase in the isolation frequency of methicillin-resistant strains was insignificant [11].

The prevalence of pathogens causing infectious complications and their resistance to antibiotics in healthcare facilities may vary. In cases where the pathogen has not yet been verified, local epidemiological data can be used to determine the optimal tactics for choosing empirical antibacterial therapy, influencing the success of treatment. According to foreign authors, the recent COVID-19 pandemic has created a predisposition to the development of concomitant diseases and coinfections in those who have recovered, which may be a manifestation of the immune burden that this virus creates for the host [12]. Increased consumption of antibacterial drugs by the population could also change the microbiological etiology of IAI and provoke an increase in antibiotic resistance.

THE AIM OF THE STUDY

To compare the spectrum of leading pathogens of implant-associated infection in the pre- (2018–2019) and post-COVID (2021–2022) period and to assess antibiotic resistance.

MATERIALS AND METHODS

A continuous retrospective comparative study was conducted at the Federal Center for Traumatology, Orthopedics and Endoprosthetics of the Ministry of Health of Russian Federation (Cheboksary).

A comparative analysis of changes in the spectrum of pathogens of intravascular inflammatory infections and antibiotic resistance was conducted in 2018–2019 and 2021–2022. The inclusion criterion in the study was the pathogen detection in a microbiological study of 4–6 samples of biomaterial from each patient (intraoperative tissue biopsies, aspirate from removed metal structures after ultrasonic treatment, synovial fluid) obtained intraoperatively and in outpatient settings in the presence of a deep or superficial infection of the surgical site. One, the most informative, of the 4–6 results was included in the analysis. Pathogens in microbial associations were not included in the analysis of monomicrobial infection.

The sample size was 548 isolated strains of microorganisms (n = 237 in 2018–2019 and n = 317 in 2021–2022) in 442 cases of infectious complications (n = 208 and n = 234, respectively).

The majority of subjects were female (52.8 %) with an average age of 58.5 ± 13.6 years (4–88 years; over 65 years – 37.5 %). According to localization, deep and superficial IAI was detected after arthroplasty – in 70.4 % (n = 311), after reconstructive plastic surgery on bones, joints (in 17.6 % of cases; n = 79) and spine (12 % of cases; n = 53).

The crops were incubated for up to 14 days with the creation of conditions for the cultivation of aerobes, anaerobes, capnophiles and fungi. Species identification of pathogens with sensitivity determination to antibacterial drugs was performed on an automatic bacteriological analyzer Vitec 2 Compact (Bio Merieux, France) and on a semi-automatic analyzer Multiskan FC (Thermo Fisher Scientific, USA) using kits and test systems Erba Lachema (Czech Republic).

Antibiotic susceptibility testing was performed in accordance with clinical guidelines[1, 2]. The disk diffusion method was used when there were criteria for assessing the inhibition zones of pathogen growth. Susceptibility determination of gram-negative bacteria to colistin and staphylococci to doxycycline and vancomycin was determined by serial dilutions in Mueller – Hinton broth or using the E-test. When resistant strains were identified by the disk diffusion method, the results were confirmed by the same methods. Despite the changes in the EUCAST (European Committee on Antimicrobial Susceptibility Testing) 2021 criteria for assessing the diameter of the inhibition zones of microorganisms with the transition from category S (susceptible) to category I (susceptible with increased exposure), we classified cases of sensitivity I of staphylococci

1 Antimicrobial susceptibility testing: clinical guidelines (version 2018-03). 2018. URL: https://www.antibiotic.ru/files/321/clrec-dsma2018.pdf [date of access: November 02, 2023].

2 Antimicrobial susceptibility testing: clinical guidelines (version 2021-01). 2021. URL: https://www.antibiotic.ru/files/321/clrec-dsma2021.pdf [date of access: November 02, 2023].

to ciprofloxacin and levofloxacin, enterobacteria – to imipenem to category S.

To obtain the production of extended emission beta-lactamases (ESBL) in the *Enterobacteriaceae* family isolates, the "double disk" method and the electronic cefazidime/clavulanate test were used. Identification of carbapenemase-producing isolates of gram-negative blasts occurs using the double disk method with ethylenediaminetetraacetic acid (EDTA) and the Hodge test.

Microbial associations included cases of isolation of more than one type of microorganism in the studied biomaterials. Antibiotic resistance was assessed taking into account all isolated pathogens, including pathogens in microbial associations. A positive result of growth of the same microorganism in two or more samples was considered diagnostically significant. The microbiological study results of the biomaterial from the fistula tract were not analyzed separately in the work.

Among gram-positive and gram-negative pathogens, an antibiotic resistance analysis of the most significant isolates (with an isolation frequency of more than 4 %) was conducted.

Statistical data processing

The sample size was determined by the type of study (continuous). The obtained data were recorded in the form of spreadsheets, the data structure visualization and data analysis were carried out using MS Office Excel 2007 (Microsoft Corp., USA). The distribution normality was determined using Kolmogorov – Smirnov test. For the categorical data analysis in the GraphPad program (GraphPad Software, USA), Fisher's exact test was used to check the relationship ($p < 0.05$). Quantitative data were assessed using frequency distribution analysis (in percent).

RESULTS

The leading causative agent of monomicrobial IAI in both study periods was *Staphylococcus epidermidis* – 37 % in 2018–2019, and 33 % in 2021–2022 (fig. 1).

There are no significant changes in the structure of IAI pathogens during the studied periods, however, an increase in the proportion of microbial associations was found – from 12.5 to 17.5 % ($p = 0.147$) – with the fungi appearance in the microbial landscape in 2021–2022.

The sample size of microbial associations was 173 isolates (49 isolates in 2018–2019, 124 isolates in 2021–2022). The most common pathogens among gram-positive microorganisms were corynebacteria (18.2 %), isolated in two or more biomaterial samples, *Staphylococcus aureus* (17.5 %) and MRSE (12.4 %); among gram-negative bacteria – *Acinetobacter baumannii* (8.7 %) and *Enterobacter cloacae* (8.0 %). At the same time, about 2/3 of cases were associations of two microorganisms ($n = 48$) and 1/3 were associations of three or more microorganisms ($n = 19$). Microbial associations were isolated from intraoperative biomaterial in 92.3 % of cases in patients with fistula infection.

The microbial spectrum, including pathogens of poly- and monomicrobial infections, is represented mainly by gram-positive bacteria with an isolation frequency of 81.0 % (2018–2019) and 73.2 % (2021–2022), respectively ($p = 0.041$) (table 1).

Despite the continuing leading role of gram-positive microorganisms in the study periods (in particular, *Staphylococcus epidermidis*), in 2021–2022 a tendency was revealed towards an increase in the number of gram-negative pathogens from 12.1 % to 16.1 % ($p = 0.219$) and other microorganisms from 6.9 % to 10.7 % ($p = 0.136$) with a decrease in the proportion of gram-positive pathogens from 81.0 % to 73.2 % ($p = 0.041$). Among

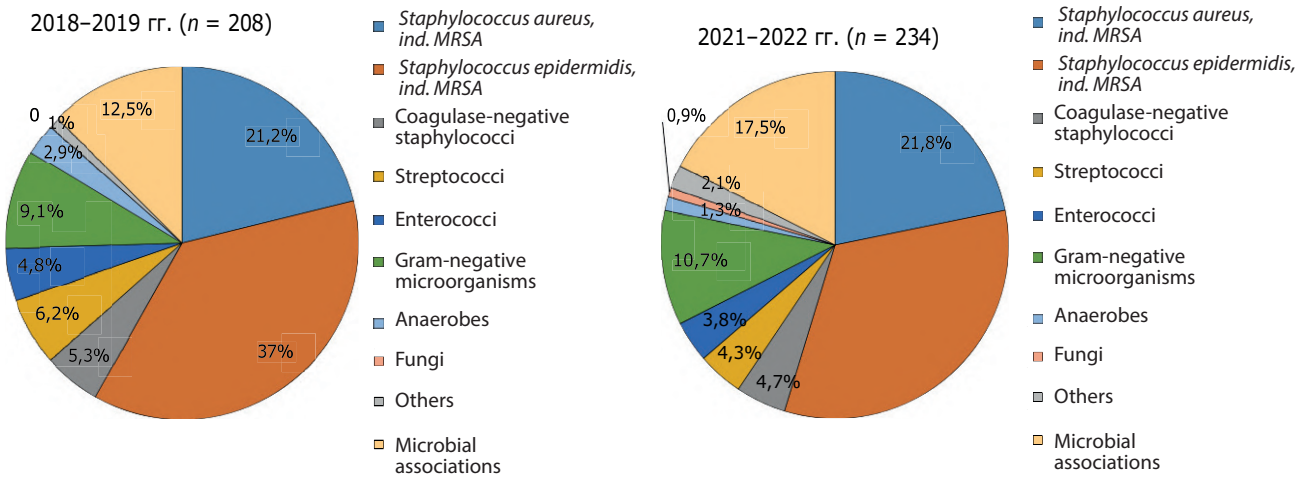

FIG. 1.
The structure of culture-positive implant-associated infection

TABLE 1

THE NUMBER OF ISOLATES OF POLY- AND MONOBACTERIAL INFECTIONS, 2018–2019 AND 2021–2022

Types of microorganisms	2018–2019, *N* (%)	2021–2022, *N* (%)	*p*
Gram-positive microorganisms	**187 (81.0)**	**232 (73.2)**	0.041
Staphylococcus aureus MSSA	51 (22.0)	70 (22.1)	1.000
Staphylococcus aureus MRSA	4 (1.7)	3 (0.9)	0.462
Staphylococcus epidermidis MSSE	17 (7.4)	18 (5.7)	0.481
Staphylococcus epidermidis MRSE	70 (30.3)	76 (24.0)	0.117
Staphylococcus lugdunensis	1 (0.4)	12 (3.8)	0.010
Staphylococcus capitis	1 (0.4)	4 (1.3)	0.403
Staphylococcus hominis	0 (0.0)	3 (0.9)	0.267
Staphylococcus haemolyticus	8 (3.5)	3 (0.9)	0.060
Staphylococcus caprae, Staphylococcus warneri, Staphylococcus xylosus	3 (1.3)	5 (1.6)	1.000
Streptococcus spp.	13 (5.6)	25 (7.9)	0.395
Enterococcus faecalis	18 (7.8)	11 (3.5)	0.033
Enterococcus facium	1 (0.4)	2 (0.6)	1.000
Gram-negative microorganisms	**28 (12.1)**	**51 (16.1)**	0.219
*Burkholderia cepacia**	10 (4.3)	5 (1.6)	0.064
Pseudomonas aeruginosa	4 (1.7)	6 (1.9)	1.000
Achromobacter xylosoxidans	1 (0.4)	2 (0.6)	1.000
Acinetobacter baumannii	5 (2.2)	8 (2.5)	1.000
Escherichia coli	1 (0.4)	10 (3.2)	0.029
Enterobacter cloacae	5 (2.2)	11 (3.5)	0.448
Klebsiella pneumoniae	1 (0.4)	5 (1.6)	0.322
Klebsiella oxytoca	1 (0.4)	1 (0.3)	1.000
Citrobacter braakii	0 (0.0)	1 (0.3)	1.000
Proteus mirabilis	0 (0.0)	1 (0.3)	1.000
Serratia marcescens	0 (0.0)	1 (0.3)	1.000
Others	**16 (6.9)**	**34 (10.7)**	0.136
Anaerobes	6 (2.6)	13 (4.1)	0.479
Corynebacterii	8 (3.5)	16 (5.0)	0.406
Candida albicans	0 (0.0)	2 (0.6)	0.511
Micrococcus spp.	1 (0.4)	1 (0.3)	1.000
Macrococcus caseolyticus	0 (0.0)	1 (0.3)	1.000
Listeria spp.	1 (0.4)	1 (0.3)	1.000
Total	**231 (100)**	**317 (100)**	

Note. * – the pathogen was isolated from patients who had undergone primary surgery in the same medical facility in another region.

gram-negative pathogens, *Burkholderia cepacia* was the leader in 2018–2019, and *Enterobacter cloacae* was the leader in 2021–2022.

In the post-COVID period, an increase in the number of *Staphylococcus aureus* resistant to tetracycline and doxycycline and an increase in sensitivity to fluoroquinolones and gentamicin were recorded; the isolation frequency of methicillin-resistant strains decreased from 4 cases (out of 187) to 3 (out of 232); 100 % sensitivity to rifampicin and cotrimoxazole remained. The isolation frequency of methicillin-resistant *Staphylococcus epidermidis* remained: 80.5 % in 2018-2019 and 80.9 % in 2021-2022. An increase in *Staphylococcus epidermidis* resistance to all tested antibiotics was revealed, including a statistically significant increase in resistance to fluoroquinolones (p = 0.002–0.003). Among coagulase-negative staphylococci in 2018–2019, resistance to methicillin was determined in 64.5 %, in 2021–2022 – in 81.0 %. All isolated staphylococcal strains remained sensitive to vancomycin and linezolid (table 2).

In the structure of gram-negative microorganisms in the post-COVID period, the frequency of *Escherichia coli* detection increased from 0.4 % to 3.2 % (p = 0.029), *Klebsiella pneumoniae* – from 0.4 % to 1.6 %, *Enterobacter cloacae* – from 2.2 % to 3.5 %. In 2018-2019, one case of *Klebsiella pneumoniae* and *Escherichia coli* was recorded, and if the first pathogen was characterized by a multidrug-resistant resistance phenotype, the second was sensitive to all tested antibiotics. In the post-COVID period, the *Enterobacteriaceae* family representatives in most cases were producers of extended-spectrum beta-lactamases with increasing resistance to co-trimoxazole. *Enterobacter cloacae* remained sensitive to fluoroquinolones, *Escherichia coli* and *Klebsiella pneumonia* were resistant from 40 to 80 %. *Enterobacter cloacae* and *Escherichia coli* remained sensitive to carbapenems and aminoglycosides in both study periods (table 3).

In the post-COVID period, an increase in resistance of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* to carbapenems and fluoroquinolones was noted. *Acinetobacter baumannii* isolates, previously sensitive to amikacin, acquired 57.1 % resistance to aminoglycosides (p = 0.018). All gram-negative microorganisms were sensitive to colistin (fig. 2, 3).

The identified increase in antibiotic resistance to fluoroquinolones may indicate unfavorable trends and further contribute to restrictions on the prescription of ciprofloxacin oral forms for long-term use.

TABLE 2

THE RESULTS OF ANTIBIOTIC RESISTANCE OF *STAPHYLOCOCCUS AUREUS* AND *STAPHYLOCOCCUS EPIDERMIDIS*, 2018–2019 AND 2021–2022

Medications	*Staphylococcus aureus* (n = 128), %			*Staphylococcus epidermidis* (n = 181), %		
	2018–2019 (n = 55)	2021–2022 (n = 73)	p	2018–2019 (n = 87)	2021–2022 (n = 94)	p
Cefoxitin	7.3	4.1	0.462	80.5	80.9	1.000
Gentamicin	12.7	4.1	0.098	32.2	42.6	0.169
Erythromycin	21.8	17.8	0.654	32.2	45.7	0.069
Clindamycin	18.2	17.8	1.000	28.7	27.8	1.000
Ciprofloxacin	10.9	5.5	0.325	29.9	52.1	0.003*
Levofloxacin	10.9	5.5	0.325	29.9	52.1	0.003*
Moxifloxacin	9.1	4.1	0.288	28.7	52.1	0.002*
Tetracycline	21.8	28.8	0.419	34.5	28.7	0.427
Fusidic acid	0	2.7	0.506	5.7	9.6	0.410
Doxycycline	1.8	11.0	0.077	13.8	20.2	0.324
Vancomycin	0	0	1.000	0	0	1.000
Rifampicin	1.8	0	0.430	2.3	8.5	0.102
Co-trimoxazole	1.8	0	0.430	16.1	26.6	0.104
Linezolid	0	0	1.000	0	0	1.000

Note. * – the differences are statistically significant at $p < 0.05$.

TABLE 3

ANTIBIOTIC RESISTANCE OF *KLESIELLA PNEUMONIA, ENTEROBACTER CLOACAE, ESCHERICHIA COLI*, 2018–2019 AND 2021–2022

Medications	*Klesiella pneumoniae* (*n* = 6)			*Enterobacter cloacae* (*n* = 16)			*Escherichia coli* (*n* = 11)		
	2018–2019 (*n* = 1)	2021–2022 (*n* = 5)	*p*	2018–2019 (*n* = 5)	2021–2022 (*n* = 11)	*p*	2018–2019 (*n* = 1)	2021–2022 (*n* = 10)	*p*
Ceftazidime	100	100	1.000	40	72.7	0.293	0	60.0	1.000
Ceftriaxone	100	100	1.000	40	72.7	0.293	0	60.0	1.000
Cefepime	100	100	1.000	40	72.7	0.293	0	60.0	1.000
Imipenem	100	40	1.000	0	0	1.000	0	0	1.000
Meropenem	100	60	1.000	0	0	1.000	0	0	1.000
Amikacin	100	60	1.000	0	0	1.000	0	0	1.000
Tobramycin	100	60	1.000	0	0	1.000	0	0	1.000
Ciprofloxacin	100	80	1.000	0	0	1.000	0	40.0	1.000
Levofloxacin	100	80	1.000	0	0	1.000	0	40.0	1.000
Co-trimoxazole	0	80	1.000	40	62.5	0.592	0	40.0	1.000
Aztreonam	100	75	1.000	20	33.3	1.000	0	40.0	1.000
Colistin	0	0	1.000	0	0	1.000	0	0	1.000

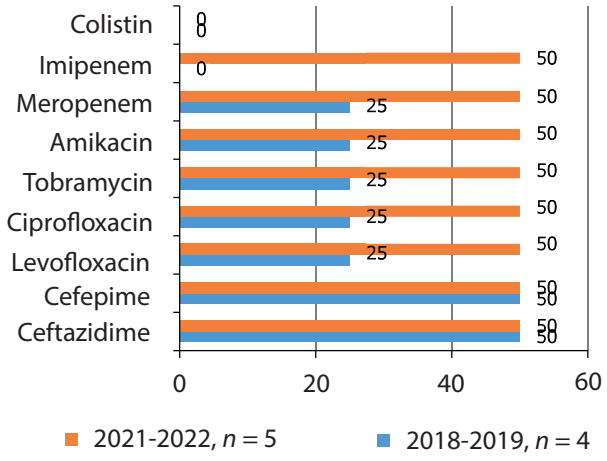

FIG. 2.
Antibiotic resistance of Pseudomonas aeruginosa, 2018–2019 and 2021–2022

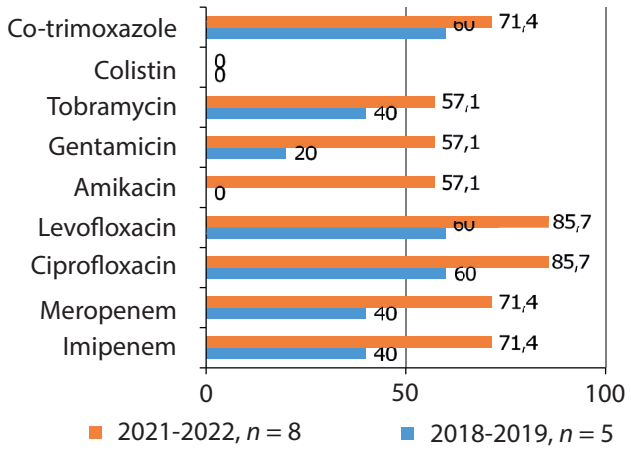

FIG. 3.
Antibiotic resistance of Acinetobacter baumannii, 2018–2019 and 2021–2022

DISCUSSION

Over the past four decades, there has been an increase in the number of antibiotic-resistant bacterial pathogens [13].

The study by F.S. Fröschen et al. (2022) shows that the most common causative agent of orthopedic implant-associated infection is coagulase-negative staphylococci, which could be detected in 44.61 % of cases, followed by *Staphylococcus aureus* (14.31 %) and enterococci (9.01 %) [3]. Our study confirms the leading role of *Staphylococcus epidermidis* in the IAI etiology with a detection rate of 37 % (2018–2019) and 33 % (2021–2022). Similar data were obtained by other foreign researchers – D.B.G. Tai et al. (2022) – for the observation period from 2010 to 2019 (2067 episodes of infection in 1651 patients), where it was demonstrated that coagulase-negative staphylococci (except *Staphylococcus lugdunensis*) were the leaders in the etiology of implant-associated infection (37 %; *n* = 761) [14].

In the work of B.T. Bjerke-Kroll et al. (2014), *Staphylococcus aureus* was indicated as the leading causative agent of IAI [15]. Similar data were published in the periprosthetic joint infection study by Y. Tsai et al. (2006–2014; *n* = 294), where the leading role in the development of hip and knee joint infection with a detection rate of up to 27 % (methicillin resistance – 21 %) belonged to *Staphylococcus aureus* [16], however, we could not confirm these results. Our study showed that in 2020–2021, only 4.1 % of *Staphylococcus aureus* isolates demonstrated methicillin resistance. The study, conducted by S.A. Bozhkova et al. (2018) at the R.R. Vreden National Medical Research Center of Traumatology and Orthopedics of the Ministry of Health of the Russian Federation (St. Petersburg), showed high activity against methicillin-resistant strains of *Staphylococcus aureus* fusidic acid and fosfomycin; in our study in the post-COVID period, we identified two strains resistant to fusidic acid [11].

In contrast to the coagulase-negative staphylococci methicillin resistance described in recent publications (76 % according to H.M. Peng et al. [17] and 60 % according to F.S. Fröschen et al. [3]), the resistance level identified in our study was even higher and amounted to 81.0 %.

According to our data, in both study periods the proportion of methicillin-resistant strains was almost half of all isolated staphylococcal isolates. Due to the targeted action of vancomycin against methicillin-resistant staphylococcal strains in cases of an unknown pathogen, it can be recommended as the drug of choice for IAI empirical therapy. Given the patient's concomitant diseases, potential alternatives to vancomycin should not be forgotten – such as teicoplanin, daptomycin or linezolid; in our study, pathogens resistant to linezolid were not detected.

A retrospective review of implant-associated infection cases in two large infectious centers (Germany (*n* = 898) and the Rothman Institute in Philadelphia (*n* = 772)) showed a low frequency of polymicrobial infection (3.4 % and 7.4 %, respectively) [18]. In contrast, the work of T. Rosteius et al. (2018; *n* = 937) notes an increase in the number of microbial associations in the development of IAI of the hip or knee joint in the period from 2003 to 2011 with a polymicrobial infection detection rate of 23.6 % [19]. Our study also shows a high polymicrobial IAI frequency with growth dynamics in the post-COVID period.

According to the study by D.B.G. Tai et al. (2022), the presence of a fistula increased the likelihood of isolating more than one microorganism by almost three times (median – 2.6; 95% confidence interval: 2.0–3.3) [14], which correlates with the data of our study.

Infection caused by gram-negative bacteria and fungi more often leads to an unfavorable outcome after sanitation due to the high virulence of these microorganisms and growing antibiotic resistance. F.D. Wang et al. (2018) believe that the very fact of the gram-negative bacteria involvement in the IAI etiology greatly complicates and prolongs its treatment [20]. In our study in 2021–2022, along with an increase in the proportion of microbial associations in the IAI etiological structure, there is a tendency towards an increase in the frequency of gram-negative pathogens (of which *Enterobacter cloacae* took the leading position in the post-COVID period) and the fungi appearance in the microbial landscape, which may be associated with the high frequency of use of antibacterial drugs during the COVID-19 pandemic and a decrease in the general immunological status of patients.

Multidrug-resistant strains of gram-negative microorganisms currently retain sensitivity only to colistin. However, when choosing empirical antimicrobial therapy, the colistin use is limited by its high cost and insufficient effectiveness as monotherapy.

The results of the microbiological examination of the biomaterial from the fistula tract were not analyzed separately in the work, since patients with the fistula form of IAI are not recommended to undergo discharge bacteriological examination taken with a swab from the fistula tract [21, 22].

A limitation of this study is the small number of isolated strains of gram-negative bacteria, which prevents a full analysis of their antibiotic resistance.

CONCLUSION

According to the study, the microbial landscape has undergone minor changes in the post-COVID period. The leading causative agent of IAI is currently *Staphylococcus epidermidis* with a predominance of methicillin-resistant strains. However, it is worth paying attention to the increased role of microbial associations in the post-COVID period and, in particular, the increase in the gram-negative bacteria proportion in their composition (by 1.6 times). Timely analysis of the sensitivity of IAI pathogens is crucial for the treatment success. The high frequency of methicillin-resistant staphylococci isolation (especially among *Staphylococcus epidermidis*) determines the tactics of choosing vancomycin as an empirical therapy to ensure an optimal antimicrobial effect in the case of an unknown pathogen. In turn,

the growth of staphylococcal resistance to the fluoroquinolone series of antibiotics may further limit the use of levofloxacin as the drug of choice for IAI oral therapy. The growth of gram-negative bacteria resistance and the narrow spectrum of antibiotics acting on carbapenemase producers may reduce the therapy effectiveness, despite the fully performed surgical sanitation. Up-to-date information on the microbiological structure of pathogens is useful for optimal treatment of IAI.

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Conflicts of interest

No potential conflict of interest relevant to this article reported.

The Ethics Committee opinion regarding the study is not required, since it was carried out without providing personal data.

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Authors' contribution

Lyubimova L.V. – developed the concept, wrote the manuscript.

Pchelova N.N. – collected and analysed the data.

Nikolaev N.S. – developed the concept, carried out scientific editing of the manuscript.

Preobrazhenskaya E.V. – developed the study design, carried out a graphical representation of the data.

Lyubimov E.A. – collected and analysed the data.

PHARMACOLOGY AND PHARMACY

NEUROPROTECTIVE EFFECT OF *OROSTACHYS SPINOSE* DRY EXTRACT IN CHOLINERGIC INSUFFICIENCY

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ABSTRACT

Background. *Orostachys spinosa (L.) Sweet. – a perennial plant of a wide habitat and contains various metabolites (amino acids, flavonoids, polysaccharides, etc.). Extracts from the aerial part of the plant are used in traditional medicine as an anticonvulsant and sedative.*
The aim of the work. *To study the neuroprotective effect of O. spinosa in cholinergic deficiency.*
Materials and methods. *The studies were carried out on 52 Wistar rats. The animals were administered scopolamine (1 mg/kg) daily for 21 days, followed by O. spinosa dry extract per os at a dose of 100 mg/kg for 14 days. On the day 32, the animals developed a conditioned passive avoidance reflex (CPAR), the integrity of which was checked after 1, 24 and 72 hours; on the day 35 they were tested in an "open field". On the day 36, biochemical and histological studies of the brain were carried out.*
Results. *It has been established that O. spinosa, against the background of scopolamine intoxication, reduces the anxiety of animals, stimulates exploratory activity in the open field test, improves the production and preservation of the CPAR, and also reduces the number of functionally inactive neurons (pyknotic and shadow cells) in the cerebral cortex. The extract reduces the lactate/pyruvate ratio by 47 %, intensifies the activity of mitochondrial complexes I and II by 54–64 %, and increases the concentration of adenosine triphosphate by 1.6 times compared to the control. O. spinosa exhibits antioxidant properties by reducing malondialdehyde and increasing the activity of catalase, glutathione peroxidase and glutathione reductase in the brain.*
Conclusion. *O. spinosa dry extract has a neuroprotective effect in cholinergic deficiency. The studied extract exhibits antioxidant properties and stimulates energy processes in the brain.*

Key words: *Orostachys spinosa (L.) Sweet, dry extract, neuroprotective effect, scopolamine hydrochloride, cholinergic insufficiency*

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ИССЛЕДОВАНИЕ НЕЙРОПРОТЕКТИВНЫХ СВОЙСТВ *OROSTACHYS SPINOSA* ЭКСТРАКТА СУХОГО ПРИ ХОЛИНЕРГИЧЕСКОЙ НЕДОСТАТОЧНОСТИ

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РЕЗЮМЕ

Обоснование. *Orostachys spinosa (L.) Sweet – многолетнее растение, имеющее широкий ареал произрастания и содержащее различные метаболиты (аминокислоты, флавоноиды, полисахариды и др.). Извлечения из надземной части растения используются в традиционной медицине в качестве противосудорожного и седативного средства.*

Цель работы. *Исследовать нейропротективные свойства O. spinosa при холинергической недостаточности.*

Материалы и методы. *Исследования проведены на 52 крысах линии Wistar. Животным ежедневно в течение 21 дня вводили скополамин (1 мг/кг), далее в течение 14 дней – per os O. spinosa экстракт сухой в дозе 100 мг/кг. На 32-е сутки у животных вырабатывали условный рефлекс пассивного избегания (УРПИ), сохранность которого проверяли через 1, 24 и 72 часа; на 35-е сутки тестировали в «открытом поле». На 36-е сутки проводили биохимические и гистологические исследования головного мозга.*

Результаты. *Установлено, что O. spinosa на фоне скополаминовой интоксикации снижает тревожность животных, стимулирует исследовательскую активность в «открытом поле», улучшает выработку и сохранность УРПИ, а также снижает количество функционально неактивных нейронов (пикнотических и «клеток-теней») в коре больших полушарий головного мозга. Экстракт снижает соотношение лактат/пируват на 47 %, интенсифицирует активность митохондриальных комплексов I и II на 54–64 %, увеличивает концентрацию аденозинтрифосфата в 1,6 раза по сравнению с контролем. O. spinosa проявляет антиоксидантные свойства, снижая содержание малонового диальдегида, повышая активность каталазы, глутатионпероксидазы и глутатионредуктазы в головном мозге.*

Заключение. *O. spinosa экстракт сухой оказывает нейропротективное действие при холинергическом дефиците. Исследуемый экстракт проявляет антиоксидантные свойства и стимулирует энергетические процессы в головном мозге.*

Ключевые слова: *Orostachys spinosa (L.) Sweet, экстракт сухой, нейропротективное действие, скополамин гидрохлорид, холинергическая недостаточность*

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According to the World Health Organization, the world is experiencing demographic aging of the population. As a result, the number of cerebral and neurodegenerative diseases is increasing, and dementia and depression are among the most common diseases of the elderly [1]. All this determines the need to develop new approaches to finding rational treatment and prevention of the nervous system diseases. In the prevention of neurological diseases, herbal medicines deserve attention, which are capable of having a polymodal effect on the body due to a significant variety of metabolites [2].

In this regard, plants of the genus *Orostachys* are of interest. Thus, extracts of *O. japonicas*, used in Japanese and Korean traditional medicine as an adaptogenic agent, have an inhibitory effect on acetylcholinesterase [3], exhibit an antioxidant effect [4], and also limit neuronal apoptosis [5]. Another perennial plant of the Crassulaceae family, *Orostachys spinosa* (L.) Sweet, has similar properties. It is used in folk and traditional medicine of different peoples for diseases of the gastrointestinal tract, respiratory and nervous systems [6]. The main active compounds of *O. spinosa* are amino acids, flavonoids, coumarins, polysaccharides, fatty acids, etc. [7]. According to experimental studies, a liquid extract from the herb *O. spinosa* exhibits anxiolytic, nootropic, antihypoxic and stress-protective properties [8–10]. A dry extract was obtained from the above-ground part of *O. spinosa*, which is characterized by a constant composition [11], exhibiting neuroprotective properties in experimental cerebral ischemia [12]. In this regard, it is of interest to evaluate the neuroprotective properties of *O. spinosa* dry extract in modeling neurodegenerative disease.

THE AIM OF THE STUDY

To study the neuroprotective effect of *Orostachys spinosa* dry extract in cholinergic deficiency.

MATERIALS AND METHODS

The studies were performed on 52 Wistar rats weighing 200–220 g. The animals were kept in accordance with the Good Laboratory Practice (GLP) and the Resolution of the Russian Government No. 855 dated June 13, 2020. The research work was carried out in accordance with the Rules adopted in the European Convention for the Protection of Vertebrate Animals (Strasbourg, 1986). The study protocol was approved by the Ethics Committee of the Institute of General and Experimental Biology of the Siberian Branch of the Russian Academy of Sciences (No. 2 dated December 1, 2020).

The object of the study was a dry extract obtained from the above-ground part of *O. spinosa* by successive three-fold extraction of crushed raw materials with 10 % ethyl alcohol at a temperature of 60 °C, followed by filtration, evaporation and vacuum drying [8]. The extract was standardized by the content of the free amino acids sum in terms of glutamic acid, which should be at least 3.0 %.

Ginkgo biloba leaf extract (tanakan, tablets; Beaufour Ipsen Industrie, France) was used as a comparison drug. The action of *G. biloba* preparations is based on antioxidant properties and the ability to normalize neurotransmitter and energy processes in the brain [13].

To model chronic cholinergic deficiency, animals in the control and experimental groups were administered scopolamine hydrochloride intraperitoneally at a dose of 1 mg/kg for 21 days [14]. Scopolamine hydrochloride is considered a non-selective muscarinic receptor antagonist that causes cognitive impairment and electrophysiological changes in the brain similar to those seen in natural aging and Alzheimer's disease [15]. Scopolamine also causes a number of cellular changes, including antioxidant defense system disruption, increased oxidative stress, mitochondrial dysfunction, apoptosis, and neuroinflammation [16].

The animals were divided into four groups. The first experimental group consisted of rats (n = 13), which after a 21-day scopolamine injection were administered intragastrically once a day for 14 days *O. Spinose* dry extract at a dose of 100 mg/kg in the form of an aqueous solution in a volume of 10 ml/kg. According to a similar scheme, the animals of the second experimental group (n = 13) were administered *G. biloba* extract at a dose of 100 mg/kg, and the animals of the control group (n = 12) were administered water in a volume of 10 ml/kg. In intact control animals (n = 14), cholinergic insufficiency was not modeled; they were administered physiological saline intraperitoneally for 21 days, then they were administered water intragastrically in a volume of 10 ml/kg for 14 days.

On the 32nd day, the animals were trained in the conditioned passive avoidance reflex (CPAR) test [14]; the conditioned reflex was tested after 1, 24, and 72 hours. On the 35th day, the animals were tested in the open field [14]. On the 36th day after the start of test extract administration, the animals were decapitated under ether anesthesia, and the brain was removed for biochemical and histological studies.

The intensity of lipid peroxidation processes was determined by the content of malondialdehyde (MDA) [17]; the state of the antioxidant system – by the activity of catalase (CAT) [18], glutathione peroxidase (GPO) and glutathione reductase (GR) [19]. The effect of the studied agent on energy processes in the brain was assessed by the content of adenosine triphosphate (ATP) [20], the activity of NADH dehydrogenase (complex I) and succinate dehydrogenase (complex II) [21, 22]. The glycolysis intensity was characterized by the activity of pyruvate kinase (PK) [23] and the content of lactate and pyruvate in the brain homogenate [20].

Brain sections for histological examination were prepared using a standard technique on an MS-2 microtome (SPECTRO LAB, Russia), then stained with cresyl violet according to Nissl [24]. In layers II–V of the brain frontal cortex, the number of neurons was counted, which were differentiated into normochromic, intensely hypochromic, intensely hyperchromic (pyknotic), and "shadow cells".

Statistical data processing was performed using Statistica for Windows 6.0 (StatSoft Inc., USA). The conformity of the analyzed features to the normal distribution law was assessed using the Shapiro – Wilk criterion. The statistical significance of differences between the data of the groups was determined using Student's *t*-test, provided that the sample has a normal distribution; using the Mann – Whitney criterion if the data do not obey the normal probability distribution. The data are presented as the arithmetic mean (M) and the arithmetic mean error (m). To compare the number of animals in the comparison groups, the Fisher's angular transformation φ-test was used. Differences between the experimental and control groups were considered statistically significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

The results of testing animals in the "open field" showed that scopolamine inhibits motor and exploratory activity in animals (fig. 1), which is reduced by the *O. spinosa* extract administration. Thus, in animals that were administered *O. spinosa* extract, the number of entries into the installation central squares increased by 2.1 times, vertical stands and peeks into the "holes" – on average 1.7 times compared to the control values. At the same time, in animals of the 2nd experimental group, the number of central squares and the hole reflex were higher than in the control, by 1.6 and 1.3 times, respectively. Against the background of long-term scopolamine administration in animals of the 1st experimental group, the number of grooming acts decreased by 1.6 times compared to the control value (fig. 1).

When *O. spinosa* extract was administered, CPAR was formed in 100 % of animals and was retained after 24 and 72 hours in 92 and 77 % of animals, respectively (fig. 2). In the 2nd experimental group, the conditioned reflex was formed in the same way as in the control group, in 75 % of animals, and by the 3rd day it was retained in all of these animals, while in the control group it was retained only in 50 %.

It was found that multiple scopolamine injections lead to a reduction in the energy potential of brain cells, which is associated with a decrease in the intensity of anaerobic and aerobic processes (fig. 3, 4). According to the data presented in Figure 3, PK activity in the brain homogenate of control animals decreased by 25 %, and the pyruvate content – by 22 % compared to the intact

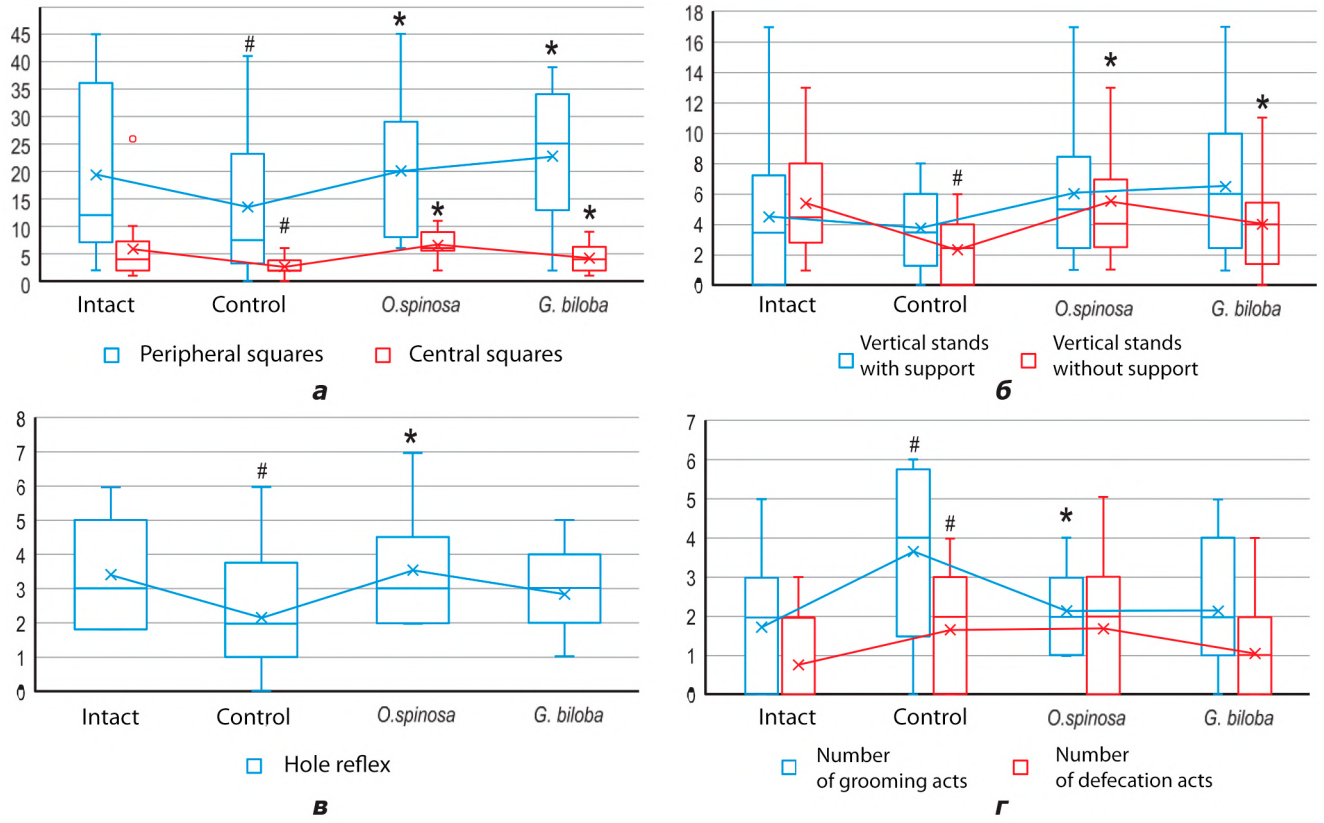


FIG. 1.
*Behavioral indicators of Wistar rats in the open field test in cholinergic insufficiency: **а** – horizontal activity; **б** – vertical activity; **в** – hole reflex; **г** – anxiety indicators. Statistical significance of differences was determined using Mann – Whitney test: # – the differences are statistically significant compared to the indicators of the intact group at $p \leq 0.05$; * – the differences are statistically significant compared to the indicators of the control group at $p \leq 0.05$*

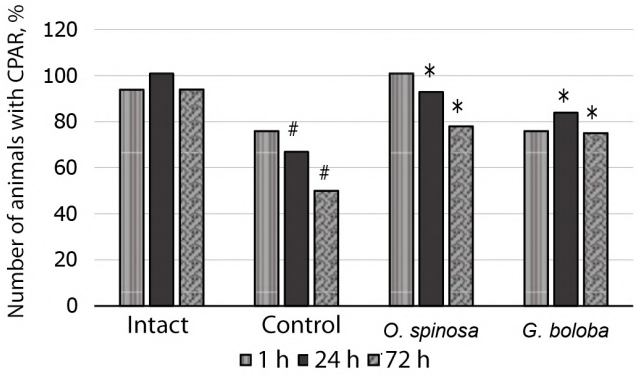


FIG. 2.
*Number of Wistar rats with a conditioned passive avoidance reflex in cholinergic insufficiency. Statistical significance of differences was determined using Fisher's φ test: # – the differences are statistically significant compared to the indicators of the intact group at p ≤ 0.05; * – the differences are statistically significant compared to the indicators of the control group at p ≤ 0.05*

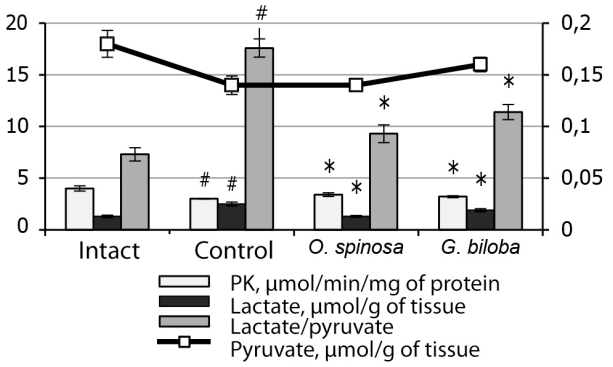


FIG. 3.
*Glycolysis indicators in the Wistar rats brain in cholinergic insufficiency. Data are presented as arithmetic mean (M) and arithmetic mean error (m). Statistical significance of differences was determined using Student's t-test: # – the differences are statistically significant compared to the indicators of the intact group at p ≤ 0.05; * – the differences are statistically significant compared to the indicators of the control group at p ≤ 0.05*

control animals. Against the background of a decrease in pyruvate concentration, the lactate level in the brain of control animals was 2.5 ± 0.19 μmol/g of tissue, which is 42 % higher than the intact value. As a result, the lactate/pyruvate ratio in the control increased by 2.4 times, reaching 17.6 ± 0.88 (fig. 3). Also, in the control, the activities of mitochondrial complexes I and II decreased twofold, as a result, the ATP concentration in the brain was 0.7 ± 0.08 μmol/g tissue, which is 2.4 times lower than the intact value (fig. 4).

Against the background of the *O. Spinose* dry extract use, the PK activity increased by only 13 %, and the pyruvate content almost corresponded to that in the control animals (fig. 3). At the same time, the lactate level in animals of the 1st experimental group was 48 % lower than the control value and corresponded to that in animals of the intact control. As a result, the lactate/pyruvate ratio in animals of this experimental group was 47 % lower than in the control, while in the 2nd experimental group it was 35 %. The activities of mitochondrial complexes I and II in rats administered with *O. spinosa* extract were higher by 57 and 64 %, and *G. biloba* extract by 87 and 20 %, respectively, than in the control (fig. 4). As a result, the ATP content in the brain of animals of the experimental groups increased on average by 1.6 times compared to the control value.

Energy metabolism disorders that develop with cholinergic insufficiency contribute to increased production of free radicals, as well as inhibition of the activity of antioxidant enzymes [16], which leads to irreversible processes and neuronal death. Thus, in animals of the control group, against the background of an increase in the MDA content (by 2.2 times), a decrease in the activity of antioxidant enzymes is observed: CAT by 42 %, GPO by 67 % and GR by 52 %, relative to the indicators in intact control animals (table 1).

Amid the administration of extracts, a decrease in the MDA concentration in the brain homogenate by 25–30 % was noted compared to the control indicator. The activity of enzymes – CAT, GPO and GR – in the brain tissue of animals of the 1st experimental group increased by 36, 78 and 32 %, the 2nd experimental group – by 67, 68 and 34 %, respectively, compared to those in the control animals.

Pathomorphological studies of the cerebral cortex showed that against the background of long-term scopolamine hydrochloride administration, most neurons in layers II–V decreased in size, the nuclei and cytoplasm appeared uniformly stained, the apical dendrite became thinner and could be traced over a long distance, "spirally twisting" (fig. 6a). On average, the number of intensely hyperchromic neurons in layers II–V was 77 % greater than the intact indicator, amounting to 10.6 ± 1.35 % of the total number of neurons (fig. 5). A greater number of "shadow cells" (by 48 %) were also observed; these neurons showed karyolysis amid homogeneous cytoplasm due to chromatolysis. Most "shadow cells" were subject to satellitosis and neuronophagy.

In animals that received the extracts under study, the pathomorphological picture of the cerebral cortex did not look as "mosaic" as in animals of the control group. Pyknotic neurons were detected singly, in most cases only in layers III and V (fig. 6 б, в). Their number was on average 2.0 times lower than in the control. "Shadow cells" and the accompanying processes of satellitosis and neuronophagy were observed much less frequently, but in all cerebral cortex layers. Due to the decrease in the total number of regressive neurons, the number of normochromic cells in animals of the 1st experimental group was 11 % higher than in the control.

Thus, *O. spinosa* dry extract against the background of long-term cholinergic insufficiency has

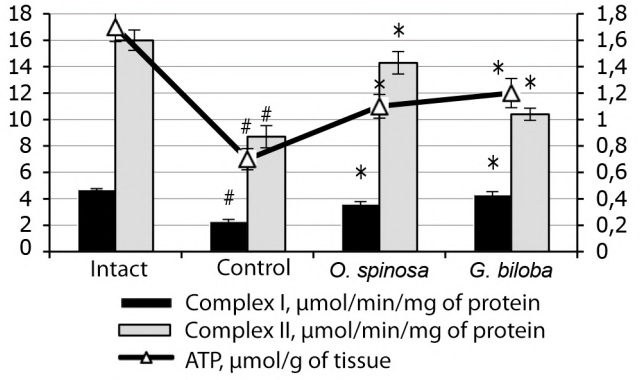


FIG. 4.
*Activity of mitochondrial complexes I and II and ATP content in the Wistar rats brain in cholinergic insufficiency. Data are presented as arithmetic mean (M) and arithmetic mean error (m). Statistical significance of differences was determined using Student's t-test: # – the differences are statistically significant compared to the indicators of the intact group at p ≤ 0.05; * – the differences are statistically significant compared to the indicators of the control group at p ≤ 0.05*

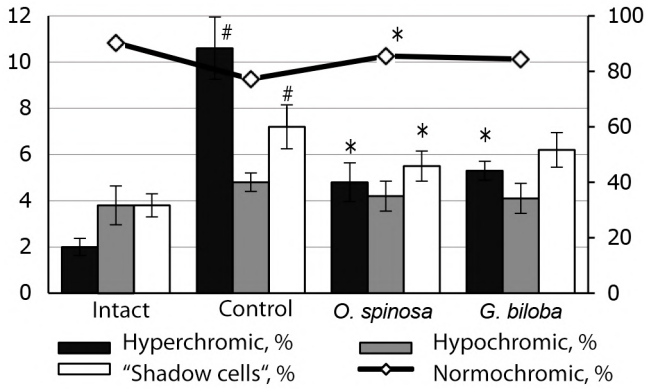


FIG. 5.
*Morphometric parameters of neurons in the cerebral cortex of Wistar rats in cholinergic insufficiency. Data are presented as arithmetic mean (M) and arithmetic mean error (m). Statistical significance of differences was determined using Student's t-test: # – the differences are statistically significant compared to the indicators of the intact group at p ≤ 0.05; * – the differences are statistically significant compared to the indicators of the control group at p ≤ 0.05*

an antiamnestic effect and normalizes the emotional state of animals; in particular, it improves the formation and preservation of conditioned reflexes, and stimulates orientation-exploratory activity. *O. spinosa* extract increases the brain resistance to the toxic effect of scopolamine, limiting the number of regressive forms of neurons (hyperchromic and "shadow cells") and increasing the number of functional neurons in the cerebral cortex. Neuroprotective effect of *O. spinose* extract is associated with its ability to influence the functional activity of NADH dehydrogenase and succinate dehydrogenase complexes of the mitochondrial respiratory chain, correct the processes of aerobic glycolysis, reduce the content of MDA products, increase the intensity of the endogenous antioxidant system by increasing the activity of enzymes (GPO, GR and CAT) in the brain.

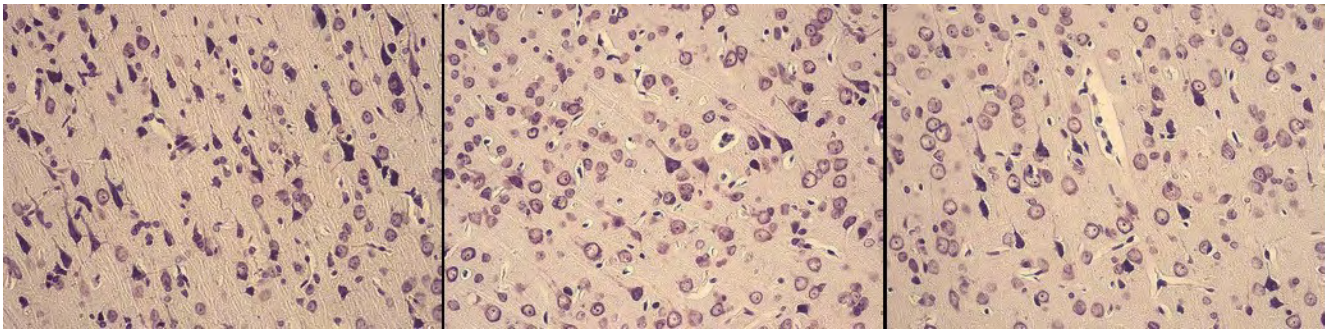
The identified neuroprotective effect of the *O. spinosa* dry extract is due to the presence of various compounds, among which flavonoids, amino acids, polysaccharides and coumarins predominate. *O. spinosa* metabolites help to inhibit dysfunction of the cholinergic system and oxidative stress. Thus, myricetin showed pronounced neuroprotective activity in the scopolamine model of Alzheimer's [25]. This flavonoid exhibits an antiamnestic effect in conditions of cognitive impairment caused by chronic stress [26], as well as amid the administration of streptozotocin [27] and D-galactose [28]. The flavonoid luteolin identified in *O. spinosa* exhibits a neuroprotective effect. Luteolin reduces cognitive dysfunction in rats with Alzheimer's disease by removing oxygen free radicals, increasing antioxidant potential, reducing NF-κB and BACE1 expression, and decreasing Aβ deposition [29]. A certain contribution to the neuroprotective effect of the studied extract is made

TABLE 1

INDICATORS OF THE PRO- AND ANTIOXIDANT SYSTEM OF THE WISTAR RATS BRAIN IN CHOLINERGIC INSUFFICIENCY, M ± M

Group	MDA, μmol/g of tissue	CAT, μmol/min/g of tissue	GPO, nmol/min/mg of protein	GR, nmol/min/mg of protein
Intact	8.4 ± 0.55	11.0 ± 0.70	49.2 ± 2.50	63.1 ± 6.36
Control	18.7 ± 0.97#	6.4 ± 0.12#	16.4 ± 1.10#	30.2 ± 2.01#
O. spinosa	14.0 ± 1.15*	8.7 ± 0.34*	29.2 ± 1.78*	40.0 ± 1.11*
G. biloba	13.0 ± 1.19*	10.7 ± 0.30*	27.5 ± 2.10*	40.4 ± 2.40

Note. The statistical significance of the differences was determined using Student's t-test: # – differences are statistically significant relative to the indicators of the intact group at $p \leq 0.05$; * – differences are statistically significant relative to the indicators of the control group at $p \leq 0.05$.



а *б* *в*

FIG. 6.
Microphotographs of Wistar rats cerebral cortex in long-term cholinergic insufficiency (Nissl cresyl violet staining, magnification ×200): ***а*** *– control;* ***б*** *– O. spinose;* ***в*** *– G. biloba*

by coumarins, which, due to their antiplatelet and anticoagulant properties, help to normalize cerebral blood flow. The neuroprotective effect of coumarins is also realized by inhibiting oxidative stress and neuroinflammation [30]. Amino acids and polysaccharides contained in the *O. spinosa* extract also have pharmacotherapeutic efficacy in the treatment of nervous system diseases through various mechanisms, including inhibition of oxidative stress, neuroinflammation, cellular apoptosis, and excitotoxicity [31, 32].

CONCLUSION

Thus, the *O. spinosa* dry extract exhibits neuroprotective properties with long-term scopolamine administration, preventing the development of "anxiety" in animals, improving learning and memory processes amid limiting changes in the cerebral cortex neurons. The neuroprotective effect of the *O. spinosa* dry extract is associated with its ability to stimulate the antioxidant system activity and metabolic processes in the brain.

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Conflicts of interest

No apparent and potential conflicts of interests relevant to this article reported.

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PHTHISIOLOGY

LEVELS OF MARKERS OF COAGULATION AND FIBRINOLYSIS SYSTEMS IN PATIENTS WITH PULMONARY TUBERCULOSIS WITH CONCOMITANT DIABETES MELLITUS AFTER COVID-19

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ABSTRACT

Background. *It is known that COVID-19 can be followed by a shift in the hemostatic system towards hypercoagulation, which is more pronounced in the presence of diabetes mellitus (DM). Tuberculosis process is often accompanied with hypercoagulation syndrome. Of great interest is the study of the state of hemostatic systems in patients with pulmonary tuberculosis (TB) with concomitant DM who have had COVID-19.*
The aim. *To study the relationship between the state of the hemostatic and fibrinolysis systems and moderate and severe COVID-19 in patients with pulmonary tuberculosis and diabetes mellitus.*
Methods. *Thirty two patients with TB and DM were divided into two groups. Group 1 included 16 patients with TB and DM who have previously had COVID-19 (TB-DM-COVID). Group 2 included 16 patients with TB and DM who did not have COVID-19 (TB-DM).*
Results. *It was found that TB-DM-COVID patients were more likely to develop a hypercoagulable shift compared to TB-DM patients. This was evidenced by a more frequent shortening of such indicator as activated partial thromboplastin time (43.7 % and 25.0 % of cases, respectively; $\chi^2 = 7.22$; $p = 0.01$), an increase in fibrinogen levels (43.7 % and 25.0 %, respectively; $\chi^2 = 7.22$; $p = 0.01$) and D-dimer (43.7 % and 18.7 %, respectively; $\chi^2 = 14.74$; $p = 0.0001$). These changes were closely associated with the systemic inflammatory response, as strong and positive correlations were found between fibrinogen and C-reactive protein levels ($r = 0.420$; $p = 0.01$), and erythrocyte sedimentation rate ($r = 0.433$; $p = 0.01$) in TB-DM-COVID patients.*
Conclusion. *In patients with pulmonary tuberculosis and diabetes mellitus after moderate and severe COVID-19, compared to patients who have not had COVID-19, a hypercoagulable shift associated with the development of more pronounced systemic inflammation develops more often.*

Key words: *pulmonary tuberculosis, diabetes mellitus, COVID-19, hemostasis, fibrinolysis, systemic inflammation*

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УРОВЕНЬ МАРКЕРОВ СИСТЕМ СВЁРТЫВАНИЯ И ФИБРИНОЛИЗА У БОЛЬНЫХ ТУБЕРКУЛЁЗОМ ЛЁГКИХ С СОПУТСТВУЮЩИМ САХАРНЫМ ДИАБЕТОМ ПОСЛЕ ПЕРЕНЕСЁННОЙ COVID-19

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РЕЗЮМЕ

Обоснование. *Известно, что при COVID-19 в системе гемостаза наблюдается сдвиг в сторону гиперкоагуляции, который носит более выраженный характер при наличии сахарного диабета (СД). Спутником туберкулёзного процесса часто является гиперкоагуляционный синдром. Большой интерес представляет изучение состояния систем гемостаза у больных туберкулёзом лёгких (ТБ) с сопутствующим СД, перенёсших COVID-19.*

Цель исследования. *Изучить взаимосвязь между состоянием систем гемостаза и фибринолиза и перенесённого COVID-19 средней и тяжёлой степени у больных туберкулёзом лёгких и сахарным диабетом.*

Методы. *32 больных ТБ и СД были разделены на две группы. В первую группу вошли 16 больных ТБ и СД, которые ранее перенесли COVID-19 (ТБ-СД-COVID). Вторая группа включала 16 больных ТБ и СД, которые не перенесли COVID-19 (ТБ-СД).*

Результаты. *Было обнаружено, что у больных ТБ-СД-COVID чаще развивался гиперкоагуляционный сдвиг по сравнению с больными ТБ-СД. Об этом свидетельствовало более частое укорочение такого показателя, как активированное частичное тромбопластиновое время (соответственно 43,7 % и 25,0 % случаев; $\chi^2 = 7,22$; $p = 0,01$), повышение уровня фибриногена (соответственно 43,7 % и 25,0 %; $\chi^2 = 7,22$; $p = 0,01$) и D-димера (соответственно 43,7 % и 18,7 %; $\chi^2 = 14,74$; $p = 0,0001$). Эти изменения были тесно связаны с системным воспалительным ответом, поскольку были обнаружены тесные и позитивные корреляциионные связи между уровнем фибриногена и уровнем С-реактивного белка ($r = 0,420$; $p = 0,01$), а также скоростью оседания эритроцитов ($r = 0,433$; $p = 0,01$) у больных ТБ-СД-COVID.*

Заключение. *У больных ТБ и СД, перенёсших COVID-19 средней и тяжёлой степени, по сравнению с больными, не перенёсшими COVID-19, чаще развивается гиперкоагуляционный сдвиг, связанный с развитием более выраженного системного воспаления*

Ключевые слова: *туберкулёз лёгких, сахарный диабет, COVID-19, гемостаз, фибринолиз, системное воспаление*

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INTRODUCTION

Currently, despite improvements in a number of epidemiological indicators for tuberculosis (TB) both globally and in the Russian Federation, the situation remains alarming. According to the global report of the World Health Organization (WHO), 10.6 million people fell ill with tuberculosis in 2022 [1]. In the Russian Federation, the incidence of tuberculosis in 2022 was 31.11 cases per 100,000 population (45,377 cases) [2]. There are several reasons for the tense situation, including the consequences of the recent coronavirus infection COVID-19 outbreak, which had a significant impact on the implementation of anti-tuberculosis measures [3, 4], as well as the significant spread of diabetes mellitus (DM), which, according to the WHO global report on tuberculosis, is one of the five main factors creating the risk of developing tuberculosis [1, 5, 6]. According to literature data, diabetes mellitus has been diagnosed in more than 15 % of the population (1.5 million people) of tuberculosis patients worldwide [7–9].

Today, the clinical burden of COVID-19 has significantly decreased [10]. Despite this, the infection consequences remain significant worldwide [11]. A certain amount of information has accumulated in the literature indicating the COVID-19 impact on the clinical, radiological and laboratory parameters of the tuberculosis process [12, 13]. It is known that with COVID-19, a hypercoagulable shift in the hemostasis system is observed, which is accompanied by intravascular blood coagulation (IVBC), and these changes are closely related to the systemic inflammatory response [14, 15]. There is also evidence that the tuberculosis development in patients with diabetes is accompanied by a hypercoagulable shift [16]. In these conditions, the study of the state of the coagulation and fibrinolytic systems in TB patients with concomitant diabetes mellitus who have a COVID-19 history is of great interest.

THE AIM OF THE STUDY

To study the relationship between the state of the hemostasis and fibrinolysis systems and the COVID-19 history in patients with pulmonary tuberculosis and concomitant diabetes mellitus.

METHODS

A prospective cohort study was performed. Thirty two patients with pulmonary TB and diabetes mellitus were divided into two groups. The first group included 16 patients with pulmonary TB and concomitant diabetes mellitus, who had a history of COVID-19 infection (TB-DM-COVID). The second group included 16 patients with pulmonary TB and concomitant diabetes mellitus who had not had COVID-19 (TB-DM). The time from recovery from COVID-19 to admission to the tuberculosis hospital in patients included in the study was up to 6 months. All patients had moderate to severe COVID-19 before admission to the clinic.

The study included: patients aged 18 to 60 years with pulmonary tuberculosis and a history of type 1 and type 2 diabetes mellitus, as well as confirmed infection caused by COVID-19, moderate or severe in accordance with the temporary guidelines of the Ministry of Health of the Russian Federation [17], as well as patients aged 18 to 60 years with pulmonary tuberculosis, a history of type 1 and type 2 diabetes mellitus, who have not had COVID-19.

The study excluded patients with HIV infection, chronic diseases in the decompensation stage, malignant neoplasms, alcoholism, drug addiction, and pregnancy.

There were 56.3 % men and 43.7 % women in each group. The patients' age ranged from 18 to 60 years (median 45.5 years). Newly diagnosed and previously treated pulmonary TB occurred in equal proportions in both groups (50.0 % and 50.0 %, respectively). The incidence of patients with different forms of pulmonary tuberculosis in both groups also did not differ statistically significantly. Patients with tuberculomas were more common (37.6 % in each group), while focal pulmonary tuberculosis was less common (25.0 % in each group). The incidence of patients with infiltrative tuberculosis was 18.7 %, and with cirrhotic tuberculosis was also 18.7 % (in each group).

In the TB-CD-COVID group, lung decay and bacterial excretion were observed in 43.8 % of patients, in the TB-CD group – in 31.3 % of patients ($p > 0.05$). Preserved drug sensitivity of Mycobacterium tuberculosis (MBT) to anti-tuberculosis drugs was observed in 56.3 % of cases in the TB-CD-COVID group and in 43.8 % in the TB-CD group. Patients excreting MBT with multiple and extensive drug resistance were observed in both groups in 31.3 % of cases.

Type 1 diabetes was detected in 11 (68.7 %) patients in the TB-DM-COVID group and in 8 (50.0 %) patients in the TB-DM group ($p > 0.05$). Type 2 diabetes was detected in 31.3 % of patients in the TB-DM-COVID group and in 8 (50.0 %) patients in the TB-DM group ($p > 0.05$). Complications of diabetes were diagnosed in the form of retinopathy (in 25.0 % of the TB-DM-COVID patients, in 18.7 % of the TB-DM group; $p > 0.05$), polyneuropathy (in 7 (43.7 %) and 6 (37.5 %) patients, respectively; $p > 0.05$), nephropathy (in 5 (31.5 %) and 4 (25.0 %) patients, respectively; $p > 0.05$), encephalopathy (in 1 (6.2 %) and 1 (6.2 %) patient, respectively; $p > 0.05$). The incidence of comorbidities in the compared groups also did not differ statistically significantly. Hypertension was observed in 4 (25.0 %) patients in the TB-DM-COVID group, and in 5 (31.2 %) patients in the TB-DM group ($p > 0.05$); cardiovascular diseases – in 4 (25.0 %) and 3 (18.7 %) patients, respectively ($p > 0.05$); viral hepatitis – in 2 (12.5 %) and 2 (12.5 %) patients, respectively ($p > 0.05$); obesity – in 2 (12.5 %) and 2 (12.5 %) patients, respectively ($p > 0.05$); chronic obstructive pulmonary disease – in 1 (6.2 %) and 1 (6.2 %) patient, respectively ($p > 0.05$).

As can be seen from the presented data, the compared groups in this study did not differ statistically significantly in terms of demographic indicators, clinical and radiological signs, as well as laboratory characteristics of the course of the tuberculosis process and diabetes.

A detailed examination of patients included in the study was conducted at the clinic of the Central Scientific Institute of Tuberculosis using clinical, radiological and laboratory methods.

To assess the plasma hemostasis system state, changes in the parameters of activated partial thromboplastin time (APTT), thrombin time (TT), prothrombin time (PT) and fibrinogen (F) were studied. The anticoagulation system state was assessed by the level of antithrombin III (AT III). The fibrinolytic system activity was judged by the content of D-dimer in the blood plasma. Along with this, some parameters of the clinical blood test were assessed, which indicate changes in the platelet link of the hemostasis system: the platelet count (PLT), the thrombocrit index (TI) and the values of the relative platelet distribution width (PDW). Of the markers of the systemic inflammatory response, the content of acute phase proteins (C-reactive protein (CRP) and F) in the blood serum was determined, and, in addition, the erythrocyte sedimentation rate (ESR) was studied.

Laboratory tests were conducted upon admission of patients to the clinic of the Central Scientific Institute of Tuberculosis before the start of anti-tuberculosis chemotherapy. To determine the reference values of the above laboratory tests, serum and plasma tests were performed in 47 healthy volunteers.

The study was conducted in accordance with the ethical principles of the World Medical Association Declaration of Helsinki ("Ethical Principles for Conducting Medical Research Involving Human Subjects"). The local Ethics Committee of the Central Scientific Institute of Tuberculosis approved this study (Protocol No. 1 dated January 18, 2021). Voluntary informed consent to participate in the study was obtained from all patients in written form.

The patient database was created using MS Excel (Microsoft Corp., USA) and SPSS Statistics, version 27 (IBM Corp., USA). The Kolmogorov – Smirnov criteria were used to assess the distribution of values. The frequency (in %) with which certain values of qualitative features occurred in the sample was used to describe qualitative data. Quantitative data were described using the median (Me) and percentiles (Q1 and Q3). The hypothesis about the equality of sample means was tested using the Mann – Whitney criterion. The Pearson's χ^2 criterion was used to assess the statistical significance of differences in the frequency of features of the compared samples depending on their size. Differences were considered statistically significant at $p < 0.05$.

THE RESULTS OF THE STUDY

The analysis of the occurrence frequency of various deviations in the parameters of the coagulation and fibrinolysis systems in patients with pulmonary tuberculosis, with concomitant diabetes mellitus, who have had COVID-19, is presented in Table 1.

As can be seen from the data provided, the APTT indicator was shortened in 43.7 % of cases in the TB-DM-COVID-19 group of patients, and in 25.0 % of cases in patients with pulmonary tuberculosis with concomitant diabetes mellitus who had not had COVID-19 ($\chi^2_{1-2} = 7.22$; $p = 0.01$). APTT shortening is one of the indicators of the hypercoagulable shift presence. PT shortening, which also indicated the hypercoagulable shift presence, was observed with the same frequency in the compared groups (25.0 % and 25.0 %, respectively; $p > 0.05$). A decrease in the AT III level, which is one of the indicators of the hypercoagulation presence, was observed only in the group of patients with pulmonary TB with concomitant diabetes mellitus who had COVID-19 (18.7 %). In the group of patients who did not have COVID-19, no such cases were detected. An increase in the F content, which is also one of the markers of the hypercoagulable shift presence, was more often detected in the group of patients with TB-DM-COVID compared to patients with TB-DM (43.7 % and 25.0 %, respectively; $\chi^2 = 7.22$; $p = 0.01$). There were no changes in the TB indicator in both groups of patients. An increase in the D-dimer level, which, on the one hand, is an indicator of the intravascular blood coagulation presence, and on the other hand, a marker of the fibrinolytic system activity, was more often observed in the group of patients with TB-DM-COVID (43.7 %). In the group of patients with TB-DM, such a shift was noted in 18.7 % of cases ($\chi^2 = 14.74$; $p = 0.0001$). There were no statistically significant changes in the PLT, TI, and PDW indicators.

The levels of coagulation and fibrinolysis system parameters in patients with pulmonary TB with concomitant diabetes mellitus who have and have not had COVID-19 infection are presented in Table 2.

As can be seen from the data provided, in the group of patients with pulmonary tuberculosis with concomitant diabetes mellitus who had COVID-19, an APTT indicator shortening was observed compared to healthy volunteers.

The PT was shortened in both groups of patients compared to healthy controls, indicating the hypercoagulation syndrome presence.

The AT III level in all study groups did not statistically differ significantly from that in healthy volunteers.

The F content in the group of patients with pulmonary TB with concomitant diabetes mellitus who had COVID-19 was statistically significantly higher compared to both healthy individuals and the group of patients with TB-DM.

The median value of the TT indicator in all compared groups did not statistically differ significantly from that in healthy individuals.

Compared with healthy volunteers, the D-dimer content was higher in the TB-DM-COVID and TB-DM groups. However, no significant differences were found between the groups.

TABLE 1

FREQUENCY OF CHANGES IN MARKERS OF COAGULATION AND FIBRINOLYSIS SYSTEMS IN PATIENTS WITH PULMONARY TUBERCULOSIS WITH CONCOMITANT DIABETES MELLITUS WHO HAVE AND HAVE NOT HAD COVID-19 (%)

Indicators	TB-DM patients who had COVID-19 infection (n = 16)						TB-DM patients who have not had COVID-19 infection (n = 16)					
	norm		decrease		increase		norm		decrease		increase	
	abs.	%	abs.	%	abs.	%	abs.	%	abs.	%	abs.	%
		1		2		3		4		5		6
APTT	6	37.5	7	43.75	3	18.75	10	62.5	4	25.0	2	12.5
Prothrombin time	12	75.0	4	25.0	–	–	12	75.0	4	25.0	–	–
Antithrombin III	10	62.5	3	18.75	3	18.75	13	81.25	–	–	3	18.75
Fibrinogen	7	43.75	2	12.5	7	43.75	10	62.5	2	12.5	4	25.0
Thrombin time	16	100.0	–	–	–	–	16	100.0	–	–	–	–
D-dimer	10	62.5	–	–	6	37.5	13	81.25	–	–	3	18.75
Platelets	13	81.25	1	6.25	2	12.5	15	93.75	1	6.25	–	–
Thrombocrit	16	100.0	–	–	–	–	16	100.0	–	–	–	–
Platelet volume distribution width	16	100.0	–	–	–	–	16	100.0	–	–	–	–

TABLE 2

LEVEL OF MARKERS OF COAGULATION AND FIBRINOLYSIS SYSTEMS IN PATIENTS WITH PULMONARY TUBERCULOSIS WITH CONCOMITANT DIABETES MELLITUS WHO HAVE AND HAVE NOT HAD COVID-19, ME (Q1; Q3)

Indicators	Healthy volunteers	TB-DM patients who had COVID-19 infection (n = 16)	TB-DM patients who have not had COVID-19 infection (n = 16)
	1	2	3
APTT	36.5 (34.5; 37.7)	33.5 (31.25; 34.4) $p_{1-2} < 0,05$	36 (32.25; 37.75)
Prothrombin time	14.0 (12.0; 16.0)	11.5 (10.6; 12.0) $p_{1-2} < 0.05$	11.2 (10.8; 11.6) $p_{1-3} < 0.02$
Antithrombin III	100 (91.7; 108.7)	102 (96; 114)	109 (103.7; 114.7)
Fibrinogen	3.10 (2.71; 3.20)	3.82 (3.21; 5.39) $p_{1-2} < 0.05$	2.88 (2.65; 3.2)
Thrombin time	20 (17.2; 22.0)	18 (17; 19)	18 (17; 19)
D-dimer	0.25 (0.19; 0.40)	0.5 (0.19; 1.31) $p_{1-2} < 0,01$	0.43 (0.24; 0.50) $p_{1-3} < 0.05$
Platelets	250.0 (220.0; 290.0)	243 (197.5; 299.7)	220 (192; 282.7)
Thrombocrit	0.19 (0.14; 0.25)	0.20 (0.17; 0.26)	0.17 (0.16; 0.20)
Platelet volume distribution width	14 (11.3; 15.2)	12.2 (11.1; 12.9)	11.3 (9.6; 12.3)

TABLE 3

LEVEL OF C-REACTIVE PROTEIN AND ERYTHROCYTE SEDIMENTATION RATE IN PATIENTS WITH PULMONARY TUBERCULOSIS WITH CONCOMITANT DIABETES MELLITUS, WHO HAVE AND HAVE NOT HAD COVID-19, ME (Q1; Q3)

Indicators	Healthy volunteers	TB-DM patients who had COVID-19 infection (*n* = 16)	TB-DM patients who have not had COVID-19 infection (*n* = 16)
	1	2	3
CRP	1.5 (1.0; 2.0)	12.6 (7.0; 22.5) $p_{1-2} < 0.01$	3 (2.0; 7.0) $p_{2-3} < 0.02$
ESR	15.0 (8.5; 24.5)	38 (30.7; 88.7) $p_{1-2} < 0.01$	7 (2.5; 36.2)

The groups of patients also did not differ from healthy ones in terms of platelet count and thrombocrit. In both groups of patients examined, the PDW indicator was lower compared to healthy ones, but there were no statistically significant differences between the groups.

The differences in the level of markers of the coagulation and fibrinolysis systems in patients with pulmonary tuberculosis with diabetes types 1 and 2 in the group of those who had COVID-19 were statistically insignificant. This was probably due to the absence of statistically significant differences in the incidence of diabetes complications in these groups.

Considering that changes in the hemostasis and fibrinolysis systems are components of the systemic inflammatory response, we studied changes in the level of CRP and ESR.

It was found that an increase in the CRP level in the group of patients with TB-DM-COVID was observed in the overwhelming majority of cases (87.5 %), and in the group of patients with TB-DM – in 31.2 % of cases ($\chi^2 = 64.82$; $p = 0.00001$).

A statistically significant increase in the ESR index was observed more often in the group of TB-DM-COVID patients compared to TB-DM patients (75.0 % and 31.2 %, respectively; $\chi^2_{1-2} = 38.86$; $p = 0.00001$).

Analysis of the study results of the CRP and ESR levels showed that their values were statistically significantly higher in the group of patients with pulmonary TB with concomitant diabetes who had COVID-19 (table 3).

Correlation analysis showed that fibrinogen levels closely and positively correlated with CRP levels (r = 0.420; $p = 0.01$) and ESR (r = 0.433; $p = 0.01$) in patients with pulmonary tuberculosis and concomitant diabetes who had COVID-19.

CONCLUSION

An analysis of the occurrence frequency of various deviations and the median of the coagulation and fibrinolysis systems indicators showed that patients with pulmonary tuberculosis with concomitant diabetes mellitus who had moderate and severe COVID-19 more often develop a hypercoagulable shift compared to patients who have not had COVID-19. This was evidenced by a frequent shortening of the APTT indicator, a decrease in the level of AT III, an increase in the level of fibrinogen and D-dimer. At the same time, the hypercoagulable shift in the group of patients who had COVID-19 was more pronounced, which was documented by statistically significantly high fibrinogen values. These changes were probably associated with damage to the vascular system after COVID-19. The above-described changes in the hemostasis and fibrinolysis system were closely associated with the systemic inflammatory response, which was observed more frequently and was more pronounced in the group of patients with pulmonary tuberculosis and concomitant diabetes mellitus and who had COVID-19. This is confirmed by the statistically significant frequent and more pronounced increase in the level of CRP, fibrinogen and ESR in these patients.

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Conflicts of interest

No potential conflict of interest relevant to this article reported.

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EXPERIMENTAL RESEARCHES

BIOCHEMICAL AND HISTOLOGICAL CHANGES IN TWO NON-ALCOHOLIC FATTY LIVER DISEASE MODELS OF DIFFERENT SEVERITY

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ABSTRACT

Background. *One of the priority areas of modern medicine, which unites the interests of various specialists (therapists, cardiologists, gastroenterologists, endocrinologists), is the study of the pathogenesis and clinical manifestations of non-alcoholic fatty liver disease (NAFLD), which is widespread and of unconditional social significance. The search for adequate experimental models of NAFLD that reflect the severity of liver damage is of paramount importance for studying its etiology and pathogenesis.*

The aim of the study. *To compare biochemical and histological changes in experimental models of NAFLD of varying severity.*

Materials and methods. *Two NAFLD model versions were used: a light one – non-alcoholic steatosis (NAS) and a severe variant – non-alcoholic steatohepatitis (NASH). The following biochemical parameters were measured: enzyme activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), alkaline phosphatase (AP), plasma glucose concentration, total protein (TP), total bilirubin (TBil) and its conjugate fraction (CB), plasma concentrations of homocysteine (HC), total cholesterol (TC), triacylglycerides (TG), catalase (Cat), superoxide dismutase (SOD) and malondialdehyde (MDA).*

Results. *When used in a model of steatohepatitis, liver function was impaired to a significantly greater extent than in the model of steatosis; this difference was manifested in a statistically significant increase in ALT, AST, AP, TC, Tbil, MDA ($p < 0.001$) and a decrease in Cat, SOD ($p < 0.05$). This is confirmed by the development of more pronounced symptoms of disorders of pigment and lipid metabolism, cytolytic and cholestatic syndromes, significant activation of lipid peroxidation and depression of the antioxidant system when modeling non-alcoholic steatohepatitis. Various degrees of severity of morphological changes in the experimental groups were revealed.*

Conclusion. *The study showed the priority of determining biochemical markers, including the levels of ALT, AST, OBIL, TG, MDA and SOD to optimize laboratory methods for diagnosing the severity of liver dystrophy.*

The practical originality of the results lies in the optimization of the methodology for laboratory diagnosis of the severity of the pathological process in NAFLD.

Key words: *non-alcoholic fatty liver disease, hepatic steatosis, steatohepatitis, metabolism, rats, lipid peroxidation, malondialdehyde, superoxide dismutase*

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БИОХИМИЧЕСКИЕ И ГИСТОЛОГИЧЕСКИЕ ИЗМЕНЕНИЯ НА ДВУХ МОДЕЛЯХ НЕАЛКОГОЛЬНОЙ ЖИРОВОЙ БОЛЕЗНИ ПЕЧЕНИ РАЗЛИЧНОЙ СТЕПЕНИ ТЯЖЕСТИ

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РЕЗЮМЕ

Введение. *Одним из приоритетных исследований современной медицины, объединяющих интересы различных специалистов (терапевтов, кардиологов, гастроэнтерологов, эндокринологов), является изучение патогенеза и заболеваний неалкогольной жировой болезни печени (НАЖБП), для которой характерна распространённость и безусловная инновационность. Поиск адекватных экспериментальных моделей НАЖБП, отражающих степень тяжести повреждения печени, имеет первостепенное значение для изучения её этиологии и патогенеза.*

Цель исследования. *Определение биохимических маркеров для определения степени тяжести неалкогольной жировой болезни печени.*

Материалы и методы. *В эксперименте использовались два варианта модели НАЖБП: лёгкий – неалкогольный стеатоз (НАС), тяжёлый – неалкогольный стеатогепатит (НАСГ). Измеряли следующие биохимические показатели: активность ферментов аланинаминотрансферазы (АЛТ), аспартатаминотрансферазы (АСТ), лактатдегидрогеназы (ЛДГ), щелочной фосфатазы (ЩФ), глюкозы в плазме, общего белка (ОБ), общего билирубина (ОБил) и его прямых соединений (ПБ), состояния в плазме гомоцистеина, холестерина (ОХ), триацилглицеридов (ТГ), каталазы (Кат), супероксиддисмутазы (СОД) и малонового диальдегида (МДА).*

Результаты. *На моделях стеатогепатита функция печени нарушается в значительно большей степени, чем при стеатозе; этот фактор проявился в динамике повышения АЛТ, АСТ, ЩФ, ОХ, ОБил, МДА ($p < 0,001$) и снижения Кат, СОД ($p < 0,05$), что способствует развитию более выраженных проявлений пигментного и липидного обмена, цитолитических и холестатических синдромов, активации ПОЛ и депрессии антиоксидантной системы при моделировании неалкогольного стеатогепатита. Также выявлена различная степень выраженности морфологических изменений в экспериментальных группах.*

Выводы. *Исследование показало приоритетность определения биохимических маркеров, в том числе уровней АЛТ, АСТ, ОБил, ТГ, МДА и СОД, для оптимизации лабораторной методики диагностики степени тяжести дистрофии печени.*

Практическая оригинальность результатов заключается в оптимизации методологии лабораторной диагностики степени тяжести патологического процесса при НАЖБП.

Ключевые слова: *неалкогольная тяжёлая болезнь печени, стеатоз печени, стеатогепатит, обмен веществ, крысы, перекисное окисление липидов, малоновый диальдегид, супероксиддисмутаза*

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a clinical and laboratory syndrome characterized by profound disorders of lipid metabolism, morphologically manifested by lipid deposition in hepatocytes [1]. The current classification of NAFLD includes three stages: non-alcoholic steatosis (NAS), non-alcoholic steatohepatitis (NASH) and liver cirrhosis.

Currently, NAFLD is not only the predominant liver pathology in the world, but also a component of the metabolic syndrome [2]. Recent screening studies in Russia found NAFLD in 27 % of people, with 80 % of these patients diagnosed with NAS, 17 % with NASH, and 3 % with cirrhosis [3]. Up to 80 % of all cases of liver cirrhosis in Russia are directly caused by NAFLD [4]. Manifestations of NAFLD and metabolic syndrome occur in 30 % of all therapeutic patients in Russia [5]. The increased incidence of NAFLD directly correlates with increased cardiovascular and endocrine pathology [6, 7]. This high prevalence of NAFLD is associated with modern trends in nutrition and the prevalence of a sedentary lifestyle among the population [8].

One theory for excess lipid accumulation in hepatocytes is a decrease in the oxidation of free fatty acids (FFA) in mitochondria, as well as an increase in the delivery of FFA to the liver. Progressive accumulation of FFAs causes direct damage to cell membranes, activation of lipid peroxidation (LPO), oxidative stress, chronic inflammation (NASH), collagenogenesis and progressive fibrosis.

The lack of effective methods for the treatment and prevention of NAFLD is due to insufficient understanding of its etiology and pathogenesis. Liver biopsy is still the gold standard for diagnosing NAFLD. But its use is not always appropriate, and it cannot be used in all patients [9]. Thus, the relevance of the study lies in the validation of existing models of liver damage, as well as elucidation of aspects of the development of the pathological process over time using a number of biochemical indicators. Considering the above, we determined the purpose of this study: to identify biochemical markers to determine the severity of non-alcoholic fatty liver disease.

EXPERIMENTAL SECTION

Materials and methods

Prior to the experiment, the study plan, standardized operating procedures and accompanying documentation were subjected for ethical review and subsequently approved by the Local Ethical Committee of the Ministry of Health of Russian Federation (protocol No. 1/1 dated January 16, 2017).

The study involved 120 male albino rats with body mass 220–240 g divided into three groups:

1. Controls (n = 24) – intact healthy animals tested for reference blood parameters. They were fed with standard food rations and had free access to water.

2. "Liver steatosis" (n = 48) – rats that were fed with standard rations identical to those of the controls but received 10 % fructose solution instead of water [10].

3. "Steatohepatitis" (n = 48) – rats that throughout the entire study were fed with food briquettes consisting of 21 % protein, 5 % animal fat, 60 % fructose, 8 % cellulose, 5 % minerals and 1 % vitamins. This routine was shown in our previous morphologic studies to cause in 3–4 weeks severe hepatic fibrosis [11].

Restrictions on access to food, diets and drinking conditions were not introduced. Throughout the study, the control group (healthy, intact animals) was fed with a complete extruded and granulated food specially designed for feeding laboratory rodents (Laboratorkorm LLC, Russia). Before feeding the animals, the food was sterilized. Water for the animals was filtered and, after filling the drinking bottle, irradiated with ultraviolet light for 5 minutes.

Blood samples (6 ml) were collected into vacutainers through a transcutaneous heart puncture into Monovette vacuum systems, after which the animals were euthanized. Samples from control animals were taken on day 1 of the experiment and from the rats of "Liver steatosis" and "Steatohepatitis" groups – on days 21, 28 and 37 of the experiment. Previously, the animals of these groups were combined into three subgroups of 16 rats each.

Biochemical blood tests were carried out using generally accepted methods using a StatFax 3300 analyzer and a set of reagents from Parma LLC (Russia). Studies included: glucose concentration (Glu), total plasma proteins (TP), total bilirubin (TBil) and conjugate bilirubin (CB), alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDG), alkaline phosphatase (AP), homocysteine (HC), total cholesterol (TC), triacylglycerides (TAG) levels. The intensity of LPO was judged by changes in the concentration of malondialdehyde (MDA), which was determined colorimetrically with thiobarbituric acid [12]. The state of the antioxidant system was assessed by catalase concentration, determined by the method of M.A. Korolyuk et al., 1988 [13] and superoxide dismutase (SOD) concentration, which was determined by the adrenaline autoxidation method [14].

Histological examination was carried out by light microscopy, hematoxylin-eosin staining, magnification 20×. A different degree of severity of morphological changes in the experimental groups was revealed. All experimental groups share signs of fatty degeneration of hepatocytes.

All the results were statistically processed with the help of SPSS for Windows 13.0 package. All the resulting data are presented as mean ± standard error (M ± SE). Kolmogorov – Smirnov criterion was used to determine the character of data distribution. To describe quantitative characteristics that do not correspond to the law of normal distribution, the nonparametric Mann – Whitney test was used. The obtained data are presented as median, lower and upper quartiles (Me, quartiles [25 %–75 %]). Using Friedman's χ^2 test (with a distribution other than normal), $p < 0.05$ (probability of at least 95 %) was accepted as a significant level of difference, which is standard for biomedical experiments.

RESULTS AND DISCUSSION

Starting from day 21, the animals from "Steatohepatitis" group displayed total bilirubin blood plasma concentration increase due to direct bilirubin fraction that demonstrated a valid constant increase during the entire experiment ($p = 0.037$). This increase reflects a progressive hepatic dysfunction alongside steatohepatitis development. The absence of statistically valid parallel increase of total bilirubin blood concentration during the entire experiment in comparison to control group ($p = 0.363$) confirms this thesis.

Liver steatosis unlike steatohepatitis had caused moderate impairment of pigment metabolism with slow but reliable total bilirubin blood concentration increase ($p = 0.040$) without substantial fluctuations of conjugate bilirubin concentration testifying to mild hepatocytes dysfunction (Table 1).

The analysis of data on the activity of cellular enzymes characterizing cytolytic liver impairment in blood of animals with steatohepatitis (ALT and AST) had revealed a synchronous reliable increase reaching a statistically valid level of difference in comparison to the control group from the very beginning of the experiment (ALT: $p < 0.001$, AST: $p < 0.001$), with a continuous increment during the entire experiment (Fig. 1).

Hepatic transaminases activity in "Liver steatosis" group demonstrated a slow increase. It was only on day 37 that they have reached statistically valid difference from the control group (ALT: $p = 0.001$; AST: $p = 0.002$). Cytolytic syndrome intensity in case of steatosis was much lower which was confirmed by a lower ALT and AST level in the animals of this group in comparison with "Steatohepatitis" group (AST level lower by 8.5 IU/l ($p = 0.011$), ALT level – by 13.4 IU/l ($p = 0.004$)) (Fig. 1). This fact confirms validity of two chosen NAFLD models of varying severity.

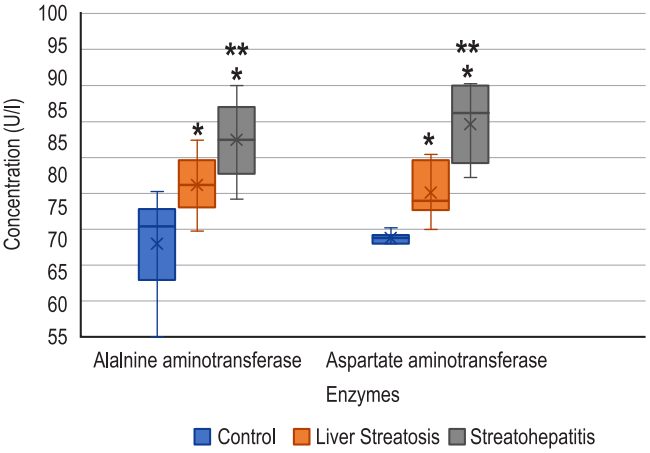


FIG. 1.

*Alanine aminotransferase (ALT), aspartate aminotransferase (AST) level changes (IU/l) in rats with liver steatosis and steatohepatitis. Boxplots showing hormonal and metabolic differences between "Liver Steatosis" groups and "Control" group: * – differences from the "Control" group are statistically significant (Mann – Whitney test); ** – differences from the "Liver Steatosis" group are significant (Mann – Whitney test)*

A significant discrepancy was revealed in the dynamics of biochemical blood parameters of experimental animals, characterizing the condition of the liver between groups with different severity of the process. Liver functions in the "Steatohepatitis" group were significantly more disturbed than in the "Liver steatosis" group: disorders of pigment and lipid metabolism, as well as cytolytic and cholestatic syndromes and hyperhomocysteinemia in the former group were much more pronounced than in the latter one. The evaluation supports the validity of fructose-induced NAFLD models.

The used high-carbohydrate (60 % fructose of the total feed mass – "Steatohepatitis" group) and lipid-rich

TABLE 1

INDICATORS OF PIGMENT METABOLISM IN RATS WITH NAFLD OF VARYING SEVERITY (ME [25 %; 75 %]) IN EXPERIMENTAL GROUPS

Groups	Observation period (days)	*n*	Indicators studied	
			Total bilirubin, μmol/l	Conjugate bilirubin, μmol/l
Control	0 (1)	24	10.0 [4.8; 15.1]	1.1 [0.69; 1.7]
	21 (2)	16	12.2 [4.4; 22.1]	1.1 [0.62; 1.6]
Liver steatosis	28 (3)	16	12.3 [8.9; 14.9]	1.6 [1.1; 2.0]
	37 (4)	16	15.4 [13.7; 16.5]	1.1 [0.62; 1.6]
	21 (5)	16	14.5 [12.8; 16.2]	1.0 [0.68; 1.2]
Steatohepatitis	28 (6)	16	19.4 [10.1; 29.2]	1.3 [1.01;2.0]
	37 (7)	16	28.1 [24.2; 33.1]	1.6 [1.1; 2.0]

Note. $^{2-7}$ – measurements within groups on days 21, 28, 37 were made using the Friedman's test ($p < 0.005$); $^{1-2,1-3,1-4,1-5,1-6,1-7}$ – measurements within groups on days 21, 28, 37 were made using the Mann – Whitney test; statistically significant differences ($p < 0.005$) were observed in the groups 1–4, 1–5, 1–6, 1–7 by the level of total bilirubin.

diet led to the rapid formation of pathological processes (5 weeks) compared to other used models [15–17]. However, such a diet also led to the formation of serious pathological conditions in the cardiovascular system and liver of rats, which is confirmed by the 30 % mortality of animals at the end of the study (day 37). In studies by other authors [18, 19], no mortality was reported.

Lethality rate, in our opinion, can be used as an integral parameter for assessing the severity of the pathological process and the intensity of the studied models. The high-carbohydrate (60 % fructose by weight), lipid-rich diet caused rapid development of pathological processes, leading to a 32 % mortality rate by the end of the experiment (day 37).

In studies by other authors, high levels of ALT and AST in the experimental groups were similar to our data [20–23]. However, the degree of disturbances in lipid and pigment metabolism is significantly higher according to our results.

LDG blood levels in "Steatohepatitis" group demonstrated a reliable moderate increase ($p < 0.001$). LDG blood levels in "Liver steatosis" group did not substantially differ from those of the control animals. Comparison of mean LDG blood levels in "Steatohepatitis" and "Liver steatosis" groups revealed that LDG values in rats with liver steatosis were reliably lower by 13.4 IU/l than in the animals with steatohepatitis ($p = 0.026$).

AP blood levels in "Steatohepatitis" group demonstrated a reliable moderate increase in comparison with the control group ($p < 0.001$) which is a cholestatic syndrome biochemical marker. Glucose levels in "Steatohepatitis" group grew slowly during the experiment and demonstrated a valid difference with control ($p = 0.015$), while Glu levels in "Liver Steatosis" group did not differ statistically from those in the control group (Fig. 2). Homocysteine blood concentration increase, an important hepatic and endothelial dysfunction marker, was statistically valid in "Steatohepatitis" group ($p = 0.001$), but not in "Liver steatosis" one.

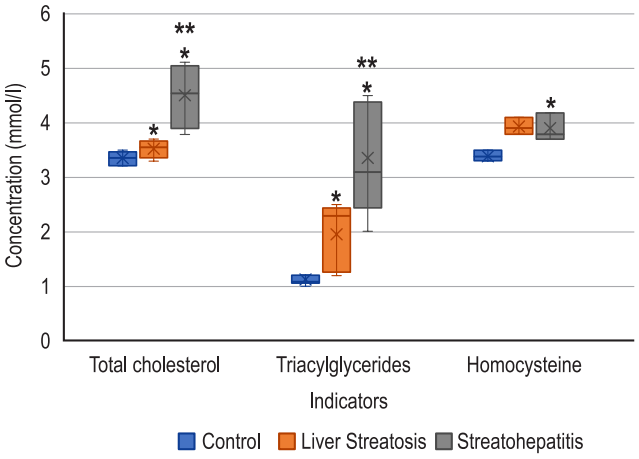


FIG. 2.

*Total cholesterol, triacylglycerides and homocysteine level changes (mmol/l) in rats with liver steatosis and steatohepatitis. Boxplots showing hormonal and metabolic differences between "Hepatic Steatosis" groups and "Control" group: * – differences from the "Control" group are statistically significant (Mann – Whitney test); ** – differences from the "Liver Steatosis" group are statistically significant (Mann – Whitney test)*

Fatty liver dystrophy in both experimental models was assessed in the present study is based on profound metabolic disorder with hypercholesterolemia and hypertriglyceridemia (Fig. 2). Total cholesterol blood concentration in "Steatohepatitis" group increased considerably in comparison with control from the very beginning of the experiment ($p < 0.001$) with a parallel even more substantial rise of TAG blood levels ($p < 0.001$); TAG/TC ratio increasing from 0.53 on day 21 up to 9.79 on day 37 (TAG/TC ratio in control group was 0.52).

The rats in "Liver steatosis" group also demonstrated an increase of TC and TAG blood levels (Fig. 2). However, the increase was slower and not as high as in "Steatohepatitis" group (TC: $p = 0.003$; TAG: $p = 0.002$). TAG/TC ratio in this group changed from 0.46 on day 21 to 0.67 on day 37 of the experiment.

It is assumed that the pathogenesis of NAFLD is based on a pronounced imbalance of lipid metabolism with the formation of hypercholesterolemia and hypertriglyceridemia [24, 25]. In our studies, on day 37 of observation, the TG level in the "Steatohepatitis" group became 300 % higher than in the "Control" group. This is slightly higher than in the experiment of Z. Ackerman [11]: on day 35 of observations, the indicator increased by 223 %. The level of TC by the end of the experiment increased by 167 %, while in studies by the same author this figure increased by 89 % [11].

Serious metabolic disorders accompanying the development of NAFLD in experimental animals were reflected by biochemical blood plasma changes causing lipid peroxidation and considerable antioxidant system depression. These disorders were represented by a progressive increase of MDA blood concentration in both NAS and NASH models with a parallel decrease of basic antioxidant system enzymes activity (catalase, SOD) (Table 2).

MDA blood concentration in "Steatohepatitis" group grew quickly and reliably ($p < 0.001$) from the very beginning of the experiment reflecting increased lipid peroxidation (Table 1). The intensity of LP in the rats from "Liver steatosis" group was way lower than in the animals with NASH: MDA blood concentrations in rats with NAS grew slowly ($p = 0.010$) but by the end of the study (day 37) MDA mean value was statistically higher by 9.8 mmol/l than in the control group ($p = 0,001$) although lower by 11 mmol/l than in rats with NASH.

Parallel to lipid peroxidation activation in both experimental groups basic antioxidant system enzymes (SOD and catalase) considerably decreased their concentration. SOD blood concentration in "Steatohepatitis" group demonstrated a precipitous drop ($p < 0.001$) with a synchronous decrease of blood catalase concentration from the very beginning of the experiment ($p = 0.001$).

The same enzymes' blood concentration in "Liver steatosis" group decreased slower (SOD: at day 28 ($p = 0.002$); catalase: at day 37 ($p = 0.009$) and not as substantial.

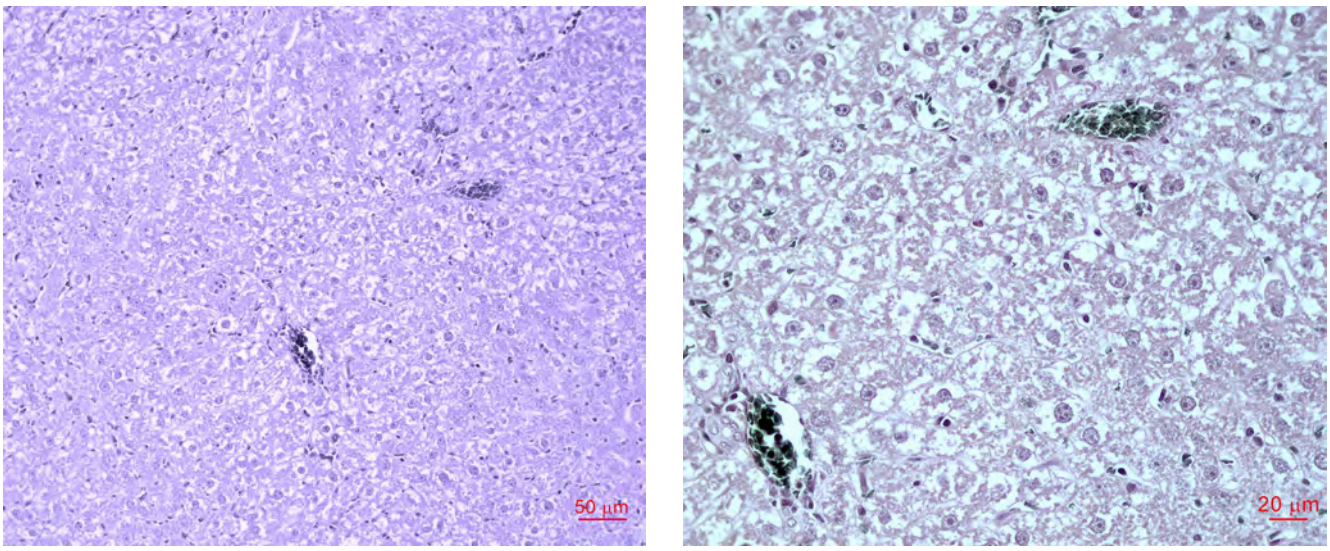
Metabolic disorders in animals that accompany the development of NAFLD in our studies lead to a decrease in the activity of the body's antioxidant system and activation of LPO [26]. This is reflected in a progressive increase

TABLE 2

ANTIOXIDANT SYSTEM ENZYMES ACTIVITY AND PEROXIDATION INTENSITY IN RATS WITH TWO NAFLD MODELS, ME [25 %; 75 %]

Groups	Day of the experiment	*n*	Biochemical parameters		
			SOD, IU/ml	Catalase, mmol/l	MDA, mmol/l
Control	0 (1)	24	6.4 [6.4; 6.6]	0.15 [0.13; 0.17]	9.5 [9.1; 9.7]
	21 (2)	16	5.9 [5.0; 7.5]	0.15 [0.14; 0.16]	13.9 [13.1; 14.7]
Liver steatosis	28 (3)	16	5.8 [3.9; 7.3]	0.14 [0.13; 0.15]	16.8 [15.6; 17.0]
	37 (4)	16	4.6 [4.4; 4.8]	0.13 [0.12; 0.14]	18.9 [18.4; 19.4]
	21 (5)	16	5.8 [3.9; 7.3]	0.14 [0.13; 0.15]	15.5 [13.9; 17.1]
Steatohepatitis	28 (6)	16	4.6 [3.7; 5.0]	0.1 [0.9; 0.11]	19.8 [16.7; 22.0]
	37 (7)	16	4.0 [2.9; 4.9]	0.07 [0.06; 0.08]	29.9 [26.3; 33.5]

Note. $^{2-7}$ – measurements within groups on days 21, 28, 37 were made using the Friedman's test ($p < 0.005$); $^{1-2,1-3,1-4,1-5,1-6,1-7}$ – measurements within groups on days 21, 28, 37 were made using the Mann – Whitney test. Statistically significant differences ($p < 0.005$) were observed in the groups 1–3, 1–4, 1–5, 1–6, 1–7 by the level of superoxide dismutase; in the groups 1–4, 1–5, 1–6, 1–7 – by the level of catalase; in the groups 1–3, 1–4, 1–5, 1–6, 1–7 – by the level of malondialdehyde.



a ***b***

FIG. 3.

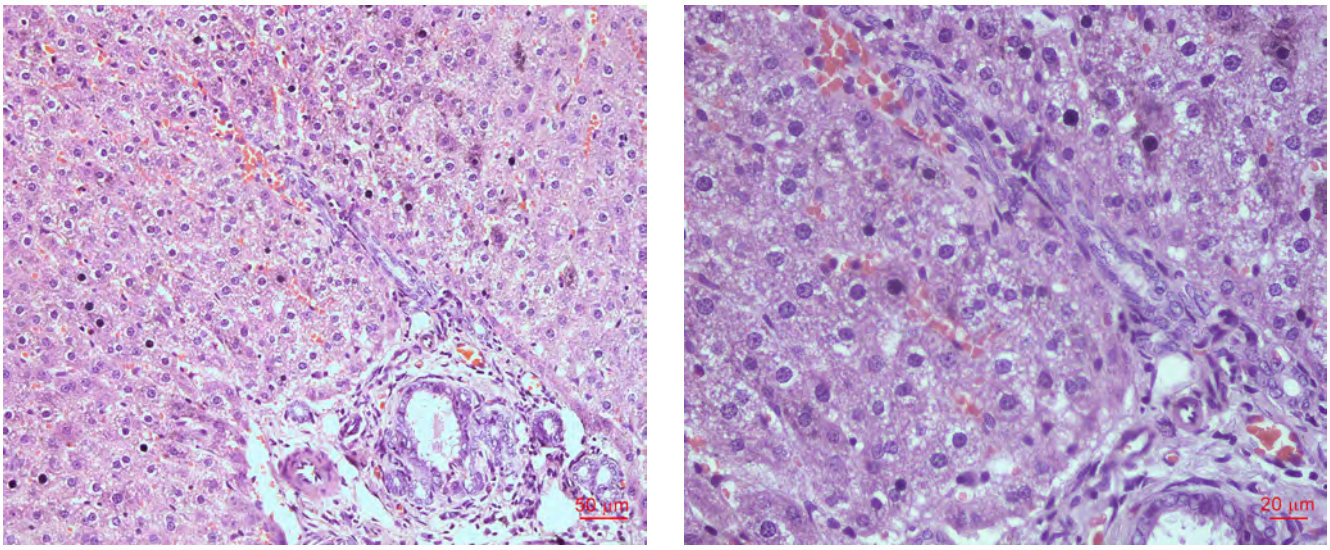
Histological changes in "Liver steatosis" group: ***a*** *– hematoxylin and eosin staining, magnification 10×;* ***b*** *– hematoxylin and eosin staining, magnification 40×*

in the level of MDA in the blood of rats in both models of NAFLD and a decrease in the content of the main antioxidant enzymes (catalase, SOD), which is comparable with the results of studies by other authors. [27]. Lujan P.V. et al. also observed a significant decrease in the level of antioxidant enzymes SOD and catalase against the background of NAFLD [28].

In the experimental "Liver steatosis" group, large droplet fatty degeneration is observed, which is characterized by the presence of large lipid droplets in the cytoplasm of hepatocytes with a displacement of the nucleus to the cell periphery (Fig. 3).

Signs of liver tissue degeneration are most pronounced in the "Steatohepatitis" group (Fig. 4). Signs of balloon dystrophy, apoptosis of hepatocytes are noticeable in comparison with the control (Fig. 5) and the "Liver steatosis" group. Small droplet fatty degeneration was revealed: there are a lot of small lipid droplets in hepatocytes, the nucleus is located in the center of the cell. Hepatocytes are also found in a state of balloon dystrophy. Focal centrilobular necrosis often develops with small droplet steatosis. Hyaline bodies of Mallory are detected with different frequency. The inflammatory infiltrate inside the lobules contains neutrophils, lymphocytes, and histiocytes.

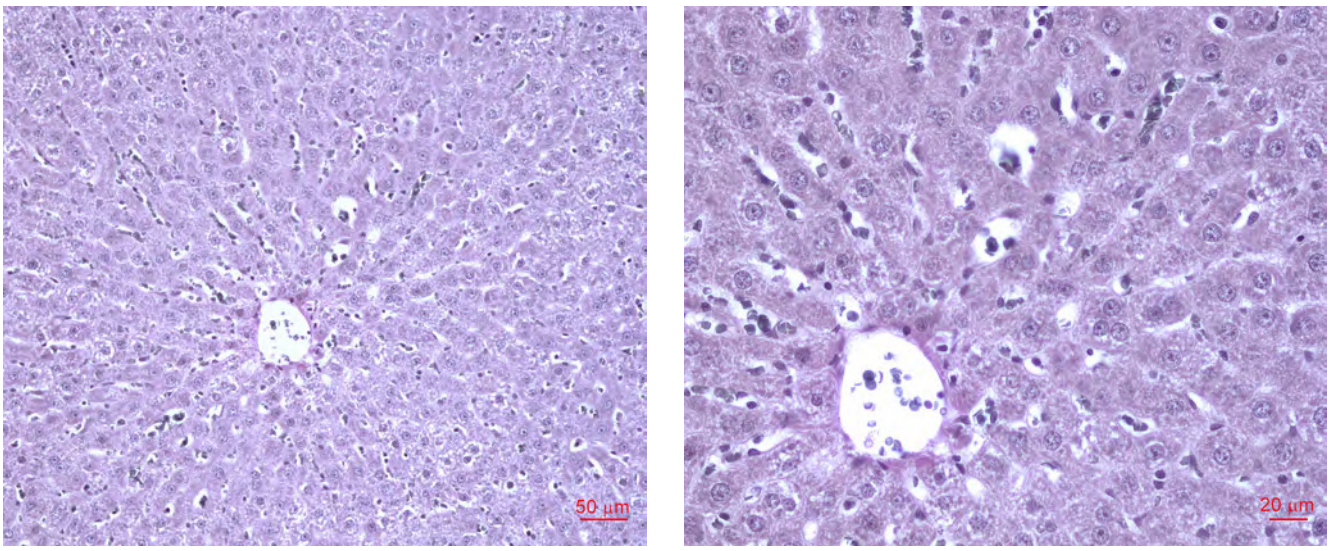
The used models of steatosis and steatohepatitis were characterized by the development of fatty liver in experimental animals, bilirubinemia, cholesterolemia, activation lipid peroxidation and suppression of antioxidant mech-



a *b*

FIG. 4.

*Histological changes in "Steatohepatitis" group: **a** – hematoxylin and eosin staining, magnification 10×; **b** – hematoxylin and eosin staining, magnification 40×*



a *b*

FIG. 5.

*Histological changes in the Control group: **a** – hematoxylin and eosin staining, magnification 10×; **b** – hematoxylin and eosin staining, magnification 40×*

anisms, cytolytic and cholestatic syndromes. The severity of metabolic disorders depended on the severity of the disease being modeled.

The results of the study prove the possibility of using biochemical markers of NAFLD (ALT, AST, TC, TAG, MDA, SOD) for more accurate diagnosis of the severity and stage of development of liver pathology, as well as for monitoring the effectiveness of therapy.

CONCLUSIONS

1. Both NAFLD models studied caused disorders of the hepatobiliary, endocrine and cardiovascular systems. The intensity of these disorders depended on the severity of the model used and was maximum when modeling steatohepatitis (60 % fructose in the diet) and less pronounced when modeling steatosis (10 % fructose solution instead of drinking water).

2. High mortality rates in both models of NAFLD confirm the adequate severity of both models of NAFLD, as well as a direct correlation of dysmetabolic changes and disorders of compensatory mechanisms.

3. In the model of steatohepatitis, liver functions were impaired to a much greater extent than in steatosis; this difference was manifested in more pronounced symptoms of disorders of pigment and lipid metabolism, the severity of cytolytic and cholestatic syndromes, significant acti-

vation of lipid peroxidation and depression of the antioxidant system.

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Conflicts of interest
The authors declare no conflict of interest.

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THE EFFECT OF ALUMINUM- AND SILICON-CONTAINING ENTEROSORBENT ON THE THYMIC CELLULAR COMPOSITION IN MICE KEPT UNDER TWO-WEEK ALL-NIGHT LIGHTING

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ABSTRACT

Background. *Continuous lighting contributes to the development of desynchronosis, which is stressful for the body. As a result, the normal functioning of the immune system is disrupted, which in turn can shift the physiological balance towards pathology and endotoxemia. It is relevant to develop innovative drugs based on a sorbentmatrix, which can be modified with biologically active molecules that extendedly leave the sorbent surface. At the same time, the sorbent retains the properties of a detoxifier, fixing toxic agents on the surface and removing them from the body, which helps restore the internal environment and normalizes the overall reactivity of the body in extreme conditions.*

The aim. *To study the effect of aluminum- and silicon-containing enterosorbent (based on aluminum oxide and polydimethylsiloxane) on the cellular composition of the thymus and the distribution of thymocytes in the organ according to the cell cycling state in C57Bl/6 mice kept under the all-night lighting.*

Materials and methods. *Animals received sorbent (0.665 g per 1 kg of body weight in 200 µl of distilled water) through an intragastric tube once a day for 14 days against the background of continuous lighting. Intact mice and placebo animals composed control group. We used flow cytometry to assess the percentage of $CD3^{hi}$ and $CD3^{low}$ lymphocytes of the thymus, the $CD3^{low}/CD3^{hi}$ ratio, viability and distribution of cells across according to the cell cycling state.*

Results. *Continuous lighting inhibited the differentiation and maturation of young $CD3^{low}$ lymphocytes into mature forms of $CD3^{hi}$, reduced the proliferation of thymic epithelial cells, and activated apoptosis of lymphocytes and epithelial cells in the organ. The introduction of the sorbent restored the content and viability of young $CD3^{low}$ lymphocytes and contributed to the preservation of the viability and proliferation of thymic epithelial cells.*

Conclusion. *Using an enterosorbent based on aluminum oxide and polydimethylsiloxane under conditions of continuous lighting helps maintain the functional activity of the thymus, preventing its involution, and is advisable against the background of circadian disruption.*

Key words: *continuous two-week lighting, aluminum- and silicon-containing sorbent, $CD3^{hi}$ lymphocytes, $CD3^{low}$ lymphocytes, thymic epithelial cells, cell cycle, apoptosis, stress*

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ВЛИЯНИЕ АЛЮМИНИЙ-, КРЕМНИЙСОДЕРЖАЩЕГО ЭНТЕРОСОРБЕНТА НА КЛЕТОЧНЫЙ СОСТАВ ТИМУСА МЫШЕЙ, СОДЕРЖАВШИХСЯ ПРИ ДВУХНЕДЕЛЬНОМ КРУГЛОСУТОЧНОМ ОСВЕЩЕНИИ

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РЕЗЮМЕ

Обоснование. *Непрерывное освещение способствует развитию десинхроноза, что является стрессом для организма. Как следствие нарушается нормальная работа иммунной системы, что в свою очередь способно сдвигать физиологическое равновесие в сторону патологии и эндотоксикоза. Актуальна разработка инновационных лекарственных средств, в основе которых находится сорбентная матрица, которая может быть модифицирована биологически активными молекулами, пролонгированно покидающими поверхность сорбента. При этом сорбент сохраняет свойства детоксиканта, фиксируя на поверхности токсические агенты и выводя их из организма, что способствует восстановлению внутренней среды и нормализует общую реактивность организма в экстремальных условиях.*

Цель исследования. *Изучить влияние алюминий-, кремнийсодержащего энтеросорбента (на основе оксида алюминия и полидиметилсилоксана) на клеточный состав тимуса и распределение тимоцитов в органе по фазам клеточного цикла у мышей C57Bl/6, содержавшихся при круглосуточном освещении.*

Материалы и методы. *Животные получали сорбент (0,665 г на 1 кг веса тела в 200 мкл дистиллированной воды) через внутрижелудочный зонд 1 раз в день в течение 14 суток на фоне непрерывного освещения. Контролем служили интактные мыши и животные плацебо. Используя метод проточной цитометрии, оценивали процентное содержание $CD3^{hi}$- и $CD3^{low}$- лимфоцитов тимуса, соотношение $CD3^{low}/CD3^{hi}$, жизнеспособность и распределение клеток по фазам клеточного цикла.*

Результаты. *Круглосуточное освещение угнетало процессы дифференцировки и созревания молодых лимфоцитов $CD3^{low}$ в зрелые формы $CD3^{hi}$, снижало пролиферацию эпителиальных клеток тимуса, активировало апоптоз лимфоцитов и эпителиальных клеток в органе. Введение сорбента восстанавливало содержание и жизнеспособность молодых $CD3^{low}$-лимфоцитов и способствовало сохранению жизнеспособности и пролиферации эпителиальных клеток тимуса.*

Заключение. *Применение энтеросорбента на основе оксида алюминия и полидиметилсилоксана в условиях непрерывного освещения способствует сохранению функциональной активности тимуса, препятствуя его инволюции, и целесообразно на фоне нарушения суточных ритмов режима освещения.*

Ключевые слова: *непрерывное двухнедельное освещение, алюминий-, кремнийсодержащий сорбент, $CD3^{hi}$-лимфоциты, $CD3^{low}$-лимфоциты, эпителиальные клетки тимуса, клеточный цикл, апоптоз, стресс*

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INTRODUCTION

Circadian desynchrony is becoming increasingly common due to the changing conditions of modern 24-hour society, including exposure to artificial lighting, shift work, and time zone changes. Suppression of melatonin synthesis due to prolonged exposure to continuous light leads to the light-induced functional desynchronosis development, which is stress for the body [1]. An unbalanced response to severe or prolonged stress can shift the physiological balance towards pathology and endotoxicosis development. It has been shown that a decrease or blockade of melatonin synthesis accompanies the development of numerous common pathologies – from aging and type 2 diabetes mellitus to neurological disorders [2, 3]. Neurohumoral mechanisms play a major role in maintaining the functional and biochemical stability of the body in changing environmental conditions, and the pituitary-adrenal axis activity is considered to be the basis of the adaptive response to stress. Long-term and/or strong exposure activates the secretion of cortisol and corticosterone by the adrenal glands, which exert their metabolic influence on cells and organs, including causing a decrease in the thymus functional activity [4]. The basis for the thymus gland involution is considered to be excessive apoptosis and proliferation inhibition of a subpopulation of cortisol-sensitive lymphocytes [5].

The thymus is a central organ of lympho-/immunopoiesis, in which proliferation and selection of antigen-specific and host antigen-tolerant lymphocytes ensures migration of mature and naive T-cells into the bloodstream and peripheral lymphoid organs, where T-cells provide both cellular and humoral immune responses by activating B-lymphocytes. Thymus involution can contribute to the risk and recurrence of cancer, increased susceptibility to infections [6], and immunocompetence loss can lead to the development of a wide range of immune and severe infectious diseases, obesity and diabetes [5, 7]. Aging and fatty degeneration of the thymus become a factor in maintaining inflammation and the development of "aging" diseases – arthritis, and cardiovascular diseases [8].

In connection with the above, there is a clinical need for non-toxic and affordable means capable of reducing the stress impact on the body. Scientific research by academician Yu.I. Borodin and his students have shown that sorbent treatment methods are directly related to the possibility of overcoming endotoxicosis syndrome. Enterally administered sorbent is capable of influencing the physiological constants of the body, not even directly related to the enterosorption process. In this case, the sorbent acts as a trigger for a cascade of reactions from the local to the organism level. There is a functional analogy in the action of the sorbing agent and the regional lymphatic apparatus. In both cases, drainage and detoxification of the pathological focus take place [9, 10].

Currently, the interest of researchers is aimed at developing innovative drugs based on a sorbent matrix modified with biologically active molecules that leave the sorbent surface for a prolonged period. The sorbent itself (as an enterosorbent) retains the properties of a detoxifier, fixing a wide class of substances on its surface – from low-molecular to high-molecular, found in excess in various diseases and poisonings (for example, bacterial toxins, bilirubin, catecholamines, kinin, bile acids), and removing them from the body. From this point of view, a promising hydrophilic-hydrophobic aluminum-, silicon-containing sorbent (enterosorbent) based on aluminum oxide and polydimethylsiloxane – Al_2O_3@PDMS with a pore volume of 0.2 cm^3/g, capable of binding molecules of different charges and different sizes – from low- to high-molecular. The adsorption activity, for example, in relation to low-molecular methylene blue, is 10 mg/g; and it absorbs *Staphylococcus aureus* from the aqueous medium by 25 % of their initial content. This sorbent, used as a carrier for lithium, has successfully passed preclinical studies as part of a drug within the framework of a state assignment. The enterosorbent itself is safe, belongs to hazard class IV. This free-flowing white powder with a particle size of 0.04 mm and a specific surface area of 100 m^2/g can be used as a component of drugs. Of interest is the study of its biological properties in relation to vulnerable thymus cells under conditions of disruption of natural day-night biorhythms.

The aim of this work was to study the effect of aluminum-, silicon-containing enterosorbent Al_2O_3@PDMS on the thymus cellular composition and the distribution of thymocytes in the organ according to the cell cycle phases in C57Bl/6 mice kept under continuous lighting.

THE METHODS OF THE STUDY

The study was conducted at the SPF Vivarium of the Federal Research Center Institute of Cytology and Genetics, Siberian Branch of Russian Academy of Sciences (RFMEFI61914X0005 and RFMEFI62114X0010) and complied with the requirements of Directive 2010/63/EU of the European Parliament and of the Council of the European Union on the protection of animals used for scientific purposes and good laboratory practice. The experimental protocol was approved by the Ethics Committee of the Research Institute of Clinical and Experimental Lymphology, a branch of the Institute of Cytology and Genetics, Siberian Branch of Russian Academy of Sciences (Protocol No. 128 dated March 15, 2017). Male C57Bl/6 mice aged 10–12 weeks were kept in controlled barrier rooms with free access to water and food.

Some mice were kept under continuous lighting conditions (CL; $n = 6$) created by Philips 18 W fluorescent lamps (Philips, Netherlands; light : dark photoperiod 24 : 0 h) for 14 days. The second group of animals (CL + Sorbent; $n = 6$) were given a sorbent composition of aluminum oxide and polymethylsiloxane (0.665 g per 1 kg of body weight in 200 µl of distilled water intragastrically daily against the background of CL for 14 days. Animals that received 200 µl of distilled water intragastrically

daily against the background of continuous lighting were selected as a placebo group.

The third group consisted of intact mice (Control; $n = 6$) kept under standard lighting and feeding conditions (light : dark photoperiod 14:10 h). The animals were removed from the experiment by craniocervical dislocation, the thymus was removed, and a cell suspension was prepared, which was examined using a CytoFlex S100 flow cytometer (Beckman Coulter, USA).

The thymus was homogenized in cold phosphate-buffered saline (PBS) in a glass homogenizer at 4°C. Thymocytes were pelleted by centrifugation (300 g, 5 min) and used for staining with CD3e-APC monoclonal antibodies (BioLegend, USA) to identify young $CD3^{low}$- and mature $CD3^{hi}$-lymphocytes. To analyze cell distribution across cell cycle phases, thymus cells (5 × 106) were fixed in cold 70 % ethanol for 24 h [11].

Cell samples (2 06) in 100 µl were stained with fluorescently labeled antibodies for 30 min at room temperature in the dark. They were then washed twice with PBS and analyzed on a flow cytometer. Cells fixed with 70 % ethanol were centrifuged (300 g, 7 min), washed with PBS and incubated in hypotonic extraction buffer for 5 min to remove low molecular weight DNA to determine the subdiploid peak. The cells washed with buffer were incubated (30 min, room temperature, dark) in staining buffer containing 50 µg/mL propidium iodide (PI; Sigma-Aldrich, USA) and 200 µg/mL RNase-A (Invitrogen, USA). PI fluorescence was determined using a flow cytofluorimeter (λEm = 670 nm). The number of cells with different DNA content in the cell cycle phases: SubG1, G0/G1, S, G2/M was estimated.

Statistical processing of the obtained results was performed in the Statistica 12.0 program (StatSoft Inc., USA). The values of the median, first and third quartiles were determined. The statistical significance of the differences in the compared values between the groups CL + Sorbent and CL, CL + Sorbent and Control was calculated using the nonparametric Mann – Whitney U-test. Differences were considered statistically significant at $p < 0.05$.

RESULTS

1. The effect of taking a sorbent composition on the numerical density of $CD3^{low}$- and $CD3^{hi}$-lymphocytes in animals with continuous lighting

In the previous work [12], it was found that continuous lighting of mice for 14 days led to a statistically significant decrease in the relative number of both young $CD3l^{low}$ ($p = 0.0051$) and mature $CD3^{hi}$ ($p = 0.0374$) T lymphocytes in the thymus compared to the control. At the same time, the $CD3^{low}/CD3^{hi}$ ratio decreased statistically significantly ($p = 0.0131$). The development of accidental thymus involution in mice with CL was not registered in our study. Intragastric administration of enterosorbent to animals against the background of CL normalized the relative number of young $CD3^{low}$ thymocytes, increasing their content to 46.70 (45.88; 47.08) % (in intact animals – 43.40 (41.85; 44.33) %). As a result, the $CD3^{low}/CD3^{hi}$ ratio increased significantly (compared to control $p = 0.0202$; compared to CL $p = 0.0051$) (fig. 1).

Such changes can include disruption of daily biorhythms, proliferation and destruction of immune system cells under altered light conditions, as well as prolonged stress leading to proliferation inhibition of immature lymphocytes in the thymus cortex and their increased apoptosis.

To determine the sorbent effect on the survival and proliferation of thymus cells under 14-day continuous lighting, we performed a cell cycle analysis for all thymus cells.

2. The effect of the sorbent composition administration on the cell cycle of thymus cells under continuous lighting conditions

Cell cycle analysis of the entire population of thymus cells in mice after CL showed a significant decrease in the relative number of cells in the phase S to 3.4 (2.3; 6.8) % compared to the control – 7.3 (7.0; 11.1) % ($p = 0.0367$), a more than threefold increase in the proportion of cells in the apoptosis stage to 2.2 (1.8; 3.2) % compared to the control – 0.7 (0.63; 0.73) % ($p = 0.01997$).

Sorbent administration to animals against the background of CL normalized the cell cycle, increasing the percentage of cells in the phase S to 7.45 (6.5; 12.75) % and reducing the number of cells in the apoptosis stage to the control level. Thus, the sorbent maintains an increased adaptive level of thymus cell proliferation and protects cells from apoptosis under prolonged continuous lighting conditions.

For a more complete analysis of the proliferation and viability of thymus cell elements on the flow cytometry histograms, all cells were divided into 3 groups (3 gates – PLym, P2Lym, P3big) depending on their size and corrected for the cell cycle (fig. 2). Thus, the PLym gate was formed by the smallest cells, some of which were in the active cell cycle phases (S + G2/M). Young $CD3^{low}$ lymphocytes were contained mainly in this gate. The P2Lym gate contained mature non-dividing lymphocytes in the G0/G1 stage. The largest cells, most of which were actively dividing, formed the P3big gate. Figure 2 shows the cell cycle of each gate.

The gate of the smallest PLym cells, containing $CD3^{low}$ lymphocytes, had the lowest cell count compared to other groups; the number of cells in the phase S was 6.8 (6.28; 7.1) %, and cells in the apoptotic stage were 2.2 (1.5; 2.75) % in control animals. Continuous lighting inhibited the phase S, resulting in a decrease in the relative number of cellular elements to 6.05 (5.55; 6.10) %, and a statistically significant increase in the number of cells in the PLym gate in the apoptotic state to 11.65 (9.05; 13.28) % ($p = 0.0051$). Sorbent administration against the background of CL contributed to the proliferation restoration of thymus lymphocytes

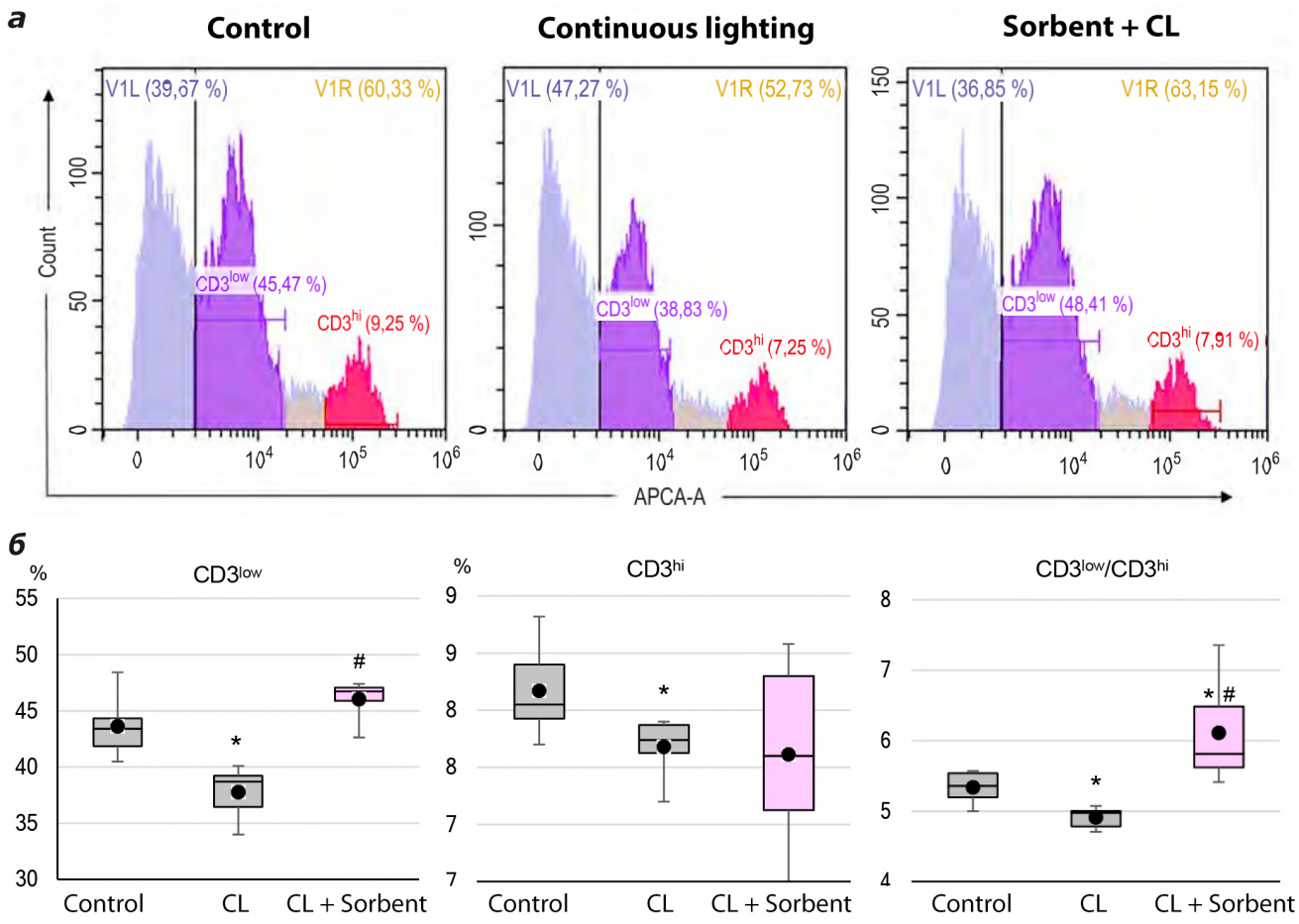

FIG. 1.
Thymus of C57Bl/6 mice treated with sorbent against the background of continuous lighting (CL): ***а*** *– histograms of distribution of $CD3^{low}$ and $CD3^{hi}$ lymphocytes;* ***б*** *– relative number of $CD3^{low}$ and $CD3^{hi}$ lymphocytes and $CD3^{low}/CD3^{hi}$ ratio; * – compared to the control; # – compared to CL ($p < 0.05$)*

and an increase in their viability, as a result the relative content of cellular elements in the phase S increased to 8.85 (7.75; 11.75) % and the percentage of cells in the apoptosis stage decreased to 4.5 (3.4; 4.85) % ($p = 0.0065$).

From Figure 2 it can be seen that long-term continuous lighting inhibited the relative number of T lymphocytes (PLym gate) in the phase S, but did not affect the content in the G2/M phase, i.e., in essence, there was an inhibition of proliferation (residual proliferation). The sorbent against the background of CL caused a significant increase in the number of cells in both phases S and G2/M (phase G2/M in the control – 0.85 (0.73; 1.05) %, in the CL group – 1.05 (0.63; 1.25) %, in the CL + Sorbent group – 1.7 (1.23; 2.63) %. Such changes are possibly associated with an adaptive response to stress. A wide range of data in the sorbent presence indirectly indicates that its action in this case is not direct and is not aimed at a specific target, but helps the body to remain at the adaptation stage and not slide to the exhaustion stage, sorbing and removing excess amounts of highly active substances.

3. The sorbent composition effect on the viability and proliferation of thymus epithelial cells under continuous lighting

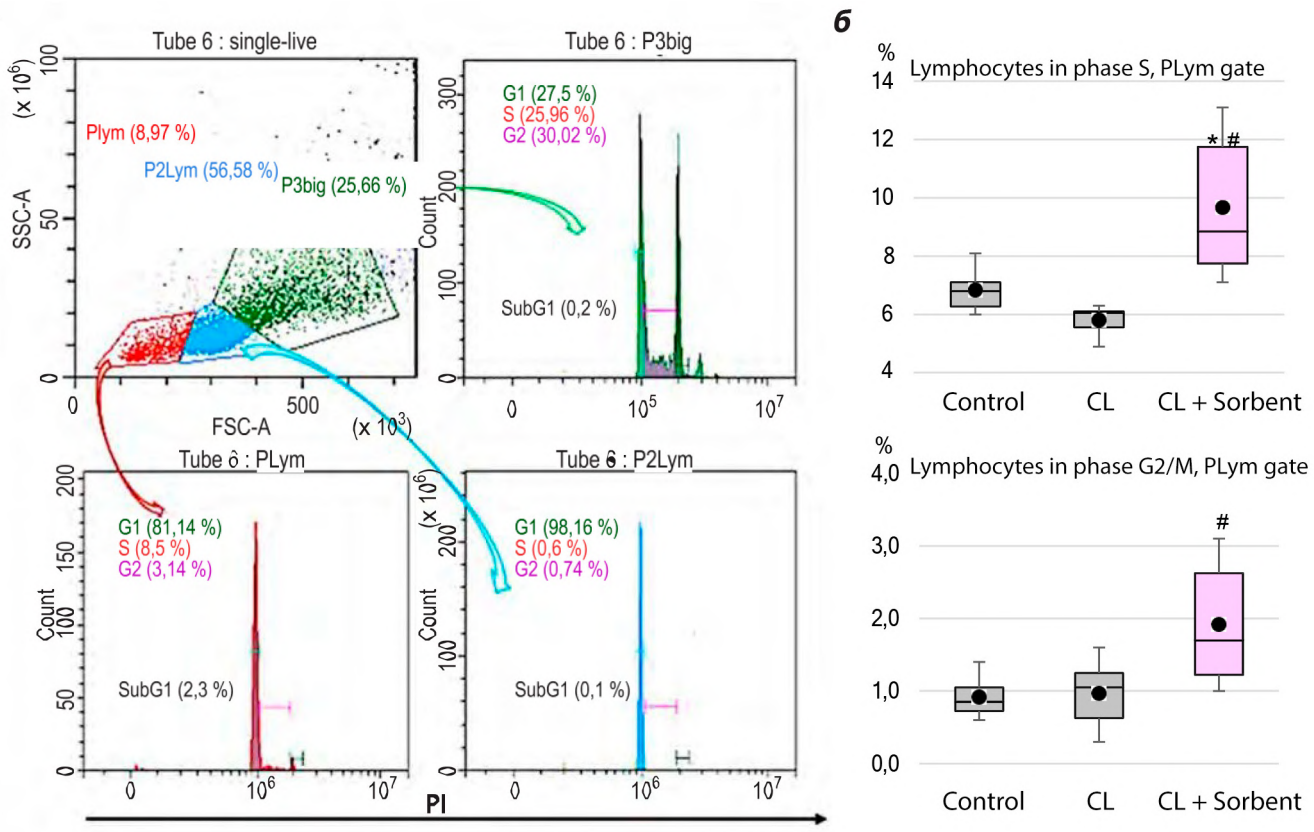

FIG. 2.
а *– flow cytometry histograms of the thymus cell cycle of C57Bl/6 mice: gating the thymus cells by size on the histogram in SSC/FSC coordinates; cell cycle histograms are presented for each selected region of thymus cells (shown by arrows): ordinate axis – the number of cells; abscissa axis – the fluorescence intensity of propidium iodide.* ***б*** *– histograms of the percentage of Plym gate lymphocytes in the S and G2/M cell cycle stages; * – compared to the control, # – compared to continuous lighting (CL) group (p < 0.05)*

Proliferation, differentiation and development of lymphocytes immunotolerant to the host with a wide range of TSR antigens occur in close contact with the epithelial cells of the thymus. In this case, negative selection of T-lymphocytes occurs in the medullary niches due to the expression of MHC-I (major histocompatibility complex) and MHC-II molecules on the surface, and cortical epithelial cells are responsible for positive selection. Maintenance of the cortical and medullary epithelial niches of the thymus is provided by epithelial cell precursors with long-term renewal and a high proliferation and differentiation rate with an estimated replacement time of one to two weeks from the first weeks of life. Maintenance of the epithelium of the thymus medulla in adults is provided by epithelial precursors that have lower self-renewal rates, but still retain a high proliferation rate [13].

In our study, the proliferative potential of the P3big gate cells was significantly higher than that of the PLym or P2Lym gate cells. The cellular elements of the P3big group are larger and can be macrophages, dendritic cells, and epithelial cells that make up the thymus stroma and provide an appropriate microenvironment for developing lymphocytes. The high proliferation rate indicates that these cellular elements are most likely epithelial cells, which are the main cells of the thymus niches. Continuous lighting had a negative effect on the organ stroma, statistically significantly reducing the numerical density of epithelial cells in the phase S to 26.55 (19.40; 27.55) % compared to the control – 32.50

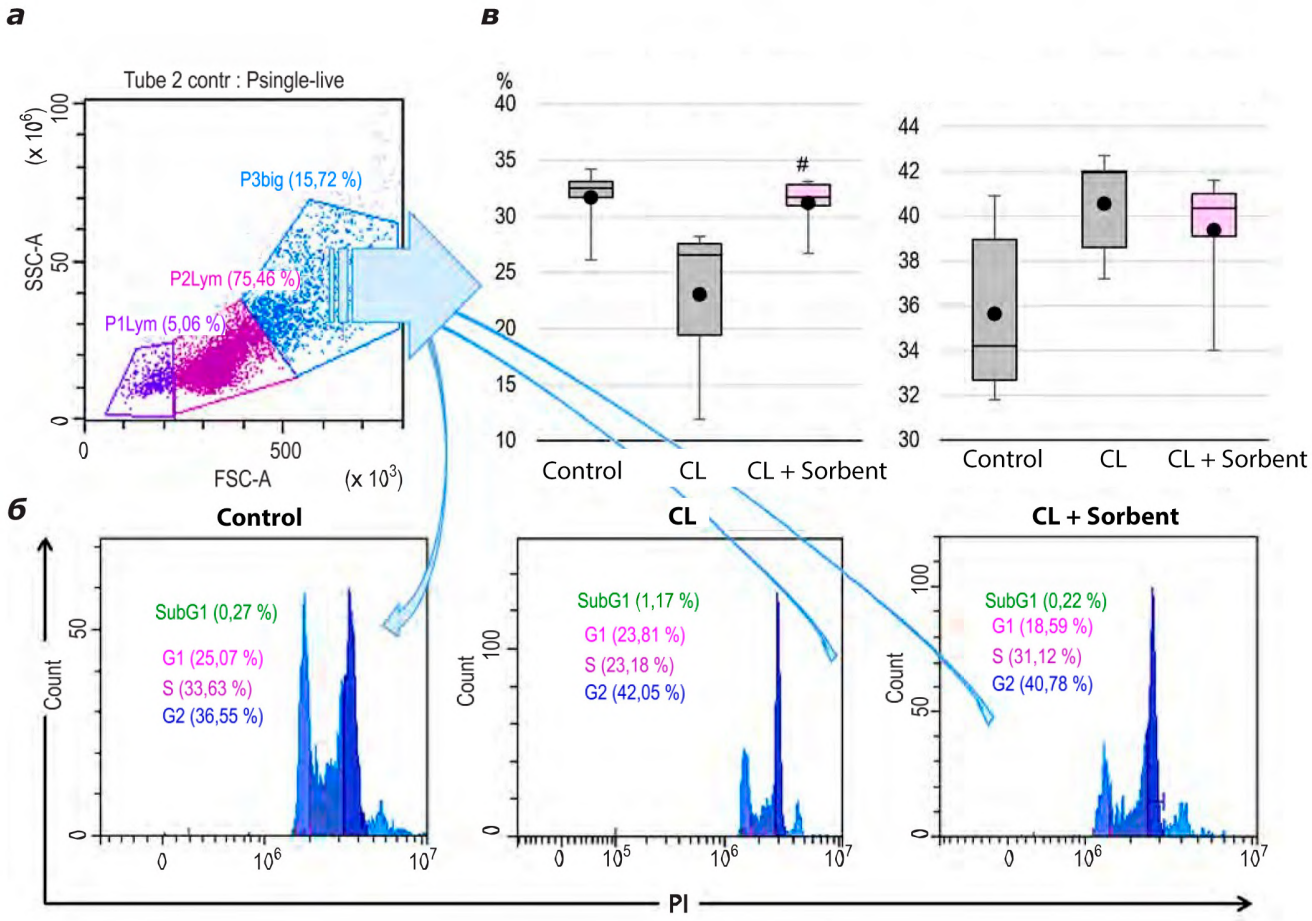

FIG. 3.
Flow cytometry histograms: ***а*** *– histogram of distribution of thymus cells in FSC/FSC coordinates, P3big gate is highlighted;* ***б*** *– histograms of distribution of P3big gate cells by phases of the cell cycle: ordinate axis – the number of cells, abscissa axis – propidium iodide;* ***в*** *– graphs of the relative number of P3big gate cells in the S and G2/M cell cycle stages: * – compared to the control, # – compared to continuous lighting (CL) group (p < 0,05)*

(31.68; 33.10) % (p = 0.0202). Sorbent administration against the background of CL normalized the proliferation of thymus epithelial cells, restoring their percentage in the phase S to 31.70 (30.95; 32.83) % (fig. 3). The content of epithelial cells in the G2/M phase did not change statistically significantly compared to the other groups of animals. Long-term and continuous lighting led to an increase in the content of epithelial cells at the apoptosis stage to 0.9 (0.73; 1.18) % (p = 0.0051). Sorbent administration against the background of continuous lighting contributed to a decrease in apoptosis to the control level (0.23 (0.22; 0.29) %; p = 0.0051).

Therefore, sorbent use against the background of long-term exposure to continuous lighting is advisable, since the sorbent supports the self-renewal of thymus epithelial cells, maintaining a high rate of epithelial cell proliferation and reducing apoptosis to the values of intact animals (fig. 3).

DISCUSSION

The results of the present study showed a connection between long-term disruption of circadian rhythms, disruption of thymus functioning and the sorbent normalizing role to support proliferation and preserve the viability of early precursors of T lymphocytes and epithelial cells of the thymus niches. A decrease in the body's resistance to any stress, in particular, with CL, is associated with insufficient functional activity of the thymus.

Previously, we showed that blockade of melatonin synthesis in mice under continuous lighting inhibits the processes of differentiation and maturation of young $CD3^{low}$ lymphocytes into mature $CD3^{hi}$ forms, leads to increased apoptosis of T lymphocytes in the thymus and, as a consequence, to leukopenia [12]. Under these conditions, the hypothalamic-pituitary-adrenal (HPA) axis is activated as part of the stress response, in which pituitary hormones stimulate the adrenal cortex to release glucocorticoids, including cortisol. Excess cortisol causes apoptosis of cortisol-sensitive early lymphocytes, which leads to T-cell immunity deficiency. This cortisol effect is considered to be a driver of thymus involution [4, 5, 14]. However, long-term continuous lighting affected not only a decrease in proliferative activity and apoptosis of young $CD3^{low}$ lymphocytes, but also a decrease in the proliferation of epithelial cells, which are the basis of both the cortical and medullary niches, which are important for maintaining the proliferation and selection of T lymphocytes. The sorption therapy use against the background of continuous lighting had a positive effect on these parameters. A possible protective mechanism of the sorbent use may be both the sorption and removal of excess cortisol, and a deeper effect mediated by the state and activity of the intestinal microbiota. Previous studies have shown that the use of the carbon-mineral sorbent SUMS-1 (based on aluminum oxide) increases the number of intestinal epithelial villi, normalizing the microbiome state [15]. Microbes have been shown to have a close relationship with the HPA axis [16]. Intervention in the intestinal microbiota can significantly affect the treatment of stress-related diseases. Stress causes changes in HPA axis hormones towards an increase in cortisol in germfree mice [17]. In addition, intestinal microbes regulate tryptophan metabolism, produce dopamine, γ-aminobutyric acid, histamine and acetylcholine, which affect the central nervous system function and the HPA axis stability [18]. On the other hand, stress itself can negatively affect the intestinal microbiome, inhibiting the vital activity of microbes, changing their secreted profile [19]. Based on the results of this study, it can be assumed that the sorbent created on the basis of aluminum oxide and polymethylsiloxane, sorbing oxidants, toxic metabolites, providing a detoxifying and lymphatic drainage effect, promotes proliferation and maintains the viability of early precursors of lymphocytes and epithelial cells of the thymus.

CONCLUSION

The conducted studies of the efficiency of porous hydrophilic-hydrophobic sorbent using based on combustion oxide and polydimethylsiloxane Al_2O_3@PDMS under conditions of long-term exposure to stress caused by continuous lighting for 14 days. The sorbent use ensures the thymus function preservation, maintaining the integrity and proliferation of young lymphocytes and epithelial cells of this section. The obtained data allow for preventive sorption therapy against the background of circadian rhythm disorders when changing time zones in people working at night or in urban northern regions with expected fluctuations in lighting throughout the year, helping to maintain the body's own stress forces, normalizing cellular immunity.

Conflicts of interest

No potential conflict of interest relevant to this article reported.

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EFFECT OF THE ORGANOSELENIUM COMPOUND 974ZH ON THE *TLR2* AND *TLR4* GENE EXPRESSION IN BLOOD AND SPLEEN CELLS OF EXPERIMENTAL ANIMALS WHEN CO-ADMINISTERED WITH *YERSINIA PESTIS* EV

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ABSTRACT

One of the important directions for increasing the immunogenic properties of vaccine strains against highly dangerous infections is the search for adjuvants that not only stimulate the immunological effectiveness of vaccination, but can also provide a metabolic correction of the vaccination process. Organoselenium compounds have immunotropic properties and an antioxidant effect, and therefore, the study of the effect of the organoselenium compound 2,6-dipyridinium-9-selenabicyclo[3.3.1]nonane dibromide (974zh) on the activity of the TLR2 and TLR4 gene expression by macroorganism cells of experimental animals immunized with Yersinia pestis EV NIIEG vaccine strain, is a current area of research.

The aim of the work. *To assess the TLR2 and TLR4 gene expression by cells of the immune phagocyte system of experimental animals immunized with the Y. pestis EV vaccine strain against the background of immunomodulation with the organoselenium compound 974zh.*

Materials and methods. *The study was carried out on 125 certified outbred white mice. Biological material (blood, spleen) was disinfected, and the spleen was homogenized. RNA isolation and reverse transcription were performed using commercial reagent kits. The expression level of the TLR2 and TLR4 genes was determined using real-time polymerase chain reaction with specific primers.*

Results. *When assessing innate immunity using the example of blood and spleen cells of animal models, features of the TLR2 and TLR4 gene expression were revealed in response to the introduction of the Y. pestis EV vaccine strain against the background of immunomodulation with the 974zh. It was found that 974zh induces a statistically significant increase in TLR2 gene expression when co-administered with Y. pestis EV at a dose of both 10^4 CFU and 10^3 CFU.*

Conclusion. *Y. pestis EV against the background of immunomodulation with 974zh, stimulates the expression of the TLR2 and TLR4 genes, which may indicate an increase in the immunogenic properties of the Y. pestis EV vaccine strain under the influence of this preparation.*

Key words: *gene expression, TLR, organoselenium compound, immunity*

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ВЛИЯНИЕ СЕЛЕНОРГАНИЧЕСКОГО СОЕДИНЕНИЯ 974ZH НА ЭКСПРЕССИЮ ГЕНОВ *TLR2* И *TLR4* В КЛЕТКАХ КРОВИ И СЕЛЕЗЁНКИ ЭКСПЕРИМЕНТАЛЬНЫХ ЖИВОТНЫХ ПРИ СОВМЕСТНОМ ВВЕДЕНИИ С *YERSINIA PESTIS* EV

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РЕЗЮМЕ

Одним из важных направлений повышения иммуногенных свойств вакцинных штаммов против особо опасных инфекций является поиск адъювантов, которые не только стимулируют иммунологическую эффективность вакцинации, но и могут оказывать метаболическую коррекцию вакцинального процесса. Селенорганические соединения обладают иммунотропными свойствами и антиоксидантным эффектом, в связи с чем изучение влияния селенорганического соединения 2,6-дипиридиний-9-селенабицикло[3.3.1]нонан дибромида (974zh) на активность экспрессии генов TLR2 и TLR4 клетками макроорганизма экспериментальных животных, иммунизированных вакцинным штаммом Yersinia pestis EV НИИЭГ, является актуальным направлением исследований.

Цель работы. *Оценка экспрессии генов TLR2 и TLR4 клетками иммунофагоцитарной системы экспериментальных животных, имунизированных вакцинным штаммом Y. pestis EV на фоне иммуномодуляции селенорганическим соединением 974zh.*

Материалы и методы. *Исследование проводили на 125 сертифицированных беспородных белых мышах. Биологический материал (кровь, селезёнка) обеззараживали, селезёнку гомогенизировали. Выделение РНК и обратную транскрипцию осуществляли с помощью коммерческих комплектов реагентов. Уровень экспрессии генов TLR2 и TLR4 определяли методом полимеразной цепной реакции в реальном времени с использованием специфических праймеров.*

Результаты. *При оценке врождённого иммунитета на примере клеток крови и селезёнки биомоделей выявлены особенности экспрессии генов TLR2 и TLR4 в ответ на введение вакцинного штамма Y. pestis EV на фоне иммуномодуляции препаратом 974zh. Установлено, что данный препарат индуцирует статистически значимое повышение экспрессии генов TLR2 при совместном введении с Y. pestis EV как в дозе 10^4 КОЕ, так и в дозе 10^3 КОЕ.*

Заключение. *Таким образом, Y. pestis EV на фоне иммуномодуляции препаратом 974zh стимулирует экспрессию генов TLR2 и TLR4, что может свидетельствовать о повышении иммуногенных свойств вакцинного штамма Y. pestis EV под влиянием этого препарата.*

Ключевые слова: *экспрессия генов, TLR, селенорганическое соединение, иммунитет*

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INTRODUCTION

The greatest contribution to the prevention and control of infectious diseases has been made by vaccination aimed at developing an immune response that provides immunity to infectious disease pathogens. Great success in the control and prevention of especially dangerous infections has been achieved thanks to the already created vaccines. Most vaccines against especially dangerous infections are attenuated strains of pathogens or inactivated pathogens that have not only immunogenic and protective activity, but also residual virulence and reactogenicity. Chemical, subunit and combination vaccines do not have sufficient immunogenic activity and cannot be compared with live vaccines in terms of effectiveness [1]. Therefore, the search for non-specific factors that can reduce the negative impact degree of live vaccines on the body and increase their immunogenic activity, allowing to reduce the dose of antigen, is relevant. Adjuvants can enhance the immune response to the vaccine administration through various mechanisms, including activation of humoral and cellular factors of innate immunity [1]. Natural immunity forms an early line of macroorganism defense against microbes with subsequent initiation of adaptive immunity. Adjuvants are able to initiate immune reactions of the innate immune system through pattern recognition receptors (PRRs) [2].

One of the PRR families includes Toll-like receptors (TLRs), which recognize the molecular structures of pathogens and are an important element in the mechanism of both innate and adaptive immune responses [3]. To date, at least 10 types of TLRs have been identified in humans and 13 in mice. TLRs are mainly expressed on tissue cells that perform immune functions and directly communicate with pathogens. The receptors differ in adapter proteins and are localized both on the cell membrane and inside the cell, ensuring the intracellular activation signal conduction [4].

Toll-like receptors of types 2 and 4 are believed to be specific for the early encounter of pathogenic bacteria with host cells. Receptors of these two types have a wide range of activating ligands localized on bacteria. *TLR2* and *TLR4* are expressed on monocytes, macrophages, neutrophils and myeloid dendritic cells, on endothelium, intestinal epithelial cells and hepatocytes. When TLR interacts with ligands, primary proinflammatory factors are induced, followed by immediate development of mechanisms of both innate and acquired immunity.

The use of immunomodulatory adjuvants makes it possible to activate innate immunity by stimulating TLR, which can be used in the development of new drugs for the treatment of infectious diseases and the creation of vaccines for their prevention [5]. In recent years, synthetic origin compounds that affect immunogenesis have been actively studied and introduced into practice as adjuvants [6, 7].

Previously conducted animal experiments have shown that the experimental synthetic organoselenium drug 2,6-dipyridinium-9-selenabicyclo[3.3.1]nonane dibromide (974zh) has immunotropic properties. Combined use with the *Y. pestis* EV NIIEG vaccine strain allows maintaining its immunogenicity while reducing the antigen load by an order of magnitude (from 10^4 CFU to 10^3 CFU) and reduces the allergic reaction during vaccination [8, 9].

Considering the positive effect of this compound on the macroorganism, studies of an experimental synthetic organoselenium compound in combination with vaccine strains as an adjuvant, which will induce an increase in the expression of Toll-like receptor genes, are promising.

THE AIM OF THE STUDY

Study of the organoselenium compound 974zh effect on the expression of *TLR2* and *TLR4* genes in peripheral blood cells and spleen of experimental animals when administered *Y. pestis* EV NIIEG.

MATERIALS AND METHODS

In this study, we used the synthetic organoselenium compound 2,6-dipyridinium-9-selenabicyclo[3.3.1] nonane dibromide (974zh) (A.E. Favorsky Irkutsk Institute of Chemistry, Siberian Branch of the Russian Academy of Sciences) and the vaccine strain *Y. pestis* EV NIIEG (Irkutsk Antiplague Research Institute, Rospotrebnadzor).

In the experiment, certified outbred white mice (125 pcs.) of both sexes, standard in weight (18–20 g) and housing conditions, were used as biomodels. Work with animals was carried out in accordance with the requirements of Directive 2010/63/EU of the European Parliament and of the Council of the European Union of September 22, 2010 on the protection of animals used for scientific purposes and the "Rules of Good Laboratory Practice" approved by Order of the Ministry of Health No. 199n dated April 01, 2016. The study was approved by the local Ethics Committee of the institute (protocol No. 3 dated June 01, 2020; protocol No. 7 dated November 15, 2021).

The animals were divided into four experimental groups and one control group (25 individuals each). The test 974zh at a dose of 2.5 mg/kg of live weight was administered to the animals subcutaneously in the left hind paw in a volume of 0.5 ml, the cell culture was administered subcutaneously in the right hind paw in a volume of 0.5 ml. Biomodels of the 1st experimental group were inoculated with the *Y. pestis* EV NIIEG vaccine strain at a dose of 10^3 CFU per individual, those of the 2nd experimental group – at a dose of 10^4 CFU, those of the 3rd experimental group – at a dose of 10^3 CFU together with the drug 974zh, those of the 4th experimental group – at a dose of 10^4 CFU in combination with 974zh. Intact white mice were used in the control (V) group. Observations were carried out for 21 days. White mice were humanely removed from the experiment. Biological material was collected

on the 1st, 3rd, 7th, 14th and 21st days. The spleen was homogenized (grinded). Whole blood was used.

The material was disinfected according to the guidelines MU 3.5.5.1034-01. Samples in a volume of 100 µl were placed in Eppendorf microcentrifuge tubes, mixed with 300 µl of lysis buffer and heated at a temperature of 65 °C for 15 min.

The RNA molecule was isolated using a reagent kit for the isolation of total RNA from whole blood, cell cultures and tissue samples RNA-EKSTRAN (OOO Sintol, Moscow). To cleave residual double-stranded DNA in the samples, the enzyme DNase (OOO Sintol, Moscow) was used according to the attached instructions.

The synthesis of the first DNA strand on the RNA matrix was carried out using a set of reagents for the reverse transcription (RT) reaction (OOO Sintol, Moscow).

The expression of *TLR2* and *TLR4* was studied using a reagent kit for real-time polymerase chain reaction (RT-PCR) in the presence of SYBS Green 1 (OOO Sintol, Moscow). RT-PCR was carried out in a thermocycler detecting DT-prime (OOO DNA Technologies, Moscow). Specific primers and their sequences used in the reaction are presented in Table 1.

Statistical processing of the obtained data was performed using the MS Excel software package (Microsoft Corp., USA). Statistical significance was assessed using the Mann – Whitney U-test. The relative concentration (RC) of TLR gene copies was calculated in the RealTime_PCR v. 7.7 program (DNA Technologies LLC, Moscow) and expressed in conventional units (c.u.). The results were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

It was established that the organoselenium compound 974zh induces an increase in the expression of *TLR2* genes in the blood on the 1st day with a subsequent increase by the 3rd and 7th days, and in the spleen – on the 3rd and 14th days when this drug is administered together with the vaccine strain *Y. pestis* EV at a dose an order of magnitude lower than the generally accepted one, i.e. 10^3 CFU. Thus, in group III of animals, the relative concentration of TLR2 in the blood was 12.8 c.u. on the 1st day of the study, 37.9 c.u. on the 3rd day, and 25 c.u. on the 7th day. Thus, the median values of TLR2 RC in blood cells were 2.8 times higher than the values of group I on the 1st day of the study, 12.3 times on the 3rd, and 22.7 times ($p < 0.05$) on the 7th day. The median value in animals of group III was 2.1 times higher on the 1st day and 7.1 times ($p < 0.05$) on the 3rd day compared to group II. In addition, the level of *TLR2* gene expression in animals of group III statistically significantly increased compared to that in group IV and was 2.8 times higher on the 1st day, 11.6 times on the 3rd, 13.2 times on the 7th, and 2.8 times ($p < 0.05$) on the 14th day of the study. In the blood cells of animals of group I, the expression of this type of Toll-like receptor did not increase and was lower than in the other groups of experimental animals (fig. 1a).

In the spleen cells of white mice of group III, the RC expression of *TLR2* genes was significantly higher compared to the same indicator in other experimental groups and in the control. On the 3rd day, the RC was 947 c.u., which is 4.7 times ($p < 0.05$) higher than in group I, 1.7 times ($p < 0.05$) higher than in group II, and 155.2 times ($p < 0.05$) higher than in group IV. On the 14th day of observation, the RC of group III was 501.1 c.u. and was statistically significantly higher than the same indicator in all other groups of experimental animals (fig. 16).

The level of *TLR4* gene expression in the blood of experimental animals immunized with *Y. pestis* EV (10^3 CFU) in combination with an organoselenium compound (Group III) was statistically significantly higher by 5.5 times ($p < 0.05$) than the expression of genes of this type of Toll-like receptor in animals that were administered only the vaccine strain *Y. pestis* EV at a dose of 10^4 CFU (Group II), and 2.4 times ($p < 0.05$) higher compared to mice immunized with *Y. pestis* EV at a dose of 10^4 CFU together with the 974zh (Group IV) on the 1st day of observation. A statistically significant increase in the median RC expression of *TLR4* genes was observed on the 7th day in blood samples of animals of Group III relative to animals of Group I and was 3.9 times higher ($p < 0.05$). In other cases, no statistically significant increase in expression occurred. On the 3rd, 14th, and 21st days, no statistically significant increase in the relative concentration of *TLR4* gene expression in blood cells was observed (fig. 2a).

Administration of the experimental 974zh in combination with *Y. pestis* EV at a dose of 10^3 CFU (Group III) to biomodels increased the expression of *TLR4* genes in spleen cells. On the 1st day, an increase in concentration was observed compared to Group V (control) and Group II, where white mice were administered only the vaccine strain *Y. pestis* EV at a dose of 10^4 CFU. Compared to other experimental groups, *TLR4* expression was lower on the 1st day. The organoselenium preparation induced *TLR4* gene expression on the 3rd and 14th days. The relative concentration of gene expression in Group III on the 3rd day was 1500 c.u. i.e., 17.8 times ($p < 0.05$) higher than the concentration in group I, 2.3 times ($p < 0.05$) higher than in group II, and 256.4

TABLE 1

SEQUENCE OF MOUSE TLR PRIMERS FOR REAL-TIME PCR

No.	Gene	Sequence (5′ → 3′)	Size (bp)
1	TLR2	F: 5′-CAGCTGGAGAACTCTGACCC-3′ R: 5′-CAAAGAGCCTGAAGTGGGAG-3′	193
2	TLR4	F: 5′-CAA CAT CAT CCA GGA AGGC-3′ R: 5′-GAA GGC GAT ACA ATT CCA CC-3′	206

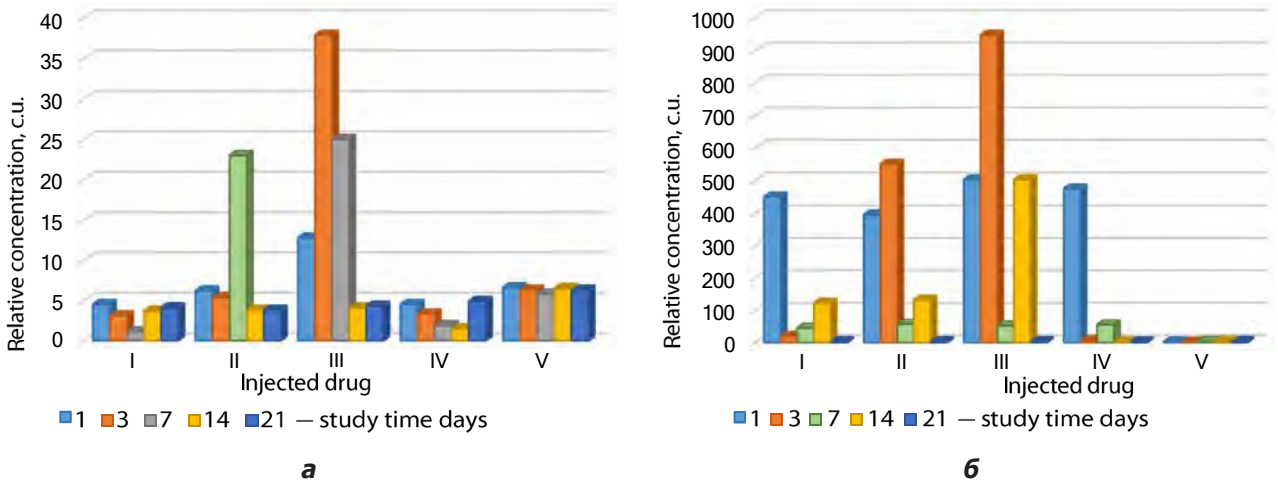

FIG. 1.
*Relative concentration of TLR2 gene expression in blood (**а**) and spleen (**б**) cells of white mice (c. u.): I – Y. pestis EV at a dose of 103 CFU; II – Y. pestis EV at a dose of 104 CFU; III – Y. pestis EV at a dose of 103 CFU + 974zh; IV – Y. pestis EV at a dose of 104 CFU + 974zh; V – control group; * – p < 0.05*

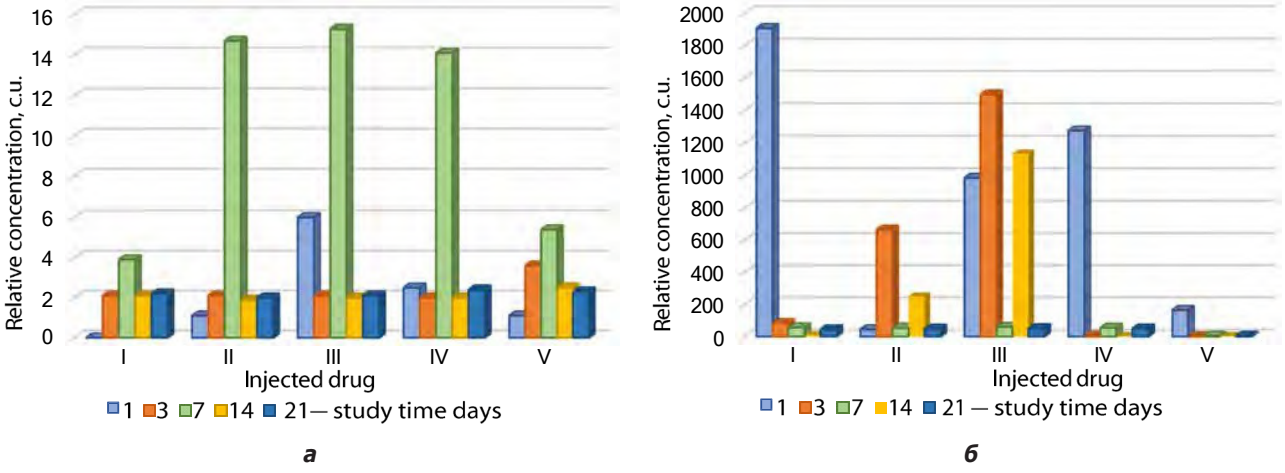

FIG. 2.
*Relative concentration of TLR4 gene expression in blood (**а**) and spleen (**б**) cells of white mice (c.u.): I – Y. pestis EV at a dose of 103 CFU; II – Y. pestis EV at a dose of 104 CFU; III – Y. pestis EV at a dose of 103 CFU + 974zh; IV – Y. pestis EV at a dose of 104 CFU + 974zh; V – control group; * – p < 0.05*

times ($p < 0.05$) higher than in group IV. On the 14th day, the level of *TLR4* gene expression in the spleen cells of animals in group III was also statistically significantly higher than in groups I, II, and IV, by 133.7, 4.5, and 283.8 times, respectively. On the 7th and 21st days, no statistically significant differences were recorded between the median values of *TLR4* gene expression in the group of biomodels immunized with *Y. pestis* EV at a dose of 10^3 CFU in combination with 974zh and other experimental groups (fig. 2б).

CONCLUSION

Thus, the revealed features of *TLR2* and *TLR4* gene expression in the innate immune cells of biomodels in response to the administration of *Y. pestis* EV NIIEG strains in terms of level and kinetics depend on the amount of the administrated vaccine strain antigen (CFU) and the presence or absence of the administrated 974zh. This experimental organoselenium compound induces an increase in the expression of *TLR2* genes when co-administered with *Y. pestis* EV at a dose an order of magnitude lower than the generally accepted one (10^3 CFU) on the 1st, 3rd and 7th days of observation in blood cells and on the 3rd and 14th days in spleen cells.

In response to the administration of the 974zh with the *Y. pestis* EV vaccine strain to experimental biomodels, an increase in *TLR4* expression was recorded in blood cells on the 1st and 7th days, in spleen cells – on the 1st, 3rd and 14th days of the experiment. Studies have shown that when administrating

a synthetic organoselenium compound in combination with the *Y. pestis* EV vaccine strain with a decrease in the immunizing dose to 10^3 CFU, there is an increase in the expression of the *TLR2* and *TLR4* genes in the blood and spleen cells of biomodels at almost all observation periods.

The *Y. pestis* EV administration both at a standard dose (10^4 CFU) and at a dose an order of magnitude lower (10^3 CFU), but without the experimental organoselenium preparation, did not cause significant and statistically significant changes in the expression of genes of the studied types of Toll-like receptors.

All of the above suggests that increased expression of *TLR2* and *TLR4* genes can enhance the immune response to the vaccine and contribute to effective combat against plague infection. Therefore, the experimental selenium-containing compound 2,6-dipyridinium-9-selenabicyclo[3.3.1]nonane dibromide (974zh) can be considered as a promising adjuvant.

Conflicts of interest

No potential conflict of interest relevant to this article reported.

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ADAPTING THE PROTOCOL FOR STUDYING THE FUNCTIONAL CAPACITY OF T LYMPHOCYTES THAWED FROM CRYOPRESERVATION

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ABSTRACT

Background. *Immunological studies are impossible without long-term storage of cryopreserved biomaterial. There are no standard procedures for working with cryopreserved mononuclear leukocytes.*

The aim of the study. *To optimize the protocol for culturing T lymphocytes thawed after cryopreservation by assessing their viability and proliferative capacity.*

Methods. *Mononuclear leukocytes were isolated from the peripheral blood of relatively healthy volunteers (n = 18). Cells were subjected to controlled freezing down to –80 °C and were transferred to liquid nitrogen. First step: after thawing, the cells were stained with CFSE (carboxyfluorescein succinimidyl ester), were divided into two parts and cultured in the presence/absence of interleukin 2 (IL-2). Cell proliferation was stimulated with phytohemagglutinin (type P). Cells were incubated for 7 days. Sample analysis was performed using flow cytometry. Second stage: thawed cells were divided into three parts. Two parts were resuspended in a full growth medium with IL-2 and were placed in a thermostat (+37 °C) to "rest" for one hour or overnight. After "resting", the cells were stained with CFSE. One third of the thawed leukocytes were stained with CFSE immediately after thawing. Cells were stimulated, cultured and analyzed the same way at both stages of the study.*

Results. *It has been established that adding IL-2 to the culture medium contributes to a better cell survival. In the presence of IL-2, stimulated $CD4^+$ and $CD8^+$ T lymphocytes produced more daughter cell generations. At the end of the 7-day incubation "rested" samples had reduced leukocyte counts compared to the samples that were cultured immediately after thawing. The number of daughter cell generations formed by stimulated $CD4^+$ and $CD8^+$ T cells decreased when the "rest" stage was included into the study protocol.*

Conclusion. *Adding IL-2 into culture medium can increase the viability and mitotic capacity of thawed T cells, making their state more similar to that of freshly isolated lymphocytes. Cell "rest" after thawing negatively affects the viability and proliferative activity of T lymphocytes during their weekly incubation.*

Key words: *T lymphocytes, cryopreservation, primary cell culture, interleukin 2, rest*

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МОДИФИКАЦИЯ ПРОТОКОЛА ИССЛЕДОВАНИЯ ФУНКЦИОНАЛЬНОЙ АКТИВНОСТИ ОТТАЯВШИХ ПОСЛЕ КРИОКОНСЕРВАЦИИ Т-ЛИМФОЦИТОВ

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РЕЗЮМЕ

Обоснование. *Иммунологические исследования невозможны без длительного хранения биоматериала в условиях криоконсервации. Стандартные методики работы с мононуклеарными лейкоцитами, подвергавшимися криоконсервации, отсутствуют.*

Цель исследования. *Оптимизировать протокол культивирования оттаявших после криоконсервации Т-лимфоцитов по оценке их жизнеспособности и пролиферативной активности.*

Методы. *Мононуклеарные лейкоциты выделяли из периферической крови относительно здоровых добровольцев (n = 18). Клетки подвергали контролируемому замораживанию до –80 °С и переносили в жидкий азот. Первый этап: после оттаивания клетки окрашивали CFSE (carboxyfluorescein succinimidyl ester), делили на две части и культивировали в присутствии/отсутствии интерлейкина 2 (ИЛ-2). Пролиферацию клеток стимулировали фитогемагглютинином-П. Клетки инкубировали в течение 7 суток. Анализ образцов проводили методом проточной цитофлюориметрии. Второй этап: оттаявшие клетки делили на три части. Две части ресуспендировали в полной питательной среде с ИЛ-2 и помещали в термостат (+37 °С) для «отдыха» на 1 час или на ночь. После «отдыха» клетки окрашивали CFSE. Третью часть размороженных лейкоцитов окрашивали CFSE сразу после оттаивания. Клетки стимулировали, культивировали и анализировали единообразно на обоих этапах исследования.*

Результаты. *Установлено, что добавление ИЛ-2 в культуральную среду способствует лучшему выживанию клеток. Кроме того, в присутствии ИЛ-2 стимулированные $CD4^+$ и $CD8^+$ Т-лимфоциты производят больше дочерних генераций. По сравнению с пробами, сразу помещёнными в культуру, в пробах, прошедших «отдых», снижено число лейкоцитов по окончании 7-суточной инкубации. Количество дочерних генераций, формируемых стимулированными $CD4^+$ и $CD8^+$ Т-клетками, снижается при включении этапа «отдых» в протокол исследования.*

Заключение. *Внесение ИЛ-2 в культуральную среду может увеличить жизнеспособность и митотическую активность размороженных Т-клеток, приближая их состояние к таковому свежевыделенных лимфоцитов. «Отдых» клеток после оттаивания оказывает негативный эффект на жизнеспособность и пролиферативную активность Т-лимфоцитов при их последующей недельной инкубации.*

Ключевые слова: *Т-лимфоциты, криоконсервация, культивирование, ИЛ-2, отдых*

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BACKGROUND

Effective research in the field of immunology is impossible without constant access to biological material, which requires its collection, accumulation and storage. Today, the only way to store peripheral blood nuclear cells for a long time is to cryopreserve them: keep them at very low temperatures (–80…–196 °C). The use of biobanks, which allow biological samples to be accumulated and stored in liquid nitrogen, significantly facilitates research aimed at studying the causes of various diseases, developing and testing drugs [1].

It should be noted that cryopreservation can affect the expression of phenotypic markers and the functional activity of peripheral blood mononuclear cells [2, 3]. To mitigate the cryopreservation effects, a number of researchers [4, 5] proposed to introduce a "rest" stage into the work protocol after the thawing procedure: placing the cells in a complete culture medium (CCM) for a period of 1 to 24 hours at a temperature of +37 °C. It was shown that this procedure has a positive effect on the cells, in particular, it promotes the functionality restoration of antigen-specific T lymphocytes when assessed by the ELISPOT method [6–10]. Other researchers [11–14] proposed enriching the culture medium with additives such as cytokines, in particular interleukin 2 (IL-2), sodium pyruvate, nonessential amino acids, β-mercaptoethanol, etc., which should promote better survival of T lymphocytes in culture and ultimately increase their functional activity. However, to date, methods for working with mononuclear leukocytes that have undergone long-term (more than 24 months) cryopreservation have not been developed.

THE AIM OF THE STUDY

To optimize the protocol for culturing T lymphocytes thawed after cryopreservation by assessing their viability and proliferative capacity.

METHODS

Study participants. Volunteers (n = 18; 39 % women; average age 37.4 ± 1.2 years) participated in the study as peripheral blood donors. The inclusion criterion for the study was age over 18 years. Exclusion criteria: acute infectious diseases less than 4 weeks before the start of the study; pregnancy.

Obtaining biomaterial. Blood was collected on an empty stomach from the cubital vein into vacuum tubes (Weihai Hongyu Medical Devices Co, Ltd., China) containing ethylenediaminetetraacetic acid. Mononuclear leukocytes were isolated by the standard method by centrifuging twice diluted blood with Dulbecco's phosphate-buffered saline (DPBS; Gibco, USA) in a Diacoll density gradient (1.077 g/ml; Diaem, Russia). Isolated cells were washed twice in DPBS solution and placed in a medium containing 90 % heat-inactivated fetal calf serum (FCS; Biowest, Columbia) and 10 % intracellular cryoprotectant dimethyl sulfoxide (AppliChem, Germany). Cells resuspended in this medium were transferred to cryovials and subjected to controlled freezing in commercial CoolCell racks (Corning, USA) with a controlled temperature decrease rate (–1 °C/min) in a freezer (–80 °C) for 24 hours to minimize cell damage. Samples were then transferred to a liquid nitrogen tank (–196 °C) and stored until further use. The average storage time of samples was 40 ± 1.4 months.

Before the study, mononuclear leukocytes were thawed at +37 °C in a water bath for 1–2 min. The cells were transferred to 15 ml tubes, after which 10 ml of CCM were added dropwise to the samples: RPMI-1640, containing 25 mM Hepes and 2 mM L-glutamine (Gibco, USA), with the addition of 10 % FBS, 100 units/ml penicillin and 100 μg/ml streptomycin (Sigma, USA). The samples were gently mixed, tilted from side to side, and centrifuged for 10 min at 400 g. The cell pellet was resuspended in CCM. The viability of thawed leukocytes, when assessed with the standard trypan blue method, was at least 92 %.

Cultivation of mononuclear leukocytes. In the first stage of the study, the cells prepared after thawing were stained with 5 μM 5,6-carboxyfluorescein diacetate-N-succinimidyl ester (CFSE, carboxyfluorescein succinimidyl ester; Biolegend, USA) and washed twice with RPMI-1640 medium containing 20 % FCS. The cells were then counted in a Goryaev chamber, divided into two parts and resuspended at a concentration of 1×10^6/ml in CCM with or without IL-2 addition (100 ng/ml; Gibco, USA) (fig. 1a). Cell proliferation was stimulated with phytohemagglutinin-P (PHA; Serva, Germany) at a final concentration of 15 μg/ml. Unstimulated cells were used as a control. The total duration of leukocyte cultivation was 7 days, with the culture medium in each sample being replaced with a medium of similar composition on the 3rd–4th days. To maintain a constant pH of the medium, the cells were incubated in a desiccator with a candle placed in a thermostat (+37 °C). At the end of the incubation time, the cells were collected, counted, and stained with anti-CD3-BV605, anti-CD4-PE, and anti-CD8-BV510 antibodies (Biolegend, USA). Zombie UV dye (Biolegend, USA) was used to assess the viability of mononuclear leukocytes.

In the second stage of the study (fig. 1b), the cells were cultured in CCM containing 100 ng/ml IL-2. The mononuclear leukocytes obtained after thawing were divided into three parts, two of which were placed in the culture medium and put into a thermostat (+37 °C) for "rest" for 1 hour (H) or overnight (O). After "resting", the cells were stained with CFSE, stimulated with PHA, and cultured according to the previously described protocol. The third part of the thawed leukocytes was stained with CFSE immediately after thawing (I) and cultured in the presence of PHA according to the above protocol. Cultivation, medium change, cell counting, and analysis of the results were carried out uniformly throughout the study.

Flow cytometry. Analysis of mononuclear leukocytes was performed using a CytoFLEX S flow cytometer (Beckman Coulter, USA). The gating approach is shown in Figure 2.

Statistical data processing. Statistical analysis and data visualization were performed using GraphPad Prism 8 software (GraphPad Software, USA). Quantitative data in the text and tables are presented as means and their standard errors. Student's *t*-test was used to compare two groups of quantitative data; one-way ANOVA was used for several groups; multiple comparisons between groups were performed using Tukey's test. The critical significance level for testing statistical hypotheses was taken to be 0.05.

RESULTS

Modification of complete culture medium by introducing IL-2. The viability of peripheral blood mononuclear leukocytes thawed after long-term (40 ± 1.4 months) cryopreservation and cultured for 7 days under various conditions was analyzed. It was shown that the number of cells sharply decreases at the end of the cultivation period: on average, 33 % of the number of initially introduced cells remained in the sample.

At the same time, it was found that the IL-2 addition to the culture medium promotes better survival of mononuclear leukocytes (fig. 3). The most pronounced positive effect on viability was noted among cells stimulated with PHA. Thus, the number of leukocytes in samples containing IL-2 was significantly higher than in samples without added cytokine ($p < 0.001$). Similar results were obtained in the study of unstimulated cells. However, under these cultivation conditions, the differences in the number of cells between samples containing and not containing IL-2 did not reach the level of statistical significance ($p > 0.05$).

A positive effect of exogenously added IL-2 on the proliferative capacity of T cells thawed after long-term cryopreservation was revealed. It was shown that in the presence of this cytokine *in vitro* stimulated $CD4^+$ and $CD8^+$ T lymphocytes produce more daughter generations (fig. 4).

Thus, it was determined that the IL-2 addition to the culture medium increases both the viability and the proliferative capacity of T lymphocytes that have undergone long-term cryopreservation.

The mononuclear leukocyte thawing protocol modification by introducing a cell "rest" stage. Three cell`s thawing protocols were studied: without "rest", with an hour or overnight "rest". In each case, the culture medium contained IL-2.

A negative effect of "rest" on the viability of mononuclear leukocytes in the culture was established. Cell counting after 7 days of incubation with PHA showed that, compared to samples "I", the number of leukocytes in samples "H" ($p < 0.05$) and "O" ($p < 0.001$) was reduced

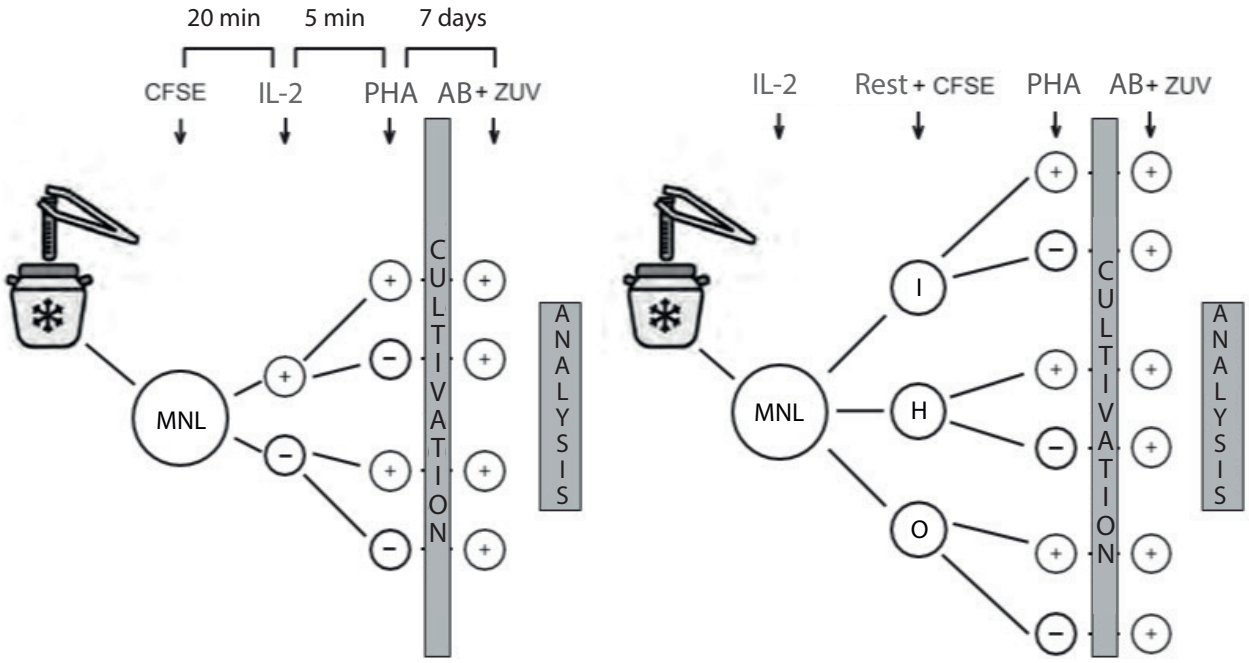

FIG. 1.
Study overview: a – first stage of the experiment; b – second stage of the experiment. MNL – mononuclear leukocytes; AB + ZUV – a cocktail of anti-CD3-BV605, anti-CD4-PE, anti-CD8-BV510 antibodies and Zombie UV vital stain. Types of "rest": I – immediately after thawing (without "rest"); H – hourly "rest"; O – overnight "rest"

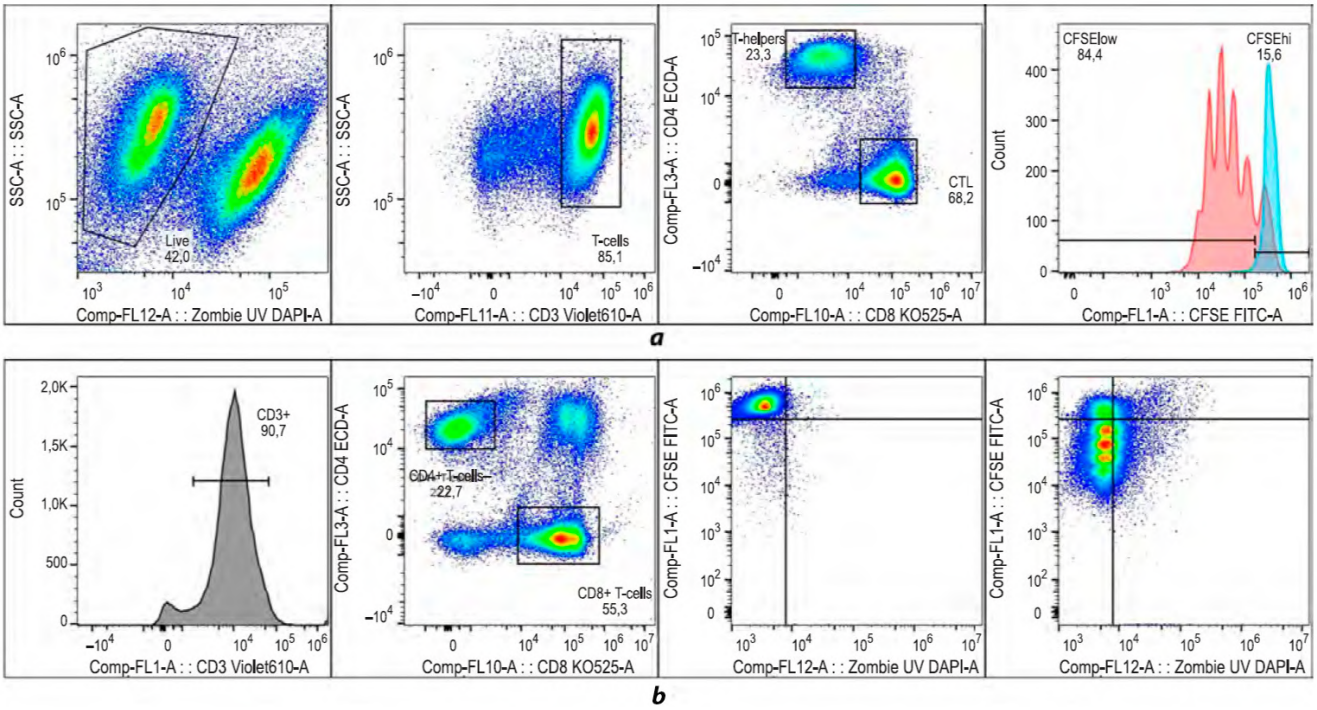

FIG. 2.
The gating approach used to distinguish mononuclear leukocytes (typical scatter diagrams). a – enumeration of daughter cell generations in stimulated T lymphocyte samples: viable elements isolation followed by CD3+ T cells gating, subsequent CD4⁺ and CD8⁺ subsets isolation, and CFSE staining analysis. b – determination of the dividing T lymphocytes viability: CD3⁺ T cells isolation with subsequent dividing into CD4⁺ and CD8⁺ T lymphocytes subsets, gating of unstimulated sample, following the simultaneous analysis of vital Zombie UV and CFSE staining of stimulated sample

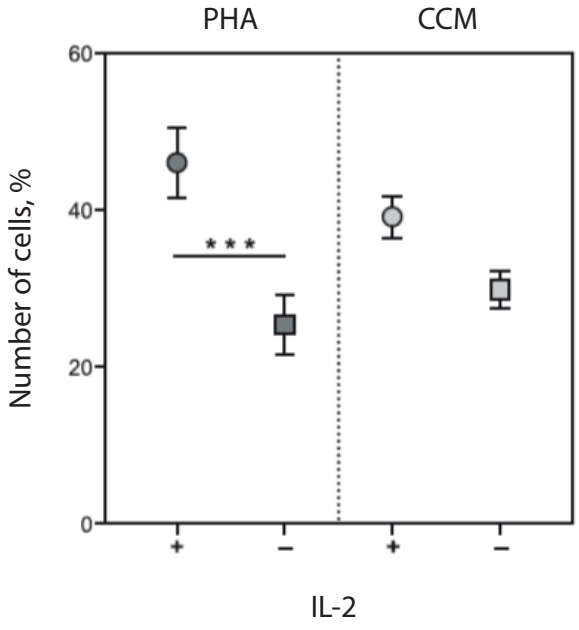

FIG. 3.
*The impact of adding IL-2 to the culture medium on the mononuclear leukocytes count following 7 days of incubation. The means and standard errors of the means are presented (n = 18 in each group). One-way analysis of variance was used to compare groups of quantitative data; multiple comparisons between groups were performed using Tukey's test; *** – p < 0.001*

(fig. 5a). It is important to note that the "rest" duration significantly affected the viability of stimulated cells: the differences between samples "H" and "O" were statistically significant ($p < 0.05$). At the same time, in samples "O" the proportion of surviving cells was reduced by more than 2 times compared to that in samples "H". In addition, it turned out that stimulated cells were more sensitive to the negative effects of "rest" than dormant elements. In cultures without the PHA addition, differences in cell numbers were revealed only between samples "I" and "O" ($p < 0.05$).

Analysis of the proliferative capacity of CD4⁺ and CD8⁺ T lymphocytes confirmed that "rest" is a negative factor. It was shown that the number of daughter generations formed by stimulated CD4⁺ and CD8⁺ T lymphocytes decreases when the "rest" stage of any time mode is included in the study protocol (fig. 5b).

It is noteworthy that the relative number of $CFSE^{low}$ cells that entered division among stimulated CD4⁺ and CD8⁺ T lymphocytes did not depend on the type of "rest" ($p > 0.05$; fig. 5c).

The introduction of an overnight "rest" into the thawing protocol significantly increased the percentage of dying cells among the CD4⁺ T lymphocytes that had entered division after being stimulated *in vitro*

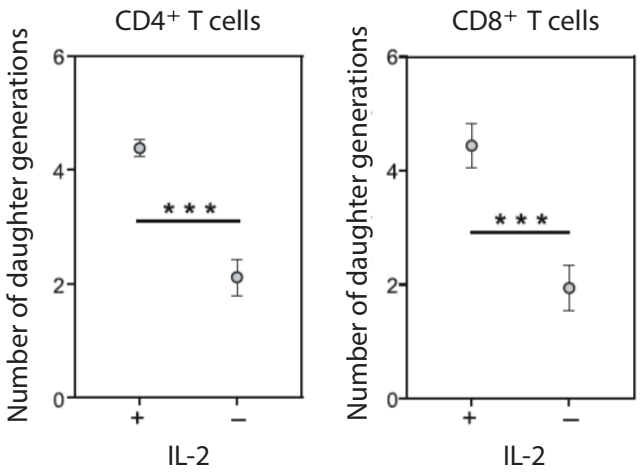

FIG. 4.
*The impact of adding IL-2 to the culture medium on the proliferation of CD4⁺ and CD8⁺ T lymphocytes during 7 days of in vitro stimulation. The means and standard errors of the means are presented (n = 18 in each group). Paired t-test was used to compare two groups of quantitative data; *** – p < 0.001*

for 7 days (fig. 5d). This phenomenon was not observed in the analysis of CD8⁺ T cells.

Thus, the inclusion of the "rest" stage in the mononuclear leukocyte thawing protocol followed by culturing stimulated cells for 7 days has a negative effect on the viability and proliferative capacity of CD4⁺ and CD8⁺ T lymphocytes. It is recommended to start working with cell cultures immediately after thawing cryopreserved mononuclear leukocytes.

DISCUSSION

This study raises a number of fundamental questions regarding the specific features of studying the functional activity of T lymphocytes using cryopreserved cells.

During the study, we determined that after 7 days of culturing cells thawed after long-term (40 ± 1.4 months) cryopreservation, on average only a third of their initially introduced number remains in the sample. Previously, when culturing mononuclear leukocytes that were not subjected to freezing, we did not note such a significant decrease in their number. An analysis of literature sources confirmed that a high tendency to die is a characteristic feature of cells that have undergone cryopreservation and thawing procedures [15].

It is known that freezing and thawing trigger processes leading to cell death – necrosis and apoptosis. Necrosis is a consequence of membrane damage by intracellular ice crystals and osmotic stress. Apoptosis can be caused by various factors, including physical stress caused by changes in cell morphology; activation of death receptors; accumulation of free radicals in the cytosol; activation of the caspase cascade [16, 17]. When using the optimal cryopreservation protocol, the main cause of death of thawed cells in culture is apoptosis [15]. It has been shown that a significant number of T lymphocytes constantly trigger the death program, which leads to a statistically significant reduction in the proportion of viable cells within 24 hours after the start of incubation. Based on the fact that stimulation of thawed cells with cytokines can significantly increase the viability of cultured cells [15], we investigated the effect of adding IL-2 to the culture medium on the functionality of T lymphocytes thawed after long-term cryopreservation.

IL-2, like other cytokines with a common gamma chain, is considered to be a survival factor for T lymphocytes *in vivo* and *in vitro* [18, 19]. It is known that IL-2 activates the tyrosine kinases Janus kinase (Jak) 1 and Jak3, which are adjacent to the cytoplasmic fragments of the cytokine receptor chains [20]. These enzymes phosphorylate tyrosine residues localized in the receptor chains, which creates sites for binding and phosphorylation of the adapter protein Shc and the transcription factor STAT5 (signal transducer and activator of transcription 5) [21]. Phosphorylated Shc activates several signaling pathways, at least one of which, PI3K/Akt (phosphatidylinositol 3-kinase/protein kinase B pathway), plays an important role in T cell proliferation [22]. In turn, phosphorylation of STAT5 molecules, their dimerization and translocation into the nucleus stimulate the expression of genes involved in division and protecting lymphocytes from apoptosis [23].

Our studies confirmed the positive effect of IL-2 added to the culture medium on the viability and proliferative activity of T cells thawed after cryopreservation. In the presence of this cytokine, we observed a less pronounced reduction in the number of leukocytes in the culture and an increase in the number of daughter generations during stimulation of T lymphocytes. However, the cultured cells still died en masse: their number significantly decreased after 7 days of incubation.

The most pronounced effect of IL-2 was on stimulated CD4⁺ and CD8⁺ T lymphocytes. Apparently, the higher sensitivity of these cells is due to the peculiarity of receptor expression that binds the cytokine. The high-affinity receptor for IL-2 is a complex of three chains: CD25, CD122 and CD132 [24]. Only two of them, CD122 and CD132, are expressed in limited quantities on resting T cells. By binding IL-2, they can form an intermediate-affinity complex capable of transmitting a signal into the cell. Stimulated T lymphocytes, in turn, induce the expression of CD25, which allows activated T cells to form a trimeric high-affinity receptor, making stimulated lymphocytes more sensitive to the cytokine action.

In the present study, we also assessed how the introduction of a "resting" step into the cell thawing protocol affects the functionality of cryopreserved T lymphocytes. Several studies [7–10], although not all [25], have previously demonstrated positive effects of "resting": a decrease in non-specific cytokine

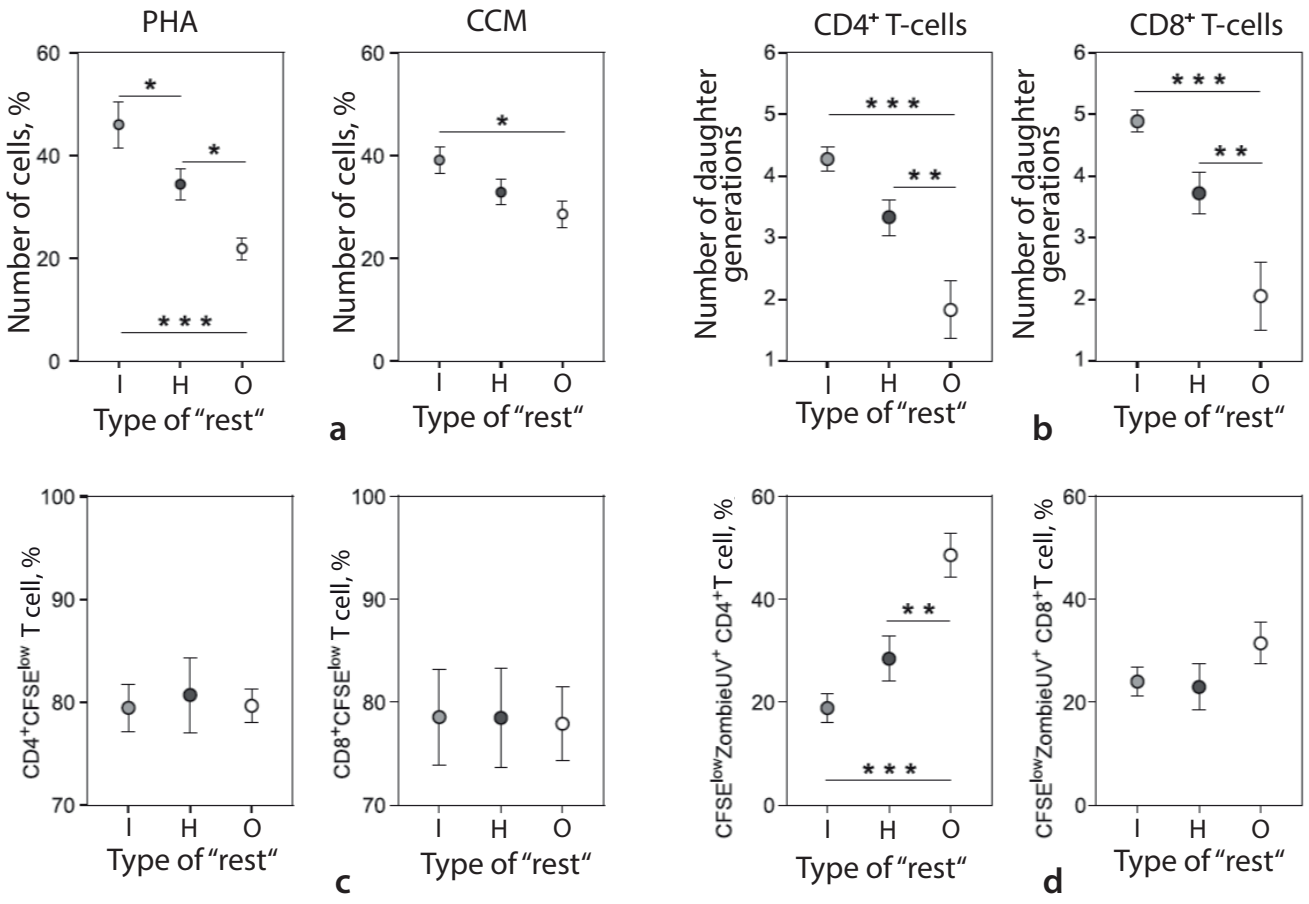

FIG. 5.

*Effect of the type of "rest" on the thawed cells parameters in a 7-day culture (the means and standard errors of the means are presented). a – number of mononuclear leucocytes in stimulated and control samples; b – number of daughter T cell generations in stimulated samples; c – number of proliferating T lymphocytes; d – number of T cells dying while dividing. Types of "rest": I – immediately after thawing (without "rest"; n = 18); H – hourly "rest" (n = 18); O – overnight "rest" (n = 18). One-way analysis of variance was used to compare groups of quantitative data; multiple comparisons between groups were performed using Tukey's test; * – p < 0.05; ** – p < 0,01; *** – p < 0.001*

production by T cells; an increase in the number of active T lymphocytes and the proportion of polyfunctional T lymphocytes. All of these phenomena were detected using the ELISPOT method. The results presented by the authors are apparently associated with the death of apoptotic T cells during the "resting" process, which contributes to an increase in the proportion of truly viable lymphocytes in the analyzed sample [9]. This is relevant given that the presence of apoptotic cells can reduce the functionality of live $CD4^+$ and $CD8^+$ T lymphocytes [26] and interfere with antigen processing [27].

As noted above, during cultivation, T cells thawed after cryopreservation constantly initiate programmed cell death processes [15], which was manifested in a gradual increase in the number of dead and dying T lymphocytes in the 7-day culture of mononuclear leukocytes that we set up. It can be assumed that the accumulation of apoptotic cells during longitudinal cultivation has a negative effect on the functional capacity of the remaining T lymphocytes, which was not noted in short-term studies using the ELISPOT method. Indeed, in our study, additional "rest" of cells (both hourly and overnight) led to a significant decrease in the number of leukocytes and an increase in the proportion of apoptotic T lymphocytes after 7 days of incubation. Moreover, we noted a decrease in the number of daughter generations among stimulated T lymphocytes that had undergone "rest" without changing the proportion of $CFSE^{low}$ cells. It is likely that in such cultures, compared to samples where the 'rest" stage was excluded from the work protocol, a greater number of cells enter into division, but they perform fewer mitoses.

Some limitations of the present study should be noted. Firstly, we did not reproduce the results of other research groups and therefore can only assume that "rest" has a positive effect in subsequent short-term studies (for example, using the ELISPOT method), but a negative

effect in further longer cell culturing (in our work, 7 days). Secondly, to modify the CCM, we used a single cytokine, IL-2, the effects of which can include not only stimulation of T lymphocyte proliferation, but also induction of regulatory T cells and initiation of activation-induced apoptosis of effector elements [28]. In further studies, we plan to introduce other cytokines of the IL-2 family into the culture medium, including IL-7 and IL-15, which are known to effectively support the viability and proliferation of T lymphocytes [29].

Despite the limitations, it should be emphasized that in the present study we evaluated for the first time the effect of "rest" on the results of proliferative capacity and viability assessment during the cultivation of T lymphocytes that had undergone long-term (40 ± 1.4 months) cryopreservation.

CONCLUSION

The obtained data encourage the development of new protocols for working with mononuclear leukocytes thawed after cryopreservation. It is obvious that the introduction of exogenous cytokines can increase the viability and functional activity of thawed T cells, bringing their state closer to that of freshly isolated lymphocytes. Optimal combination selection of cell culture medium components will allow obtaining data that more objectively reflect the processes occurring *in vivo*. It is also noteworthy that the "rest" of mononuclear leukocytes after thawing have a negative effect on the viability and proliferative activity of stimulated T lymphocytes during a week of incubation. This fact emphasizes the need to optimize research protocols.

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Conflicts of interest

No potential conflict of interest relevant to this article reported.

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Compliance with ethical standards

The study plan was approved by the Ethics Committee (registration no. IRB00008964; protocol no. 31 dated March 03, 2017). Each blood donor provided written informed consent. All procedures performed in the studies involving humans complied with the ethical standards of the national research ethics committee and the World Medical Association Declaration of Helsinki (1964) and its later amendments or comparable ethical standards.

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EPIDEMIOLOGY

PARENTERAL CHRONIC VIRAL HEPATITIS IN THE ARCTIC ZONE OF THE REPUBLIC OF SAKHA (YAKUTIA) AS THE MOST IMPORTANT MEDICAL AND SOCIAL PROBLEM

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ABSTRACT

Background. *The severe course of parenteral viral hepatitis and their further chronicity are associated with the presence of immunodeficiency disorders, frequency of which increases significantly in harsh climate. The article discusses the spread of parenteral viral hepatitis in the Arctic zone of the Republic of Sakha (Yakutia) and the issues of organizing medical care for patients with chronic viral hepatitis at the regional level.*
The aim of the study. *To analyze the incidence rates of parenteral viral hepatitis in the Arctic regions of Yakutia in order to improve the health care system using the example of remote areas of hard access.*
Methods. *The work uses materials from official statistics of the territorial department of Rospotrebnadzor for the Republic of Sakha (Yakutia) for 2000–2022 and information from the "Chronic viral hepatitis in the Republic of Sakha (Yakutia)" register.*
Results. *In the Arctic regions of Yakutia, problems are observed in chronic forms of viral hepatitis B, C and D, as well as in their outcomes, such as cirrhosis and liver cancer, leading to early disability and mortality. In the general structure, hepatitis B infection prevails, which indicates the presence of family foci of infection. All this requires a complex of not only therapeutic, but also advanced anti-epidemiological measures.*
Conclusion. *The difficult epidemiological situation regarding parenteral viral hepatitis, caused by extreme natural and climatic conditions, genetic characteristics of the indigenous population and the lack of medical institutions specializing in the treatment of chronic viral hepatitis, dictates the need to strengthen systematic on-site monitoring studies and telemedicine consultations in the Arctic zone of Yakutia. Thanks to this, residents of hard-to-reach areas of the Arctic zone of the Republic of Sakha (Yakutia) will be able to receive targeted subsidized care for the treatment of chronic hepatitis without traveling to Yakutsk.*

Key words: *parenteral viral hepatitis, cirrhosis, cancer, Yakutia, Arctic zone, health-care organization*

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ПАРЕНТЕРАЛЬНЫЕ ХРОНИЧЕСКИЕ ВИРУСНЫЕ ГЕПАТИТЫ В АРКТИЧЕСКОЙ ЗОНЕ РЕСПУБЛИКИ САХА (ЯКУТИЯ) КАК ВАЖНЕЙШАЯ МЕДИКО-СОЦИАЛЬНАЯ ПРОБЛЕМА

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РЕЗЮМЕ

Обоснование. *Тяжёлое течение парентеральных вирусных гепатитов и дальнейшая их хронизация связаны с наличием иммунодефицитных состояний, частота которых значительно возрастает в условиях сурового климата. В данной статье рассматривается распространение парентеральных вирусных гепатитов в Арктической зоне Республики Саха (Якутия) (РС(Я)) и вопросы организации медицинской помощи больным хроническими вирусными гепатитами (ХВГ) на уровне региона.*

Цель исследования. *Провести анализ показателей заболеваемости парентеральными вирусными гепатитами в арктических районах Якутии для совершенствования мероприятий системы здравоохранения на примере труднодоступных отдалённых территорий.*

Методы. *В работе использованы материалы официальной статистики территориального управления Роспотребнадзора по РС(Я) за 2000–2022 гг. и сведения из регистра «Хронические вирусные гепатиты в РС(Я)».*

Результаты. *В арктических районах Якутии отмечается неблагополучие по хроническим формам вирусных гепатитов B, C и D, а также по их исходам, таким как цирроз и рак печени, приводящим к ранней инвалидизации и смертности. В общей структуре превалирует инфекция, вызванная вирусом гепатита B, что свидетельствует о наличии семейных очагов инфекции. Всё это требует комплекса не только лечебных, но и углублённых противоэпидемиологических мероприятий.*

Заключение. *Сложная эпидемиологическая ситуация по парентеральным вирусным гепатитам, обусловленная экстремальными природно-климатическими условиями, генетическими особенностями коренного населения и отсутствием медучреждений, специализирующихся на лечении ХВГ, диктует необходимость усиления в Арктической зоне Якутии систематических выездных мониторинговых исследований и телемедицинских консультаций. Благодаря этому жители труднодоступных районов Арктической зоны РС(Я) смогут получать целевую субсидированную помощь для лечения ХВГ без выезда в Якутск.*

Ключевые слова: *парентеральные вирусные гепатиты, цирроз, рак, Якутия, Арктическая зона, организация здравоохранения*

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BACKGROUND

Viral hepatitis B, C and D remain one of the urgent problems of practical health care. In the Russian Federation, the total number of patients with chronic hepatitis B (CHB) is more than 3 million people, chronic hepatitis C (CHC) – from 1.5 to 2.5 million people, hepatitis D has been recorded in more than 10 million people [1-3].

The total number of patients with chronic viral hepatitis (CVH) in the region is almost 15 thousand people, about 1.4 thousand people (9.5 %) of which live in the Arctic zone (AZ). At the same time, a significant part of the AZ population is made up of representatives of the North indigenous peoples.

The Yakutia AZ is characterized by severe natural and climatic conditions, a low level of social infrastructure (including insufficient development of the health care system) and poorly developed transport accessibility [4]. All this determines the severe course of these diseases and their high chronicity [5]. It is important to note that hepatitis B, C and D viruses are important etiological factors for the development of primary cancer [6–8].

THE AIM OF THE STUDY

To conduct an analysis of viral hepatitis incidence rates in the Arctic regions of the Republic of Sakha (Yakutia) to improve health care measures in the hard-to-reach and remote areas of Yakutia.

METHODS

The study uses official statistics from the territorial Rospotrebnadzor administration in the Republic of Sakha (Yakutia) for 2000–2022 and information from the register of patients with viral hepatitis developed by the Reference Center for Monitoring Viral Hepatitis based at the Central Research Institute of Epidemiology of Rospotrebnadzor [9].

RESULTS AND DISCUSSION

The Arctic zone of the Republic of Sakha (Yakutia) is characterized primarily by extreme climatic conditions, an extremely vast territory (1.6 million km^2), focal nature of industrial and economic development, a significant share of small and medium-sized rural settlements (with a population of up to 1 thousand people) and poorly developed social and transport infrastructure. All this creates significant problems in organizing medical care. Currently, the Arctic zone of the Republic of Sakha (Yakutia) includes 13 districts (Abyisky, Allaikhovsky, Anabarsky, Bulunsky, Verkhnekolymsky, Verkhoyansky, Zhigansky, Momsky, Nizhnekolymsky, Olenyoksky, Srednekolymsky, Ust-Yansky and Eveno-Bytantaysky), in which about 64 thousand people live, i.e. 6.4 % of the region›s population.

The incidence of acute hepatitis B (AHB) in Yakutia, including the Arctic zone, is steadily declining. From the early 2000s to the present, this figure has dropped to levels that do not cause particular concern (fig. 1). Of course, this is due to the large-scale vaccination of the population. Thus, if in 2000, 2.6 % of citizens subject to vaccination (under 55 years of age) were vaccinated against hepatitis B in the region, then since 2011 this figure has not dropped below 95 %. For example, in 2022, 764,238 people (96.6 %) were vaccinated.

Over the entire observation period, the greatest increase in the incidence of acute respiratory viral infections was noted in 2002. During this period, acute respiratory viral infections were registered in almost all districts, with the exception of Anabarsky and Srednekolymsky, although in 2003–2005 and 2008–2009, the incidence rate also exceeded the national average.

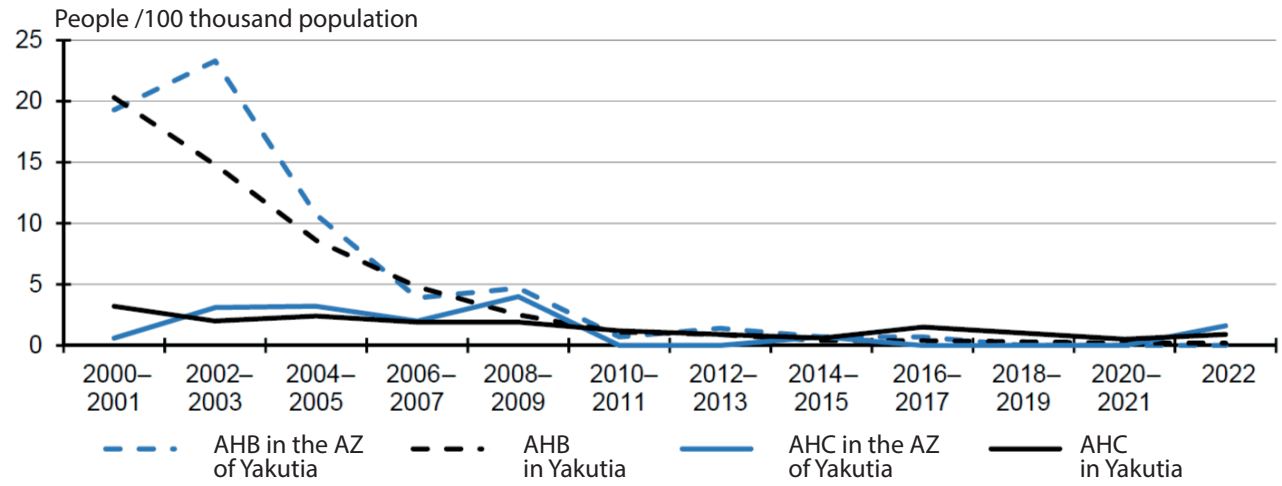

FIG. 1.
Incidence of acute hepatitis B (AHB) and acute hepatitis C (AHC) in 2000–2022

The incidence of acute hepatitis C (AHC) in the Republic of Sakha (Yakutia) has also decreased. AHC rarely occurs in a manifest form, when it can be detected in the disease phase, and the chronic course is characterized by latent forms, detected only with specific diagnostics. Thus, due to insufficiently complete examination of the Arctic population, the official figures for AHC may be somewhat underestimated.

As noted above, the incidence of chronic hepatitis B in Yakutia over a long-term period, and especially in the Arctic zone, is significantly higher than the national average (fig. 2). Thus, in 2016–2017, this indicator in the Arctic Zone of the Republic of Sakha (Yakutia) was 63.5 people/100 thousand people, while in the Russian Federation this value was at the level of 10.2 people/100 thousand population ($p < 0.05$). Currently, about 64 thousand people live in the Arctic Zone of the Republic of Sakha (Yakutia), i.e. about 6.4 % of the total population of the region. Nevertheless, out of 101 new cases of chronic hepatitis B identified in the region in 2022, 15.2 % were recorded among residents of the Arctic.

In 2023, the high frequency of the hepatitis B marker detection among the local AZ population of the Republic of Sakha (Yakutia) was once again confirmed by the employees of the Republican Center for Public Health and Medical Prevention. As a result of the analysis of rapid testing data ($n = 1776$), CHC was detected in 0.6 % of cases, CHB – in 3.4 %. Worsening of the disease course and a high degree of chronicity in hepatitis B causes super-infection with the hepatitis D virus.

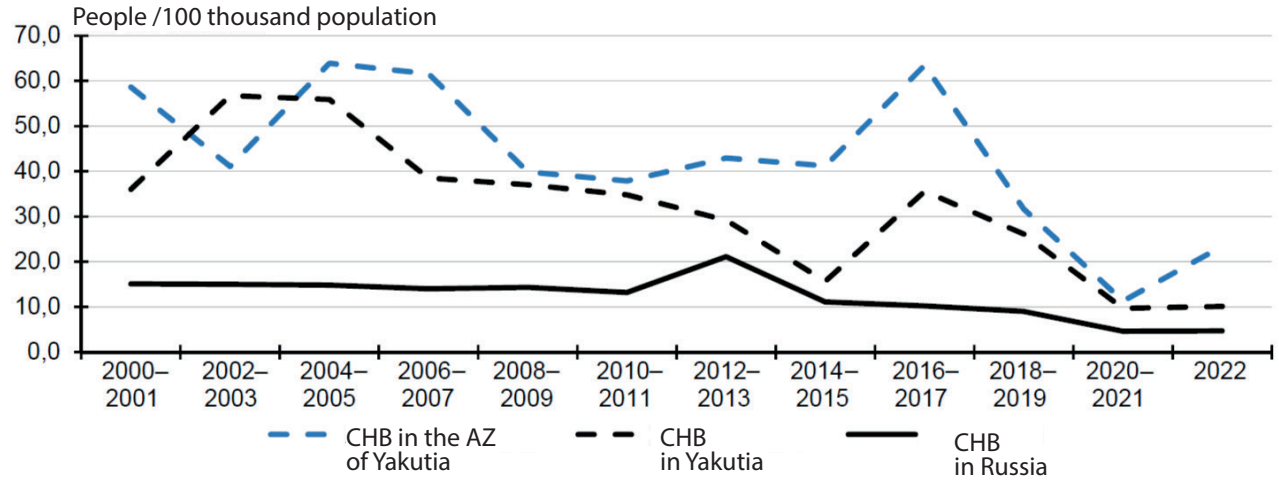

FIG. 2.
Incidence of chronic hepatitis B in Yakutia and Russia

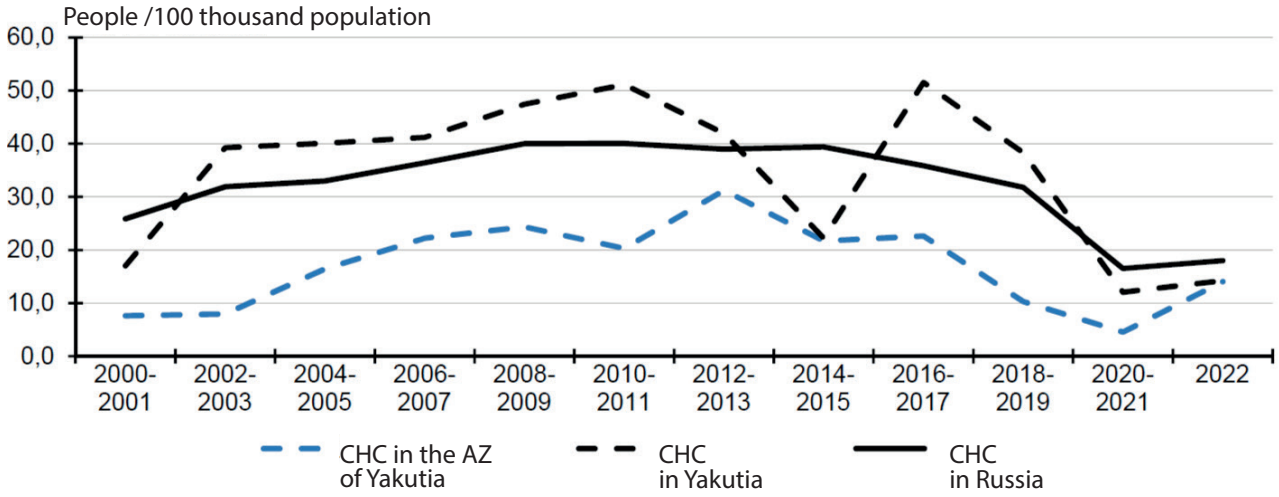

FIG. 3.
Incidence of chronic hepatitis C in Yakutia and Russia

Unfortunately, official statistics do not take into account data on the chronic hepatitis D (CHD) incidence, although its chronicity leads to the stage of cirrhosis much faster than with other forms of this disease. Studies conducted in different regions of Yakutia have shown an exceptionally high frequency (17.2–31.7 %) of detection of antibodies to the hepatitis delta virus [10]. As a result, an approximate estimate of the CHD prevalence in the region can only be given based on data from infectious disease departments in Yakutia and the results of individual screening studies.

The intensity of the CHC epidemic process in the Republic of Sakha (Yakutia) varies greatly. Nevertheless, almost every year regional rates of hepatitis C incidence exceed similar rates in the country (fig. 3).

It is important to note that the relatively low number of newly detected cases of chronic hepatitis C in the AZ of the Republic of Sakha (Yakutia) is mainly due to the fact that the disease is latent in most infected people, and large-scale screening has not been carried out in these territories. The data on the incidence of chronic hepatitis C for 2020–2021 also do not reflect the true picture, and the reason for this is the COVID-19 pandemic.

In almost half of patients with CHC, immune activation leads to a chronic inflammatory condition that can affect a number of organs outside the liver [11]. According to our earlier studies, extrahepatic manifestations are more often diagnosed in women (58.8 %), representatives of indigenous peoples (67.4 %), and in patients who did not receive antiviral treatment (65.6 %) [12]. The most common were: joint syndrome – in 56.4 %; cognitive impairment – in 35.2 %; type 2 diabetes mellitus – in 31.7 %; cardiovascular disease (coronary heart disease, arterial hypertension) – in 37.6 %, etc. (fig. 4).

In 2001–2003, studies on the prevalence of viral hepatitis B, delta, and C in the Arctic zone were conducted by the staff of the Institute of Health. Schoolchildren, conscripts, and employees of educational and medical institutions (n = 4,049) from the Abyisky, Verkhoyansky, Zhigansky, and Eveno-Bytantaysky uluses were examined. The largest number of HBsAg-positive individuals was detected in the Eveno-Bytantaysky (*n* = 128) and Zhigansky (*n* = 96) uluses. Of the 1,150 examined, antibodies to hepatitis C were detected in 23 (2 %) people, mainly from the Zhigansky District (12 people). Of the total number of those examined with hepatitis D (*n* = 106), antibodies were detected in 8.5 %, mainly in those examined from the Zhigansky and Abyisky Districts. According to the author, the data obtained indicate a problem with hepatitis D in the Arctic zone [10].

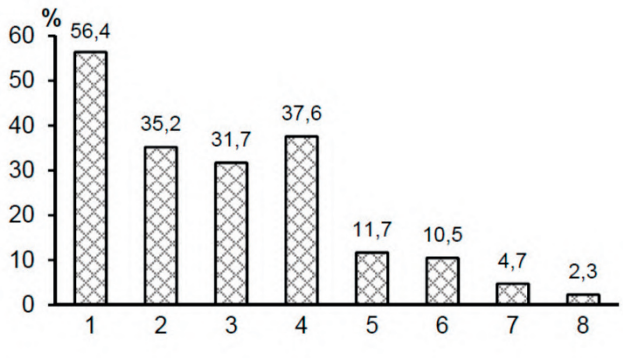

FIG. 4.
Extrahepatic complications in patients with HCV infection: 1 – joint syndrome; 2 – cognitive impairment; 3 – diabetes mellitus type 2; 4 – cardiovascular disease; 5 – hypo- and hyperthyroidism; 6 – vision impairment; 7 – hearing impairment; 8 – glomerular nephritis

One of the achievements of the infectious disease service of the Republic of Sakha (Yakutia) is the electronic register (ER) launch "Chronic viral hepatitis of the Republic of Sakha (Yakutia)", introduced into the regional healthcare system as a pilot project in November 2012. Unlike the existing statistical forms of recording, the ER provides an opportunity for a more comprehensive analysis of the incidence and adverse outcomes of chronic viral hepatitis both in the republic as a whole and separately by district. All this allows for a significant improvement in the system of medical care.

As of October 2023, the ER contains information on 14,781 people (table 1). There are 484 patients registered with viral liver cirrhosis in the region, including 75 (15.5 %) with CHB without delta agent, 207 (42.8 %) with CHD, 186 (38.4 %) with CHC, and 16 (3.3 %) with mixed infection. There are 47 people registered with primary liver cancer, including 9 (19.1 %) with CHB, 10 (21.3 %) with CHD, 26 (55.3 %) with CHC, and 2 (4.3 %) with mixed infection.

Of the total number of people in the ER, 9.5 % are the AZ residents. The largest number of people with CVH in the AZ was registered in the Bulunsky, Zhigansky, Verkhoyansky, Oleneksky and Srednekolymsky districts, the smallest – in the Momsky, Nizhnekolymsky and Eveno-Bytantaysky districts. Liver cirrhosis was recorded in 54 (11.2 %) people; in the Zhigansky district, a patient with hepatocellular carcinoma as a result of hepatitis D was also identified. In general, as studies have shown, CVH complications develop more often in men (76.0 %). Liver cirrhosis in 51.8 % (28 people) is caused by the hepatitis D virus, in 27.8 % (15 people) – by the hepatitis B virus, in 16.7 % (9 people) – by the hepatitis C virus. Mixed hepatitis caused the liver cirrhosis development in 3.7 % (2 people). Most often, cirrhosis was diagnosed in patients from the Verkhoyansky, Zhigansky and Oleneksky districts. There are no cases of cirrhosis in the Allaikhovsky, Anabarsky, Nizhnekolymsky and Eveno-Bytantaysky districts.

Among the persons registered in the AZ of the Republic of Sakha (Yakutia), 22 people died from hepatitis and its complications in 2021–2022, including 9 people from hepatitis B, 7 people from hepatitis C, 6 people from hepatitis D. A significant portion of the deceased were residents of the Srednekolymsky (6 people),

TABLE 1

DISTRIBUTION OF PATIENTS WITH CHRONIC VIRAL HEPATITIS BY ETIOLOGY ACCORDING TO THE REGISTRY DATA

Diagnosis	The Republic of Sakha (Yakutia)		The Arctic zone of the Republic of Sakha (Yakutia)		
	people	%	people	%	in % of the number of people in the register
Hepatitis B	6260	42.4	910	66.4	14.5
Hepatitis C	6779	45.9	296	21.7	4.4
Hepatitis D	1241	8.4	125	9.1	10.1
Mixed hepatitis	501	3.3	37	2.8	7.4
Total	14781	100	1368	100	9.5

Verkhoyansky (4 people), Abyysky (4 people) and Zhigansky (3 people) uluses.

In the structure of chronic viral hepatitis in the AZ, hepatitis B has the largest share (66.4 %), followed by hepatitis C (21.7 %). It is interesting that the ratio of infection caused by hepatitis B and C viruses is the same on average in the region. This fact indirectly indicates the epidemiological relevance of hepatitis B for the indigenous population of the republic. In the infection spread caused by the hepatitis B virus, the main route of transmission is intrafamilial. Based on this, it is necessary to adjust the preventive and anti-epidemic measures taken, as well as to strengthen comprehensive monitoring studies in hard-to-reach Arctic settlements.

Thus, the AZ of the Republic of Sakha (Yakutia) is one of the disadvantaged territories of the region in terms of chronic viral hepatitis, and this is directly related to the living conditions of the population, the genetic characteristics of the indigenous population, as well as the remoteness of their residence from large settlements. All this complicates full laboratory and instrumental diagnostics and provision of a complex of medical and social assistance.

A significant issue is the inequality in access to medical care in the region. For example, insufficient staffing of medical organizations with infectious disease specialists and epidemiologists in the Republic of Sakha (Yakutia) is one of the most problematic issues of regional healthcare. As of January 1, 2023, there are 89 infectious disease specialists in Yakutia, of which only 31 people work in the uluses. It is important that out of 13 Arctic uluses, where there are about 100 settlements on a total area of more than 1.6 million km^2, only 5 districts have infectious disease doctors (Verkhnekolymsky, Verkhoyansky, Zhigansky, Nizhnekolymsky, Oleneksky). Limited access to medical services, a lack of medical equipment and personnel are currently a serious obstacle to ensuring an adequate level of healthcare for all Arctic residents.

Based on the above, in order to reduce the incidence and mortality from viral hepatitis, the Ministry of Health of the Republic of Sakha (Yakutia) has prepared a draft target regional program "Improving the methods of providing medical care to patients with chronic hepatitis B, C and D for 2022-2024 in the Republic of Sakha (Yakutia)". Since 2021, the Republican Hepatology Center has been operating on the basis of the Yakutsk Republican Clinical Hospital, through which patients with chronic viral hepatitis from all over Yakutia have undergone treatment (as of January 01, 2024 – 870 people). A number of other medical institutions in the region are also involved in the treatment of patients with hepatitis in the Republic of Sakha (Yakutia), but all of them are located outside the AZ of the Republic of Sakha (Yakutia). Therefore, providing assistance to patients from remote and hard-to-reach areas of Yakutia requires closer attention. Based on this, since 2024, for the first time in the region, targeted subsidies have been allocated specifically for the AZ residents of the Republic of Sakha (Yakutia) for the purchase of antiviral drugs for the chronic hepatitis C treatment. Currently, patient selection is carried out through telemedicine consultations conducted by the chief freelance infectious disease specialist of the Ministry of Health of the Republic of Sakha (Yakutia). During 2024, it is planned to treat 150 AZ residents of the Republic of Sakha (Yakutia).

Given the extremely specific conditions of the region, the decentralization principle is the key to improve the availability and quality of medical services in the AZ of the Republic of Sakha (Yakutia). First of all, it is also necessary to introduce systematic specific serological and virological examinations of the population and pay due attention to providing the AZ with highly qualified specialists from among the graduates of the North-Eastern Federal University in Yakutsk as the main source of personnel in the republic. It is worth noting that in 2022 alone, 80 doctors were trained in the republican hepatology school as part of the continuous medical education program, including 5 people from the Arctic regions. This event has been held annually for 22 years with the invitation of leading Russian specialists. Particular attention in the region is paid to raising awareness

of viral hepatitis issues not only among health workers, but also among patients and their relatives.

CONCLUSION

The complex epidemiological situation with chronic viral hepatitis, caused by extreme natural and climatic conditions, genetic characteristics of the indigenous population and the lack of medical institutions specializing in the treatment of chronic viral hepatitis, dictates the need to strengthen systematic mobile monitoring studies and telemedicine consultations in the Arctic zone of Yakutia. Thanks to this, residents of AZ hard-to-reach areas of the Republic of Sakha (Yakutia) will be able to receive targeted subsidized assistance for the chronic viral hepatitis treatment without traveling to Yakutsk.

Important measures include eliminating the personnel shortage by increasing the prestige of the infectious disease doctor profession, creating comfortable working conditions, increasing wages, improving the system of training personnel in infectious diseases, and increasing the number of target referrals. It is also necessary to conduct medical and social surveys of the population on the satisfaction with medical care in order to assess and adjust the measures taken by the Russian Ministry of Health to improve the epidemiological situation and services provided to the population.

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Conflicts of interest

No potential conflict of interest relevant to this article reported.

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