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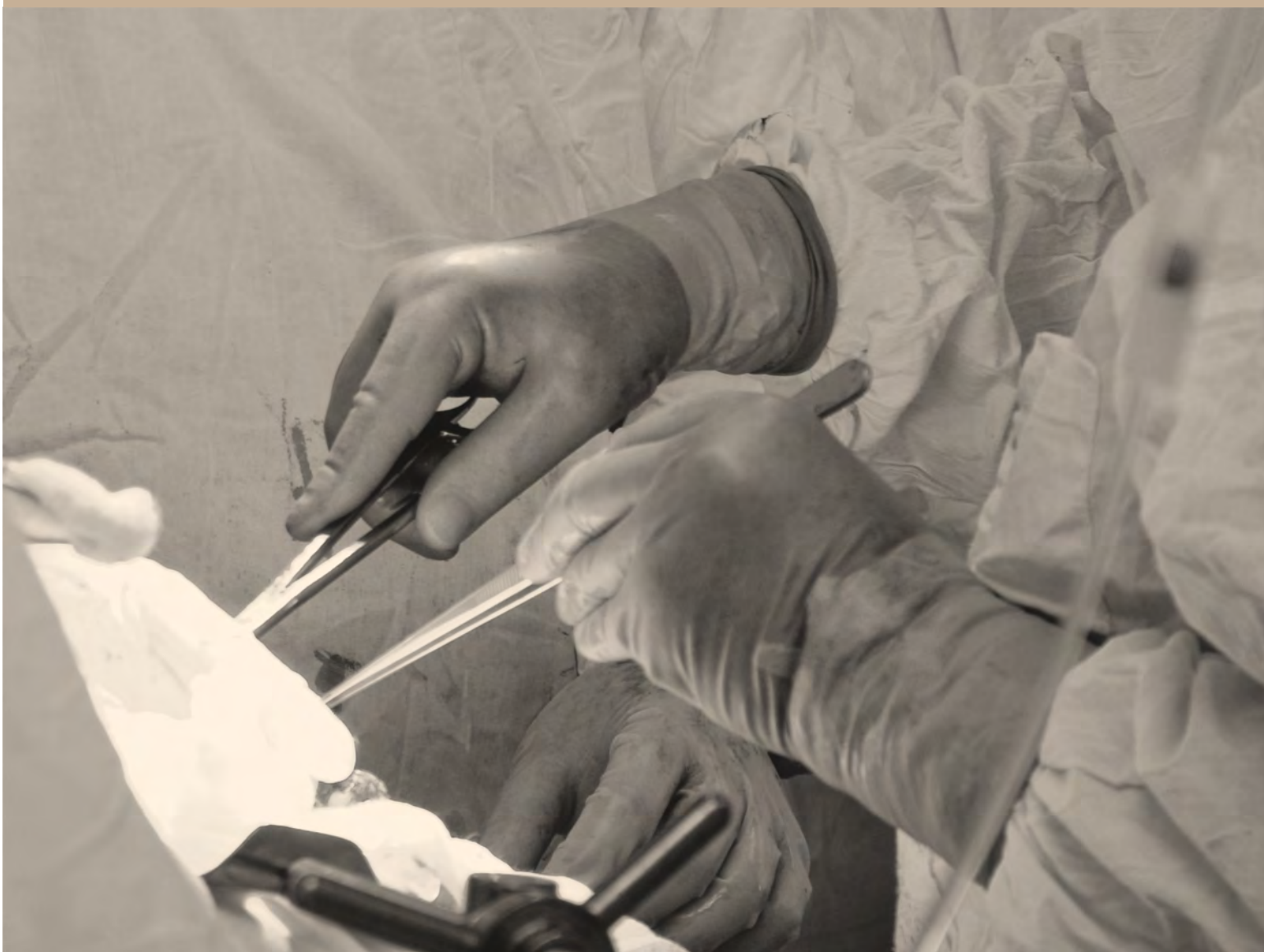

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До апреля 2017 г. журнал имел название «Бюллетень Восточно-Сибирского научного центра Сибирского отделения Российской Академии медицинских наук» (Бюллетень ВСНЦ СО РАМН). Основан в 1993 году.

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EDITOR-IN-CHIEF'S PREFACE

EDITOR-IN-CHIEF'S PREFACE TO ISSUE 4, 2023

Sergey I. Kolesnikov

Member of RAS

Dear readers!

The leading article in this issue, in my opinion, is the article by O.B. Ogarkov et al. (Irkutsk), who isolated a lipophilic representative of the *Corynebacterium tuberculostearicum* species complex from a tuberculosis lesion and sequenced it. The authors put forward an important hypothesis that favorable conditions for the development of secondary anaerobic lipophilic microbiota can be created inside the tuberculosis focus.

The second article is an interesting and promising *in vitro* study of the structure and characteristics of five microbial consortia with probiotic properties from naturally fermented milk products, performed by A.S. Pendyukhova et al. (Irkutsk). The authors showed that such consortia can be neutral and bactericidal against multidrug-resistant isolates of opportunistic bacteria, which is important for the creation of probiotics with antibacterial properties.

The third work that I would like to highlight is the study of N.V. Korotaeva, L.I. Ippolitova and E.S. Pershina (Voronezh) on the using neural network in ultrasound diagnostics for the early determination of intrauterine fetal distress based on the analysis of facial descriptors.

The search for diagnostic and prognostic criteria for COVID-19 and its consequences (including post-COVID syndrome) continues. Article by Zh.P. Vasneva et al. (Samara) demonstrates a change in the ratio of lymphocyte subpopulations in 67.7 % of patients with COVID-19 and lung damage of less than 30 %. Correlations between the content of NK cells and a wide range of T lymphocyte subpopulations were revealed, which may be a predictor of the severity of the disease. V.A. Vasilyev et al. (Petrozavodsk) showed in their research that changes in the phase structure of transmitral blood flow may be an early manifestation of intracardiac hemodynamics disorders in persons who have recovered from COVID-19.

A series of articles is devoted to the visual organ pathologies and their correction. In this section I would like to highlight the article by O.V. Pisarevskaya, A.G. Shchuko et al. (Irkutsk), who, through mathematical modeling, developed a technology for calculating the parameters of the effective SMILE surgery for the correction of high myopia with an optimal refractive effect. Another article of the authors from the Irkutsk Branch of the S. Fyodorov Eye Microsurgery Federal State Institution (Iureva T.N., Pisarevskaya O.V.) has shown that keratorefractive surgery can cause the transition of latent forms of the disease into an active process with the formation of glaucomatous neuropathy and corneal edema. In the work of M.V. Sinicin and N.A. Pozdeyeva (Novosibirsk), it was proven that rigid gas-permeable scleral lenses are preferable to the MyoRing implantation method for the correction of postkeratoplastic astigmatism.

Several works are devoted to current problems of zoonotic and infectious diseases, including socially significant ones. Of undoubted interest is the observation of E.K. Doroschenko et al. (Irkutsk) of the spread of the previously considered relict tick *Haemaphysalis concinna* in the territory of the Irkutsk region and the Republic of Buryatia, which makes it necessary to systematically monitor it in the Baikal region. Yu.V. Bazhenova et al. (Irkutsk) have shown that in case of comorbidity of tuberculosis and HIV infection, multislice computed tomography provides detailed information on the pathological process in the lungs and its dynamics during treatment.

Traditionally, our journal pays attention to various aspects of metabolic syndrome and obesity both in clinical practice and in experiment. In the study

by L.V. Belenkaya et al. (Irkutsk), a universal critical value of age (34.5 years) was determined which is associated with a significant increase in the prevalence of metabolic syndrome in women (regardless of their ethnicity), and the ranking determined the prevalence of a decrease in high-density lipoproteins. T.A. Bairova et al. (Irkutsk), when sequencing the leptin genome, has shown for the first time that 13 single nucleotide polymorphisms registered in the GenBank database and 14 unregistered single nucleotide substitutions of the *LEP* gene which may be one of the causes of obesity were identified in adolescents with overweight and obesity in contrast to adolescents with normal body weight. T.I. Dergacheva et al. (Novosibirsk) revealed in the experiment the corrective effect of a sorbent composition of aluminum oxide and polydimethylsiloxane on prelymphatics, blood and lymphatic vessels in the uterus and ovaries in *db/db* mice with obesity and type 2 diabetes mellitus, which is promising for further researches.

A.Yu. Maryanyan et al. (Irkutsk) assessed the course of pregnancy in women who consumed alcohol in the prenatal period, which was both indicated by them during the survey and, most importantly, was laboratory-confirmed (using a study of the phosphatidylethanol level), and found that 24.2 % of women did not stopped consuming alcohol. This led to intrauterine growth retardation and premature birth.

Several articles in the issue are devoted to experiment and morphological studies. Thus, in the work of I.V. Bibik et al. (Lugansk, Stavropol), new derivatives of thienopyridine and 1,4-dihydropyridine with high anti-inflammatory and analgesic activity, promising for further preclinical studies, were synthesized and studied by virtual bioscreening. V.M. Vdovin et al. (Barnaul) experimentally proved the local hemostatic effect of exogenous fibrin monomer when administered systemically after injury and the suppression of platelet aggregation function in comparison with the use of tranexamic acid. I.A. Kulikov et al. (Moscow) has found the appearance and increase in areas of myometrial necrosis at the abnormal placentation in response to an increase in the depth of placental villi ingrowth, which can stimulate the development of abnormal vascularization.

As always, scientists, clinicians and young scientists might be interested in literature reviews and lectures by L.M. Lazareva (Irkutsk), K.Yu. Krasner et al. (Novosibirsk), D.D. Morikov et al. (Irkutsk) and K.B. Lelyavin (Irkutsk).

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ПРЕДИСЛОВИЕ ГЛАВНОГО РЕДАКТОРА К № 4 (2023)

Колесников Сергей Иванович

академик РАН

Уважаемые читатели!

Передовой статьёй данного номера, на мой взгляд, является статья О.Б. Огаркова и соавт. (Иркутск), выделивших из туберкулёзного очага и секвенировавших липофильного представителя видового комплекса *Corynebacterium tuberculostearicum*. Авторы выдвинули важную гипотезу о том, что внутри туберкулёзного очага могут создаваться благоприятные условия для развития вторичной анаэробной липофильной микробиоты.

Вторая статья – интересное и перспективное исследование *in vitro* структуры и характеристик пяти микробных консорциумов с пробиотическими свойствами из кисломолочных продуктов естественного брожения, выполненное А.С. Пеньдюховой и соавт. (Иркутск). Они показали, что такие консорциумы могут быть нейтральными и бактерицидными в отношении полирезистентных изолятов условно-патогенных бактерий, что важно для создания пробиотиков с антибактериальными свойствами.

И третья работа, которую хотелось бы выделить, – исследование Н.В. Коротаевой, Л.И. Ипполитовой и Е.С. Першиной (Воронеж) использования при ультразвуковой диагностике нейрональной сети для своевременного определения внутриутробного дистресса плода по анализу лицевых дескриптов.

Продолжается поиск диагностических и прогностических критериев COVID-19 и его последствий (в т. ч. постковидного синдрома). Статья Ж.П. Васневой и соавт. (Самара) демонстрирует у больных COVID-19 с поражением лёгких менее 30 % изменения соотношения субпопуляций лимфоцитов в 67,7 % случаев. Выявлены корреляционные зависимости содержания НК-клеток с широким спектром субпопуляций Т-лимфоцитов, что может быть предиктором тяжести заболевания. В.А. Васильевой и соавт. (Петрозаводск) показано, что изменение фазовой структуры трансмитрального кровотока может быть ранним проявлением нарушений внутрисердечной гемодинамики у лиц, переболевших COVID-19.

Серия статей посвящена патологиям органа зрения и их коррекции. В этом разделе я выделил статью О.В. Писаревской, А.Г. Щуко и соавт. (Иркутск), которые путём математического моделирования разработали технологию расчёта параметров эффективной операции SMILE для коррекции миопии высокой степени с оптимальным рефракционным эффектом. В другой статье авторов из Иркутского филиала ФГАУ «НМИЦ «МНТК «Микрохирургия глаза» имени академика С.Н. Фёдорова» Минздрава России (Iureva T.N., Pisarevskaya O.V.) показано, что кераторефрактивное оперативное вмешательство может вызвать переход латентных форм заболевания в активный процесс с формированием глаукомной нейропатии и отёка роговицы. В работе М.В. Синицына и Н.А. Поздеевой (Новосибирск) доказано, что жёсткие газопроницаемые склеральные линзы предпочтительнее метода имплантации кольца MyoRing для коррекции посткератопластического астигматизма.

Несколько работ посвящены актуальным проблемам природно-очаговых и инфекционных заболеваний, в т. ч. социально значимых. Несомненный интерес представляет наблюдение Е.К. Дорощенко и соавт. (Иркутск) о распространении ранее считавшегося реликтовым клеща *Haemaphysalis concinna* на территории Иркутской области и Республики Бурятия, что делает необходимым систематическое наблюдение за ним на территории Байкальского региона. Ю.В. Баженовой и соавт. (Иркутск) показано, что при коморбидности туберкулёза и ВИЧ-инфекции мультиспиральная компьютер-

ная томография даёт детальную информацию о патологическом процессе в лёгких и о его динамике при лечении.

Традиционно журнал уделяет внимание различным аспектам метаболического синдрома и ожирения как в клинической практике, так и в эксперименте. В исследовании Л.В. Беленькой и соавт. (Иркутск) определено универсальное критическое значение возраста (34,5 года), с которым у женщин (независимо от этнической принадлежности) ассоциировано существенное увеличение распространённости метаболического синдрома, причём ранжирование определило превалирование снижения показателя липопротеинов высокой плотности. Т.А. Баировой и соавт. (Иркутск) при проведении секвенирования генома лептина впервые показано, что, в отличие от подростков с нормальной массой тела, у подростков с избыточной массой тела и ожирением идентифицировано 13 однонуклеотидных полиморфизмов, зарегистрированных в базе данных GenBank, и 14 не зарегистрированных однонуклеотидных замен гена *LEP*, которые могут быть одной из причин ожирения. Т.И. Дергачева и соавт. (Новосибирск) экспериментально выявили корригирующий эффект сорбентной композиции из оксида алюминия и полидиметилсилоксана на прелимфатики, кровеносные и лимфатические сосуды в матке и яичниках у *db/db* мышей с ожирением и сахарным диабетом 2-го типа, что перспективно для дальнейшей разработки.

А.Ю. Марянян и соавт. (Иркутск) оценили течение беременности у женщин, употреблявших алкоголь в пренатальном периоде, что было как указано ими при анкетировании, так и, что особенно важно, подтверждено лабораторно (с помощью исследования уровня фосфатидилэтанола), и выявили, что 24,2 % женщин не прекращали употреблять алкоголь. Это приводило к задержке внутриутробного развития плода и преждевременным родам.

Несколько статей номера посвящены эксперименту и морфологическим исследованиям. Так, в работе И.В. Бибика и соавт. (Луганск, Ставрополь) синтезированы и исследованы виртуальным биоскринингом новые производные тиенопиридина и 1,4-дигидропиридина с высокой противовоспалительной и болеутоляющей активностью, перспективные для дальнейших доклинических исследований. В.М. Вдовин и соавт. (Барнаул) в эксперименте доказали локальный гемостатический эффект экзогенного фибрин-мономера при его системном введении после травмы и подавлении агрегационной функции тромбоцитов в сравнении с применением транексамовой кислоты. И.А. Куликовым и соавт. (Москва) при аномальном прикреплении плаценты найдено появление и увеличение зон некроза миометрия в ответ на увеличение глубины врастания ворсин плаценты, что может стимулировать развитие аномальной васкуляризации.

Как всегда, должны заинтересовать учёных, клиницистов и молодых учёных обзоры литературы и лекции Л.М. Лазаревой (Иркутск), К.Ю. Краснер и соавт. (Новосибирск), Д.Д. Морикова и соавт. (Иркутск) и К.Б. Лелявина (Иркутск).

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DISCUSSION PAPERS, LECTURES, NEW TRENDS IN MEDICAL SCIENCE

ISOLATION AND WHOLE GENOME SEQUENCING OF A LIPOPHILIC ANAEROBIC BACTERIUM, A REPRESENTATIVE OF THE SPECIES COMPLEX *CORYNEBACTERIUM TUBERCULOSTEARICUM*, FROM A TUBERCULOSIS FOCUS

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ABSTRACT

Background. *The study of the lower respiratory tract microbiome has been actively developed in recent years with the help of whole genome sequencing (WGS) methods. Due to this, it became clear that the nature of the lungs microbiota is very different from other microbial communities inhabiting the human body. One of the important directions in the study of pathological lungs biocenosis is the study of the role of the satellite microbiota of the tuberculosis focus.*
The aim of the work. *To isolate and characterize oxygen-tolerant anaerobes from the necrotic contents of tuberculomas.*
Materials and methods. *Biopsy material from 5 patients with pulmonary tuberculosis was obtained during a planned surgical treatment of tuberculoma. A pure culture was isolated from one sample during anaerobic cultivation. Lipase activity of strain was determined by plating on brain heart infusion agar (HIMEDIA, India) supplemented with 0.1 % Tween-80 and 10 mM of $CaCl_2$. Antibiotic susceptibility was determined by RAPMYCO u SLOWMYCO of TREK Diagnostic Systems (Thermo Fisher Scientific, USA). DNA from the sediment of the broth culture was isolated by the CTAB chloroform method. Whole genome sequencing was performed on a DNBSeq-G400 NGS sequencer by Genomed (Russia).*
Results. *Based on WGS results and phylogenetic analysis, the strain was identified as Corynebacterium kefirresidentii. The strain was characterized by high lipase activity and resistance only to Isoniazid, Ethionamide and Trimethoprim/Sulfamethoxazolin.*
Conclusion. *The isolation of a lipophilic anaerobic representative of the Corynebacterium tuberculostearicum species complex from a tuberculous focus indicates a possible role of the non-tuberculous microbiota in the liquefaction of caseous necrosis. We assumed that in some cases, favorable conditions are created inside the tuberculous focus for the development of satellite anaerobic lipophilic microbiota.*

Key words: *microbiome of tuberculosis focus, tuberculoma, WGS, Corynebacterium kefirresidentii*

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ВЫДЕЛЕНИЕ И ПОЛНОГЕНОМНОЕ СЕКВЕНИРОВАНИЕ ЛИПОФИЛЬНОЙ АНАЭРОБНОЙ БАКТЕРИИ, ПРЕДСТАВИТЕЛЯ ВИДОВОГО КОМПЛЕКСА *CORYNEBACTERIUM TUBERCULOSTEARICUM*, ИЗ ТУБЕРКУЛЁЗНОГО ОЧАГА

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РЕЗЮМЕ

Обоснование. *Исследование микробиома нижних дыхательных путей активно развивается последние несколько лет за счёт применения методов полногеномного секвенирования (WGS, whole genome sequencing). Благодаря этому стало понятно, что природа микробиоты лёгких сильно отличается от других микробных сообществ, населяющих тело человека. Одним из важных направлений исследования патологических биоценозов в лёгких является изучение роли сателлитной микробиоты туберкулёзного очага.*
Цель работы. *Выделение и характеристика толерантных к кислороду анаэробов из некротического содержимого туберкулом.*
Материалы и методы. *Биопсийный материал от 5 больных туберкулёзом лёгких был получен в процессе плановой операции по иссечению туберкулом. Из одного образца при анаэробном культивировании была выделена чистая культура. Липазную активность штамма определяли посевом на сердечно-мозговой агар (HIMEDIA, Индия) с добавлением 0,1 % Tween-80 и 10 мМ $CaCl_2$. Чувствительность к антибиотикам определялась в RAPMYCO и SLOWMYCO TREK Diagnostic Systems (Thermo Fisher Scientific, США). ДНК из осадка бульонной культуры выделяли CTAB-хлороформным методом. Полногеномное секвенирование осуществлено на NGS-секвенаторе DNBSeq-G400 компанией «Геномед» (Россия).*
Результаты. *По результатам WGS и по данным филогенетического анализа штамм был идентифицирован как Corynebacterium kefirresidentii. Штамм характеризовался высокой липазной активностью и устойчивостью только к изониазиду, этионамиду и триметоприму/сульфаметоксазолу.*
Заключение. *Выделение из туберкулёзного очага липофильного анаэробного представителя видового комплекса Corynebacterium tuberculostearicum свидетельствует о возможной роли нетуберкулёзной микробиоты в процессах разжижения казеозного некроза. Нами выдвигается гипотеза о том, что внутри туберкулёзного очага в некоторых случаях создаются благоприятные условия для развития вторичной анаэробной липофильной микробиоты.*

Ключевые слова: *микробиом туберкулёзного очага, туберкулома, WGS, Corynebacterium kefirresidentii*

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The nature of the microbiota of the lower respiratory tract is very different from other microbial communities of the human body, for example, the gut microbiota and even the microbiota of the upper respiratory tract (URT), which are significantly more colonized by bacteria compared to the lower respiratory tract (LRT). In general, the microbiota of healthy lungs is characterized by low biomass and dynamic diversity [1]. Oligotrophic living conditions are created in the respiratory tract for microorganisms in comparison with the rich environment of the gastrointestinal tract. URTs are colonized by bacterial, viral and fungal communities, which are the main source of microbiota for the lower parts of the lungs. In healthy people, the lung microbiota seems to consist mainly of transient microorganisms, and its composition is determined by the balance between microbial immigration and elimination [2]. Despite the transitivity of the URT microbiota, it is possible that its composition has a protective effect against pathogens, preventing their penetration into the lower parts of the lungs [2]. The expediency of maintaining low bacterial contamination in the deep parts of the lungs is determined by the need to ensure effective gas exchange in the alveoli [1]. Therefore, the deep sections of the lungs have hundreds and thousands of times lower bacterial load than the URT. The microbial biomass is only 10^3–10^5 colony-forming units per 1 g of mammalian lung tissue (CFU/g) [3] or approximately 2.2×10^3 bacterial genomes per 1 cm^2 of the surface of human lungs [4]. For comparison, the lower parts of the human gastrointestinal tract are inhabited by 10^{11}–10^{12} CFU/g of tissue [5]. The microbiota of the URT of an adult is dominated by various representatives of the genera *Prevotella*, *Veillonella*, *Streptococcus*, *Leptotrichia*, *Rothia*, *Neisseria*, *Haemophilus*, *Moraxella*, *Staphylococcus*, *Corynebacterium*, *Fusobacterium*, etc. [6]. Our previous studies [7] indicate that the microbiota of the tuberculosis focus is divided into at least 2 types of communities: 1) mycobacterial caseoma (tuberculoma), in which more than 70 % of the genomes belong to *Mycobacterium tuberculosis*; 2) a polybacterial community in which the concentration of *Mycobacterium tuberculosis* varies from 0 to 30 %. According to our data, clinical strains of *Mycobacterium tuberculosis* are mostly unable to form biofilms [8], at the same time, in an *in vitro* experiment, the causative agent of tuberculosis significantly increased its number in the composition of a polymicrobial biofilm [9]. In other words, the study of polymicrobial communities of caseous contents can shed light on the microbial component of pathological processes occurring in the center of a tuberculous focus.

Granuloma (tuberculoma) was recognized as the leading pathology in pulmonary tuberculosis for more than 100 years ago [10]. The modern definition of pulmonary tuberculoma (caseoma) is a volumetric caseous – necrotic formation separated from the adjacent tissue by a capsule [11]. In the framework of this study, we postulate the hypothesis that the formation of the microbiota of a tuberculous focus occurs due to instability, compartmentalization and the transient nature of microbial communities in the lungs. It should be borne in mind that the conditions created by the patient's immune system in a tuberculous focus, capsule formation and curd necrotization of the contents with a predominance of lipids [12] should selectively stimulate the development of anaerobic microorganisms capable of multiplying due to the utilization of lipids. *Mycobacterium tuberculosis* is unable to reproduce under these conditions and survives in most cases in a dormant state [10]. Based on the above, the **aim of this study** was to isolate and characterize oxygen-tolerant anaerobes from the necrotic contents of tuberculosis.

MATERIALS AND METHODS

The study was approved by the Ethics Committee of the «Scientific Centre for Family Health and Human Reproduction Problems» (Protocol No. 4 dated November 16, 2020).

TABLE 1

CHARACTERISTICS OF THE EXAMINED SAMPLES

Sample #	Sex	Year of birth	CT diagnosis	Fraction, size of CT focus (mm)	Calcination
2201	F	1995	Tuberculoma of the left lower lobe with destruction	S6; up to 18	Yes
2202	F	1979	Tuberculoma of the upper lobe of the left lung in the insemination phase	S1; up to 21	Yes
2203	M	1993	Tuberculoma of the upper lobe of the right lung in the insemination phase	S1,2; up to 30	Yes
2204	F	1986	Tuberculoma of the lower lobe of the right lung in the insemination phase	S6; up to 19	No
2208	F	1984	Tuberculoma of the lower lobe of the right lung in the insemination phase	S6; up to 43	No

Note. CT – computed tomography.

Biopsy material from 5 patients with pulmonary tuberculosis was obtained during a surgical treatment of tuberculoma at the Irkutsk Regional Clinical Tuberculosis Hospital in 2022 (Table 1).

The caseous contents of the foci were cut out of the surgical biopsy with a sterile disposable scalpel in the bacteriological laboratory of a tuberculosis hospital and transfered to in 5 ml of LB broth under sterile vaseline oil in a volume of 1–2 g per tube. After 2 weeks, 0.1 ml of LB-broth was sieved onto LB-agar. The incubation of the cups was carried out in an anaerostat with Anaerogaz gas-generating packages (INKO, Russia). Isolated colonies were sown in 5 ml of LB broth under sterile vaseline oil to accumulate biomass. Antibiotic sensitivity was determined using TREK Diagnostic Systems (Thermo Fisher Scientific, USA) test systems: RAPMYCO for fast-growing mycobacteria and SLOWMYCO for slow–growing mycobacteria, according to the manufacturer's protocol. The assessment of resistance or sensitivity depending on the minimum inhibitory concentrations (MIC) was carried out in accordance with international recommendations [13]. Brain Heart Infusion Broth (HIMEDIA, India) was used as a broth for culture breeding, incubation was performed at 37 °C for three days in an anaerostat with Anaerogaz gas generating packages (INKO, Russia).

Lipase activity was determined by plating on Brain Heart Infusion agar (HIMEDIA, India) supplemented with 0.1 % Tween-80 and 10 mM of $CaCl_2$ (final concentrations). After incubation at 37 °C for three days in an anaerostat with Anaerogaz gas generating packages (INKO, Russia), the cups were incubated for a day at 4 °C. The presence of exogenous lipase activity was assessed by the formation of halo insoluble calcium salts of free fatty acids in the agar thickness around the colonies. DNA from the sediment of the broth culture was isolated by the CTAB chloroform method, as described earlier [14]. Whole genome sequencing was performed on a DNBSeq-G400 NGS sequencer by Genomed (Russia). The primary genome sequences are located at the National Center for Biotechnology Information of the USA (NCBI), project PRJNA971334.

The assembly of genomic readings into scaffolds was carried out using the Spades v. 3.11.1 software [15]. Genomic annotation and identification of the genes encoding *16S* rRNA and *23S* rRNA of the studied strain 2204 in the assembly was carried out using the SqueezeMeta software package [16]. The search for the nearest strains and species of bacteria with decoded complete genomes was carried out using the Type (Strain) Genome Server[1] using the algorithm of «genome wide hybridization» of strains *in silico* [17]. Cassettes of *16S* rRNA and *23S* rRNA genes were extracted from the complete genomes of identified closely related strains (*Corynebacterium kefirresidentii SB, Corynebacterium tuberculostearicum DSM, Corynebacterium curieae c8Ua, Corynebacterium yonathiae c21Ua, Corynebacterium marquesiae c19Ua)* according to information from the NCBI annotation. The *16S* rRNA and *23S* rRNA gene sequences were aligned in the MAFFT v. 7 program [18] and used to calculate genetic distances and determine the species of strain 2204 by the phylogenetic method (ML method; IQTREE v. 2 program [19] with an assessment of tree topology supports by the «ultrafast bootstrap» method – 1000 replicas). 99 % was considered the threshold of species identity for the complete *16S* rRNA gene [20].

[1] https://tygs.dsmz.de

RESULTS

Isolation and microbiological characteristics of the culture

The search for literary sources that can help in the development of methods for cultivating the satellite microbiota of the tuberculosis focus, both in Russian and in English language literature, has not led to any significant results. For example, the PubMed search for keywords «caseum + tuberculosis + cultivation + microbiota» returned a zero result. A search for «caseum + tuberculosis + cultivation» returned 20 publications, but none of them related to this topic. Based on this, it was decided to produce anaerobic cultivation under the most standardized conditions – LB-broth under vaseline oil.

As a result of incubation for 2 weeks, pronounced growth in a liquid LB medium was detected in one of the five tubes in the form of turbidity of the entire thickness and sediment on the surface of the biopsy. Sieving 0.1 ml of all five broth cultures on LB-agar followed by incubation in an anaerostat in only one case gave visible growth on Petri dishes.

FIG. 1.
Lipase activity of strain 2204. A halo is observed around the bacterial growth, indicating a high lipase activity of the isolated strain.

TABLE 2

TESTING OF THE SUSCEPTIBILITY OF STRAIN 2204 TO ANTITUBERCULOSIS AND ANTIMYCOBACTERIAL DRUGS WITH DETERMINATION OF MINIMUM INHIBITORY CONCENTRATIONS

No.	Antibiotics	MIC (μg/ml)	Result
1	Clarithromycin	0.06	Susceptibility
2	Rifabutin	0.25	Susceptibility
3	Ethambutol	4.0	Susceptibility
4	**Isoniazid**	**> 8**	**Resistance**
5	Moxyflixacin	0.12	Susceptibility
6	Rifampin	0.12	Susceptibility
7	**Trimethoprim/Sulfamethoxazolin**	**4/76**	**Resistance**
8	Amikacin	1.0	Susceptibility
9	Linezolid	1.0	Susceptibility
10	Ciprofloxacin	0.12	Susceptibility
11	Streptomycin	4	Susceptibility
12	Doxycicline	0.25	Susceptibility
13	**Ethionamide**	**> 20**	**Resistance**
14	Cefoxitin	4.0	Susceptibility
15	Tigecyclin	0.25	Susceptibility*
16	Imipenem	2.0	Susceptibility
17	Cefepime	1.0	Susceptibility
18	Amoxicillin/Clavulanic acid	2.0/1.0	Susceptibility
19	Ceftriaxon	4.0	Susceptibility
20	Minociclin	1.0	Susceptibility
21	Tobramicin	1.0	Susceptibility

Note. * – expected result (there are no international standards for necessary and sufficient MIC).

As can be seen from Figure 1 and Table 2, the isolated strain 2204 was characterized by high lipase activity and a relatively small spectrum of resistance to first-line antituberculosis and antimicobacterial drugs.

Whole genome sequencing and species identification of strain 2204

The primary short reads were reorganized into 6 scaffolds with a total length of 2,428,638 base pairs, among which 4 gene cassettes of the ribosomal operon *16S* rRNA – ITS – *23S* rRNA were found. Analysis using the algorithm of «genome-wide hybridization» of strains *in silico* for strain 2204 identified 5 of the closest reference genomes of the NCBI database: *Corynebacterium kefirresidentii SB; Corynebacterium tuberculostearicum DSM; Corynebacterium curieae c8Ua; Corynebacterium yonathiae c21Ua; Corynebacterium marquesiae c19Ua*. The genome length of strain 2204 corresponded to the genomes of closely related strains (lengths from 2,348,605 to 2,830,499 base pairs). The completeness of decoding the genome of strain 2204 by the presence of all necessary single-copy genes was 99.49 %. The *16S* rRNA and *23S* rRNA gene sequences of all the above genomes were used for phylogenetic identification of strain 2204. Fig. 2 shows the resulting phylogenetic tree with the above-mentioned nucleotide sequences. The shaded bush contains the tested nucleotide sequence of strain 2204. The calculation of the number of nucleotide substitutions relative to the nearest taxa showed more than 99 % identity of the strains (0.004 % substitutions). According to the strictest criteria to date,

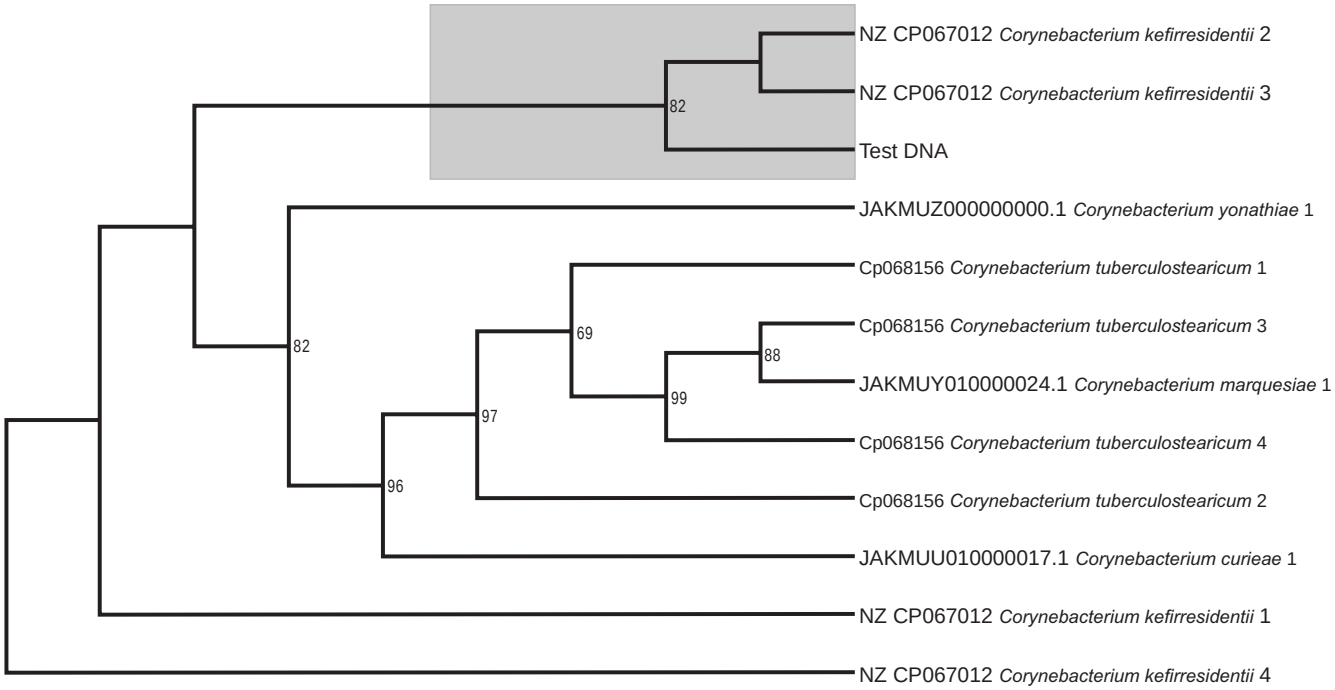

FIG. 2.

Phylogenetic relationships of strain 2204 with reference species. At the bottom of the nodes there are the bootstrap support values for branching. The shaded bush contains the tested nucleotide sequence of strain 2204

99 % should be considered the threshold of species identity for the complete *16S* rRNA gene [20]. Thus, the isolated strain can be uniquely identified as a species of *Corynebacterium kefirresidentii*.

DISCUSSION

The *Corynebacterium* genus of gram-positive rod-shaped bacteria belongs to the large family Corynebacteriaceae, was first proposed by Lehmann and Neumann in 1896 and currently has 177 species, some of which are of medical, veterinary or biotechnological interest [21]. Species of this genus are widespread and potentially pathogenic – primarily *C. diphtheria*. Corynebacteria are the dominant member of the human skin microbiota. Bacteria of the *Corynebacterium* genus account for 30 % of the total number of bacterial inhabitants of human skin [22]. The most common skin-dwelling species are represented by lipophilic *Corynebacterium tuberculostearicum*, *Corynebacterium kefirresidentii* and *Corynebacterium aurimucosum* type E, which form a narrow species complex [23]. It is of interest to note that for the first time, *C. tuberculostearicum* was isolated from focal skin lesions in leprosy [23]. An important feature of this group of species is the lack of the ability to biosynthesize *de novo* fatty acids [24], while the ability to produce specific mycolic acids, partly similar to those produced by mycobacteria, is observed. The peculiarities of lipid metabolism may explain drug resistance to isoniazid and ethionamide (Table 2), since this group of corynebacteria lacks the *InhA* gene [24, 25]. In other words, resistance to two anti–tuberculosis drugs – isoniazid and ethionamide – in this group of corynebacteria is constitutive.

CONCLUSION

The isolation of a lipophilic anaerobic *Corynebacterium tuberculostearicum* narrow species complex from a tuberculous focus, together with the results of our previous studies [7], indicates a possible role of the non-tuberculous microbiota in the liquefaction of caseous necrosis. This is important for understanding the pathological mechanisms of the formation of tuberculosis foci in the late stages of infection. We hypothesize that the formation of the microbiota of a tuberculous focus occurs due to instability, compartmentalization and the transient nature of microbial communities in the lungs. However, the conditions inside the tuberculous focus create favorable situation for the development of a secondary anaerobic lipophilic microbiota. Apparently, representatives of the species complex *C. tuberculostearicum* can play a negative role in pathological processes inside anaerobic tuberculoma, possibly provoking processes in the liquefaction of caseous masses.

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Conflict of interest

The authors of this article declare the absence of a conflict of interest.

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PROBIOTIC CONSORTIUMS: STRUCTURE AND ANTAGONISTIC ACTIVITY AGAINST OPPORTUNISTIC BACTERIA AND HUMAN NORMOBIOTA (USING THE EXAMPLE OF *ESCHERICHIA COLI*) *IN VITRO*

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ABSTRACT

Background. *Using probiotic preparations based on consortia of microorganisms not only helps to restore the balance of the intestinal microbiota, but also increases the therapeutic effect of probiotics. Promising sources for obtaining probiotic consortia are milk products that have undergone natural fermentation with the help of spontaneously formed microbial consortia.*

The aim. *To study the structure of five microbial consortia with probiotic properties from naturally fermented milk products and to assess in vitro their antagonistic activity against opportunistic bacteria and a representative of the human normobiota – Escherichia coli.*

Materials and methods. *The structure of bacterial consortia was analyzed by sequencing methods. The antagonistic activity of the consortia was assessed by the disk diffusion method.*

Results. *It has been established that the studied microbial consortiums are represented by Enterococcus spp. and Streptococcus spp. bacteria. In consortiums No. 1, No. 2, and No. 3, Enterococcus bacteria dominated, while in consortiums No. 4 and No. 5, Streptococcus dominated. Antagonistic activity was shown against four isolates of opportunistic bacteria: Klebsiella pneumoniae No. 493, Enterobacter hormaechei No. 372, Staphylococcus aureus No. 4 and Pseudomonas aeruginosa No. 25 IMB, as well as against one representative of the human normobiota – Escherichia coli No. 495. The highest growth delay zone is found in E. coli No. 495 isolate. Three test cultures (K. pneumoniae No. 509, E. coli ATCC25922 and P. aeruginosa No. 3 IMB) exhibited more dense growth around probiotic consortia.*

Conclusion. *The results of the study showed that the effect of probiotic consortia differing in the composition of microorganisms can be neutral and bactericidal. The presence of antagonistic activity in the studied microbial consortia against multiresistant isolates of opportunistic bacteria is a prospect for creating probiotics with antibacterial properties.*

Key words: *probiotics, structure of microbial consortia, ribosomal taxonomy, opportunistic pathogens, antagonistic activity*

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ПРОБИОТИЧЕСКИЕ КОНСОРЦИУМЫ: СТРУКТУРА И АНТАГОНИСТИЧЕСКАЯ АКТИВНОСТЬ В ОТНОШЕНИИ УСЛОВНО-ПАТОГЕННЫХ БАКТЕРИЙ И НОРМОБИОТЫ ЧЕЛОВЕКА (НА ПРИМЕРЕ *ESCHERICHIA COLI*) *IN VITRO*

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РЕЗЮМЕ

Актуальность. *Применение пробиотических препаратов на основе консорциумов микроорганизмов не только способствует восстановлению баланса кишечной микробиоты, но и увеличивает терапевтический эффект пробиотиков. Перспективными источниками получения пробиотических консорциумов являются кисломолочные продукты, подвергшиеся естественному сквашиванию при помощи спонтанно сформировавшихся микробных консорциумов.*

Цель работы. *Изучение структуры пяти микробных консорциумов с пробиотическими свойствами из кисломолочных продуктов естественного брожения и оценка их антагонистической активности в отношении условно-патогенных бактерий и представителя нормобиоты человека – Escherichia coli – in vitro.*

Материалы и методы. *Анализ структуры бактериальных консорциумов проводили методами секвенирования. Антагонистическую активность консорциумов оценивали диско-диффузионным методом.*

Результаты. *Установлено, что исследуемые микробные консорциумы представлены бактериями Enterococcus spp. и Streptococcus spp. В консорциумах № 1, № 2 и № 3 доминировали бактерии рода Enterococcus, в то время как в консорциумах № 4 и № 5 – Streptococcus. Показана антагонистическая активность в отношении четырёх изолятов условно-патогенных бактерий: Klebsiella pneumoniae № 493, Enterobacter hormaechei № 372, Staphylococcus aureus № 4 и Pseudomonas aeruginosa № 25 ИМБ, а также одного представителя нормобиоты человека – Escherichia coli № 495. Наибольшая зона задержки роста отмечена у изолята E. coli № 495. У трёх тест-культур (K. pneumoniae № 509, E. coli ATCC 25922 и P. aeruginosa № 3 ИМБ) наблюдался более плотный рост вокруг дисков с пробиотическими консорциумами.*

Заключение. *Результаты исследования показали, что влияние пробиотических консорциумов, отличающихся составом микроорганизмов, может быть нейтральным и бактерицидным. Наличие антагонистической активности у исследуемых микробных консорциумов в отношении полирезистентных изолятов условно-патогенных бактерий – перспектива для создания пробиотиков с антибактериальными свойствами.*

Ключевые слова: *пробиотики, структура микробных консорциумов, рибосомная таксономия, условно-патогенные бактерии, антагонистическая активность*

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OBJECTIVES

An actual way to restore the balance of the microbiota of the gastrointestinal tract is the use of probiotic preparations [1]. Recently, much attention has been paid to consortia of probiotic microorganisms, the use of which makes it possible to achieve the expected positive effects from taking probiotics [2]. In a symbiotic consortium, the biological properties of individual strains are mutually enhanced, which makes it possible to create a single biological system with protective properties against the influence of other microorganisms. Representatives of such genera as *Lactobacillus, Bifidobacterium, Streptococcus, Propionibacterium* and *Enterococcus* are most often of commercial interest among microorganisms with probiotic activity [1].

One of the directions of obtaining multistain preparations is the formation of a natural population of microorganisms. According to research, fermented milk products obtained by natural fermentation can be promising sources of such populations [3–5]. In this case, microorganisms independently form consortia with certain functional properties. Naturally formed populations of microorganisms can have a high degree of stability and synergistic effect, which makes them attractive for use as probiotics. An example of the formation of such populations can be a significant change in the species diversity of starter cultures of microorganisms, which, nevertheless, make it possible to obtain products conforming to GOST [6].

In the screening process of some commercial ferments intended for milk fermentation, despite compliance with the technology and instructions, microbial consortia were obtained, which differ from the declared ones on microscopic examination. At the same time, these consortia retained their probiotic properties, which was an important factor for studying their taxonomic structure and antagonistic activity.

THE AIM OF THE STUDY

To study the structure of five microbial consortia with probiotic properties from naturally fermented milk products and to assess *in vitro* their antagonistic activity against opportunistic bacteria and a representative of the human normobiota – *Escherichia coli*.

METHODS

The objects of research

The objects of the study were five microbial consortia with probiotic properties obtained from fermented dairy products of natural fermentation. 56 bacterial isolates were used as test cultures, of which 16 belong to the intestinal normobiota; 4 are reference strains; 36 are isolates of opportunistic bacteria with multiple antibiotic resistance (ADB), included in the Collection of Human Microbiota of the Irkutsk Region of the Scientific Centre for Family Health and Human Reproduction Problems [7]. Species composition of bacterial test cultures is shown in Table 1.

TABLE 1

SPECIES COMPOSITION OF BACTERIAL TEST CULTURES

Type of microorganism	Number of isolates, abs.
Human normobiota	
Escherichia coli NEA	16
Reference strain	
Escherichia coli ATCC 25922	1
Opportunistic bacteria	
Enterobacter cloacae	4
Enterobacter hormaechei	1
Citrobacter amalonaticus	1
Klebsiella oxytoca	1
Klebsiella pneumoniae	6
Klebsiella pneumoniae	2
Proteus mirabilis	1
Pseudomonas aeruginosa	12
Staphylococcus aureus	3
Escherichia coli WEA	3
Escherichia coli HA	2
Reference strains	
Enterococcus faecalis ATCC 29212	1
Pseudomonas aeruginosa ATCC 10145	1
Staphylococcus aureus ATCC 25923	1

Note. NEA – normal enzymatic activity; WEA – weak enzymatic activity; HA – hemolytic activity.

Study design

1. Study of the structure of five probiotic consortia:

a) sequencing of next generation amplicons (NGS, next generation sequencing);

b) sequencing of the 16S fragment of the ribosomal operon using the Sanger method.

2. Testing of the antagonistic activity of five probiotic consortia against test cultures of opportunistic bacteria and a representative of the human normobiota – *Escherichia coli – in vitro*.

Methods of study

1a. The structure of bacterial consortia was analyzed by high-throughput V3–V4 sequencing of variable fragments of the *16S* rRNA gene. DNA from storage cultures was isolated using a commercial set of Quick-DNA Miniprep Kits (Zymo Research, USA). Amplification of the target fragment was performed on highly conserved bacterial primers NGS318L and NGS806R with adapters (Table 2). The resulting amplicons were purified from primer dimers using AMPure XP (Beckman Coulter, USA) according to the manufacturer's protocol.

Sequencing using Illumina technology was carried out at the Genomic Technologies, Proteomics and Cell Biology of the All-Russian Research Institute of Agricultural Meteorology.

The NGS results were processed and taxonomically annotated using the QIIME2 v. 2022.2 platform and the SILVA 138 nucleotide sequence database.

1b. Identification of the dominant bacteria belonging to the consortium was carried out using ribosomal phylogeny using a site including V3–V8 variable regions of the *16S* rRNA gene. Primers 500F and 1350R were used to amplify this fragment (Table 2). Amplicons obtained by polymerase chain reaction (PCR) were purified in 1% agarose gel and embedded in the pJET1.2 vector according to the manufacturer's protocol (Thermo Fisher Scientific, USA). The annular plasmid was transformed into competent *E. coli* XL-1 cells [8] and direct screening of all grown colonies was performed for the presence of an insert of the required length on plasmid primers pJET1.2-F and pJET1.2-R (Table 2).

The sequence reaction was carried out using reagents Brilliant Dye Cycle Sequencing Kit v. 3.1 (NimaGen, the Netherlands) according to the manufacturer's protocol.

TABLE 2
THE STRUCTURE OF PRIMERS USED IN THE STUDY

Name	Structure (5'-3')	PCR conditions
NGS318F	TCGTCGGCAGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG	Step 1, Cycle = 01 T1 = 95 °C; t = 3 min Step 2, Cycle = 25 T1 = 95 °C; t = 30 s T2 = 55 °C; t = 30 s T3 = 72 °C; t = 30 s Step 3, Cycle = 01 T1 = 72 °C; t = 5 min
NGS806R	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCMGGGTATCTAATCCKGTT	
500F	GTGCCAGCAGCCGCGGTAA	Step 1 Cycle = 01 T1 = 95 °C; t = 5 min Step 2, Cycle = 25 T1 = 94 °C; t = 30 s T2 = 60 °C; t = 30 s T3 = 72 °C; t = 1 min Step 3, Cycle = 01 T1 = 72 °C; t = 5 min
1350R	GACGGGCGGTGTGTACAAG	
pJET1.2-F	CGACTCACTATAGGGAGAGCGGC	Step 1, Cycle = 01 T1 = 95 °C; t = 3 min Step 2, Cycle = 25 T1 = 94 °C; t = 30 s T2 = 60 °C; t = 30 s T3 = 72 °C; t = 1 min Step 3, Cycle = 01 T1 = 72 °C; t = 5 min
pJET1.2-R	AAGAACATCGATTTTCCATGGCAG	

Amplicons were sequenced by Sanger on the Nanophor-05 device at the Center for the Development of Progressive Personalized Health Technologies of the Scientific Centre for Family Health and Human Reproduction Problems.

Sequents were adjusted visually in the Bioedit v. 7.2.5 software. Species identification was performed by comparing the nucleotide sequence with the NCBI NR and EMBL-ENA Sequences bases using BLAST and FASTA, respectively.

2. The antagonistic activity of probiotic consortia was determined by the disc diffusion method, according to the standard methodology for determining the sensitivity of microorganisms to antimicrobial preparations [9]. Borosilicate glass filters Glass Microfiber Filters (GF/F) (Whatman plc., UK) with a diameter of 0.5 cm were used as discs, on which suspensions of the tested consortia in a volume of 15 µl were pipetted. The total content of microbial cells in each consortium was 10^{10}–10^{11} CFU/cm^3. Dishes were incubated at 37°C for 24 hours.

The measurement of growth retardation zones (GRZ) of test crops, taking into account the filter diameter, was carried out according to the results of two separate experiments using the ImageJ v. 1.5.3 graphical editor, which allows for image analysis. At GRZ of 6.0 mm and above, the consortium was considered to exhibit antagonism. The data are presented in the form of the arithmetic mean diameters of the growth suppression zones of test cultures (*M*) and the RMS deviation (*m*).

RESULTS AND DISCUSSION

According to the results of high-performance sequencing, *Enterococcus* spp entered the microbial consortia as dominant bacteria. and *Streptococcus* spp. (Table 3).

TABLE 3

STRUCTURE OF MICROBIAL CONSORTIA WITH PROBIOTIC PROPERTIES, %

Characteristics of the taxon	No. of the microbial consortium				
	1	2	3	4	5
Enterococcus	89.8	69.2	51.7	0.2	0.6
Streptococcus	7.2	25.2	36.9	86.6	88.3
Other	2.22	5.32	8.88	10.48	9.95

The proportion of concomitant bacteria ranged from 2.22 to 10.48 %. Consortia No. 1 and No. 2 were dominated by representatives of the genus *Enterococcus*, in consortia No. 4 and No. 5 – *Streptococcus,* while in consortium No. 3 the relative content of representatives of these genera was 51.7 % and 36.9 %, respectively.

Identification of the dominant bacteria based on ribosomal taxonomy showed that two types of enterococci were identified in Consortium No. 1 – *Enterococcus durans* and *Enterococcus thailandicus* (Table 4). Consortium No. 2 turned out to be richer in the diversity of enterococci: in addition to the above-mentioned species, *Enterococcus faecium* was identified in it. In addition, streptococci made up 25.2 % of the structure of the microbial consortium. They have been identified as *Streptococcus salivarius* and *Streptococcus thermophilus*. In consortium No. 3, enterococci are represented by the species *E. durans*, and streptococci by the species *S. salivarius* and *S. thermophilus.* The composition of consortium No. 4 is represented by the *S. thermophilus* monoculture, while *Lactobacillus brevis* was identified in addition to *S. thermophilus* in consortium No. 5.

Phylogenetic analysis showed that for bacteria of the genus *Enterococcus*, the sequences formed either independent branches or together with sequences of typical strains (Fig. 1).

E. durans is represented in the structure of consortia No. 1, No. 2 and No. 3; *E. faecium* is defined in consortium No. 2; *E. thailandicus* – in consortium No. 1.

To assess the ecological and genetic characteristics of enterococci that are part of the studied consortia, a variety of biotopes of homologous strains were analyzed, among which fermented dairy products, saliva, gastrointestinal tract (GIT) of humans and animals, faeces were found (Fig. 2).

Representatives of consortia No. 1, No. 2 and No. 3 showed phylogenetic affinity with isolates isolated from the human intestinal biotope (Fig. 2, highlighted in green), human breast milk and food dairy products (Fig. 2, highlighted in blue) and from the vaginal biotope (Fig. 2, highlighted in red).

The separation of the studied sequences and homologous strains from the NCBI database into fecal, lactic and vaginal biotopes indicates the genetic differences of microorganisms from different ecological groups. Such differences may cause a different degree of manifestation of probiotic properties. The study of the ecological and genetic characteristics of microorganisms with probiotic properties will help to form balanced microbiocenoses for specific human biotopes [10].

Figure 3 shows a phylogenetic tree based on V3–V8 variable fragments of the *16S* rRNA gene for phylotypes assigned to the genus *Streptococcus* and homologous isolates from different biotopes.

There is a difference between representatives of consortia No. 2, No. 4 and No. 5, which have separated into independent branches. The tree shows that the representatives of consortium No. 2 had homology with the *S. salivarius* H4 isolate. Also, all the studied consortia were represented by phylotypes that showed homology with *S. thermophilus isolates.* Homologous isolates were obtained from various dairy products and biotope of the human oral cavity.

TABLE 4

RIBOSOMAL TAXONOMY OF BACTERIA DOMINATED IN MICROBIAL CONSORTIA WITH PROBIOTIC PROPERTIES

Labelling of a clonal sequence	The nearest bacterial homologue	Percentage of homology, %
	Consortium No. 1	
1.10; 1.27	*Enterococcus durans* HBUAS54304	99.4; 100
1.19; 1.28	*Enterococcus durans* IPLA 655	99.5; 99.5
1.20; 1.21; 1.23	*Enterococcus thailandicus* LM4-1	99.4–99.5
1.25	*Enterococcus thailandicus* Marseille-AA00296	99.8
	Consortium No. 2	
2.1	*Enterococcus thailandicus* LM4-1	100
2.2	*Enterococcus thailandicus* Colony540	99.4
2.6	*Enterococcus durans* HBUAS54304	100
2.8	*Streptococcus salivarius* H4	99.4
2.10	*Enterococcus faecium* HBUAS66260	99.6
2.11	*Streptococcus thermophilus* ST106	99.9
2.13	*Enterococcus durans* ABRIINW.N3	99.6
	Consortium No. 3	
3.1	*Streptococcus salivarius* H4	99.1
3.3	*Enterococcus durans* ULAG	98.7
3.4; 3.7; 3.11	*Streptococcus thermophilus* c 21.5	97.8–98.4
3.8	*Enterococcus durans* HBUAS54304	99.4
3.10	*Streptococcus thermophilus* STN57	97.9
	Consortium No. 4	
4.1;	*Streptococcus thermophilus* ASR-1	98.8
4.2; 4.3	*Streptococcus thermophilus* IMAU:80427	99.1; 98.8
4.5	*Streptococcus thermophilus* ChR-I-str19	99.8
4.6; 4.7; 4.8	*Streptococcus thermophilus* BL13-10	99.0–99.8
	Consortium No. 5	
5.1	*Streptococcus thermophilus* ChR-I-str19	99.6
5.3; 5.2; 5.4; 5.5; 5.6; 5.8	*Streptococcus thermophilus* BL13-10	97.9–99.5
5.7	*Streptococcus thermophilus* PT110	84.4

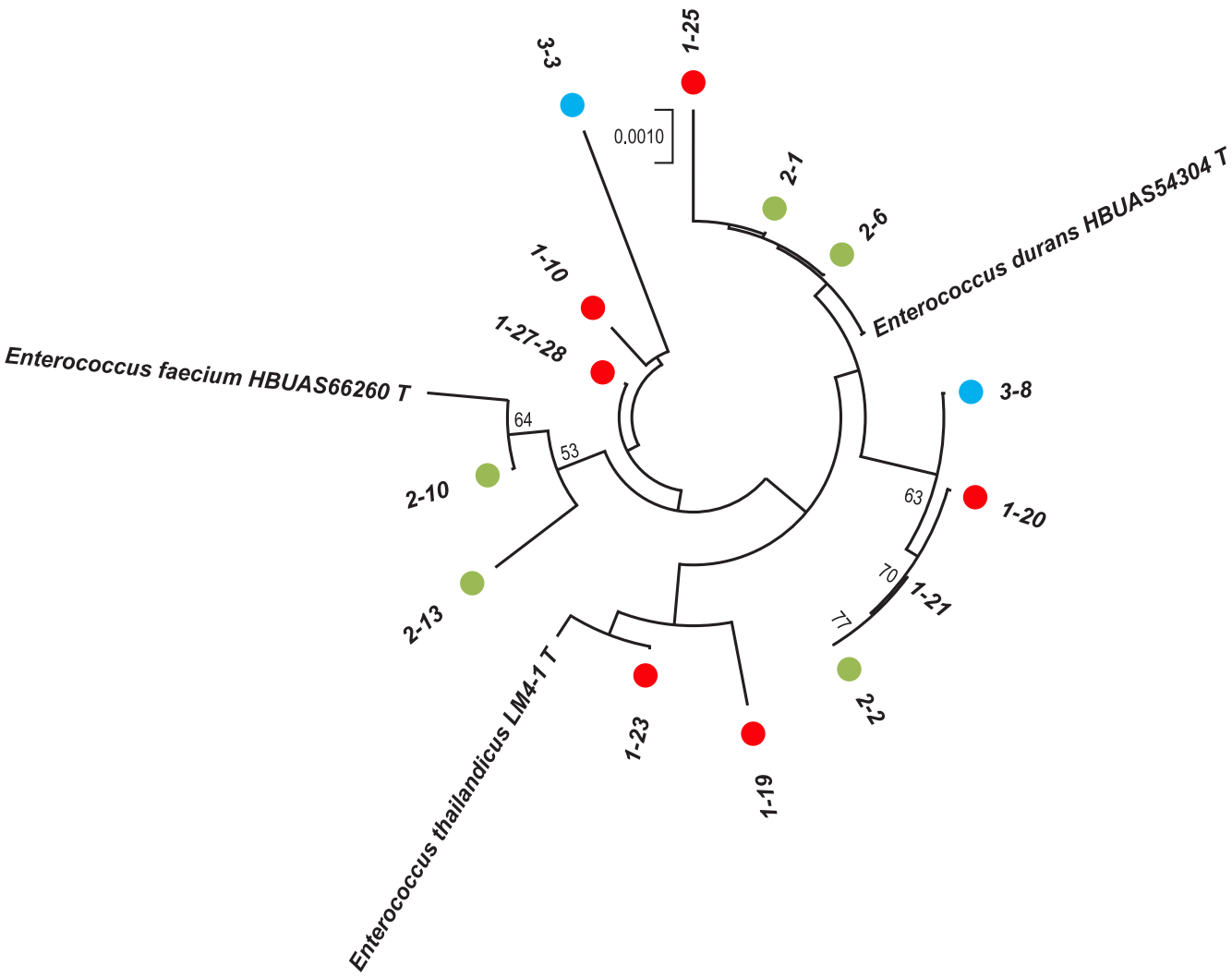


FIG. 1.

Phylogenetic tree based on V3–V8 variable fragments of the 16S rRNA gene for phylotypes assigned to the genus Enterococcus and type strains: the nodes show values of bootstrap support (%) above 50

The presence of antagonistic activity against pathogenic and opportunistic microorganisms (OM) is one of the main characteristics of the strain for classifying it as a probiotic species. The results of the study showed that the effect of the probiotic consortia under study, which differ in the composition of microorganisms, can be neutral and bactericidal: antagonistic activity was noted against five isolates of test cultures (Table 5).

All probiotic consortia suppressed the growth of two isolates: OM – *K. pneumoniae* No. 493 and a representative of the intestinal normobiota – *E. coli* No. 495. The maximum growth suppression zone was noted in *E. coli* isolate No. 495 with consortium No. 3. Consortia No. 1, No. 2, No. 4 and No. 5 exhibited antagonistic activity against *E. hormaechei* No. 372, consortia No. 1, No. 3 and No. 5 – against *S. aureus* No. 4, consortium No. 3 – against *P. aeruginosa* No. 25 IMB.

Three test cultures exhibited more dense growth around filters with probiotic consortia: *K. pneumoniae* isolate No. 509 and the reference strain of *E. coli* ATCC 25922 have discs with all five consortia, isolate *P. aeruginosa* No. 3 IMB – around probiotic consortia No. 1, No. 2, No. 3 and No. 4 (Fig. 4). This fact requires further detailed study using techniques designed to evaluate the growth-stimulating properties of bacteria.

Recently, quite a lot of papers have been published on the use of probiotics based on *Streptococcus* spp. and *Enterococcus* spp. and their beneficial properties for the human body. Probiotic strains of enterococci and streptococci are widely used to correct human intestinal dysbiosis, as well as in chronic gastrointestinal diseases [11, 12]. *S. thermophilus* strain belongs to the group of lactic acid bacteria that ferment sugars to lactic acid, exerting an acidifying effect and providing a bactericidal effect against many pathogenic microorganisms [13]. Enterococcal cultures have long been used for cooking meat, milk and vegetables. The low content of enterococci in meat and dairy products does not allow pathogenic staphylococci and *E. coli* to multiply [14]. It is known that the antibacterial activity of enterococci is associated with their ability to synthesize specific proteins – enterocins A, B, L50A/B, P, Q and Xa/β. The presence of such proteins determines the antagonistic activity of enterococci against infectious

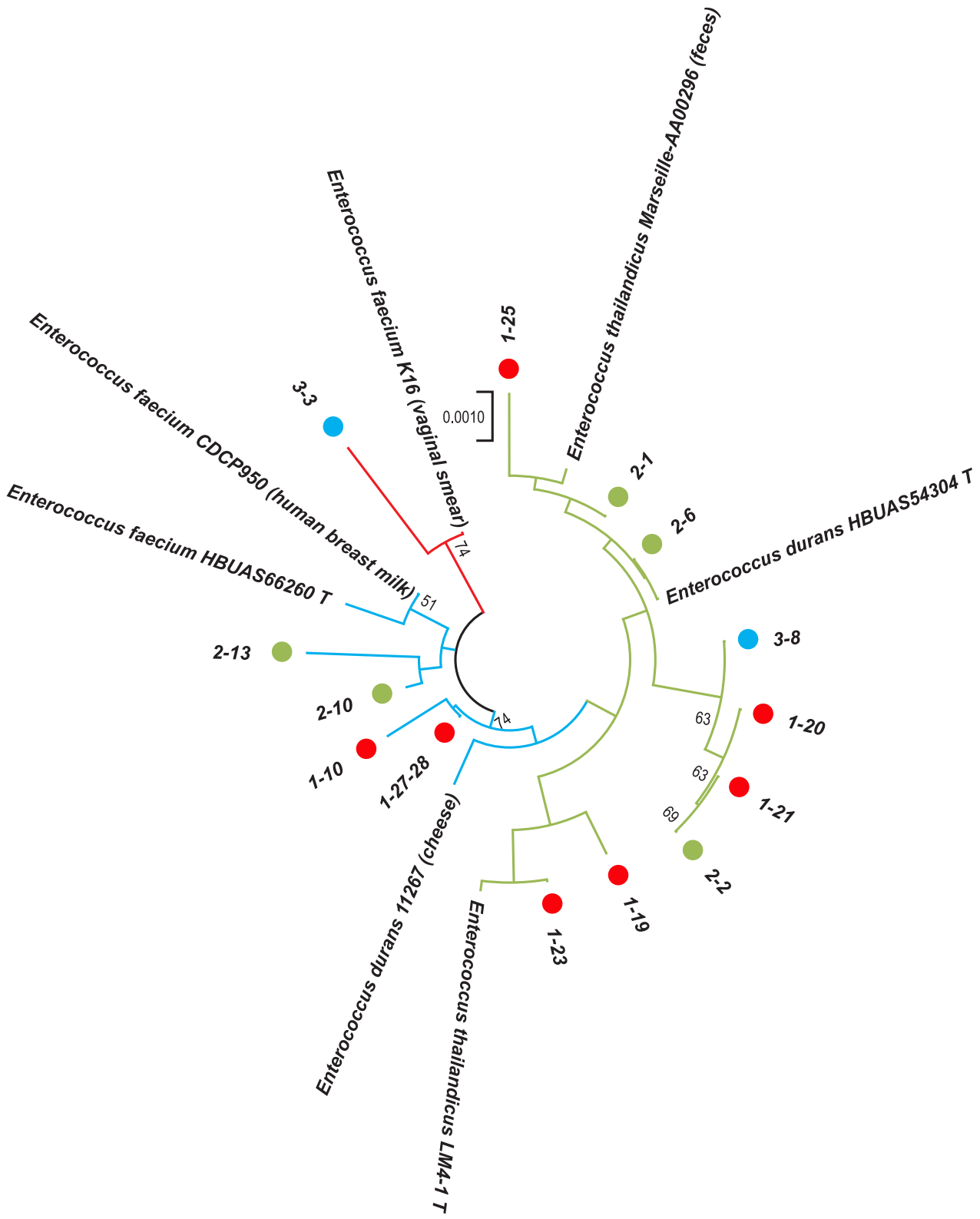


FIG. 2.

Phylogenetic tree based on V3–V8 variable fragments of the 16S rRNA gene for phylotypes assigned to the genus Enterococcus, type strains and homologous strains from various biotopes: the nodes show values of bootstrap support (%) above 50

agents such as *S. aureus*, *E. coli*, as well as *Klebsiella* spp., *Enterobacter* spp., *Streptococcus* spp., *Listeria* spp. and *Clostridium* spp. [15, 16]. The data obtained by us on the antagonistic activity of probiotic consortia based on *Streptococcus* spp. and *Enterococcus* spp. correspond to the data found in the literature. However, the tested isolates have multiple antibiotic resistance, which means they are more adapted to the effects of negative environmental factors. Probably, this can explain the selective sensitivity of resistant isolates of one type of OM to the action of probiotic consortia.

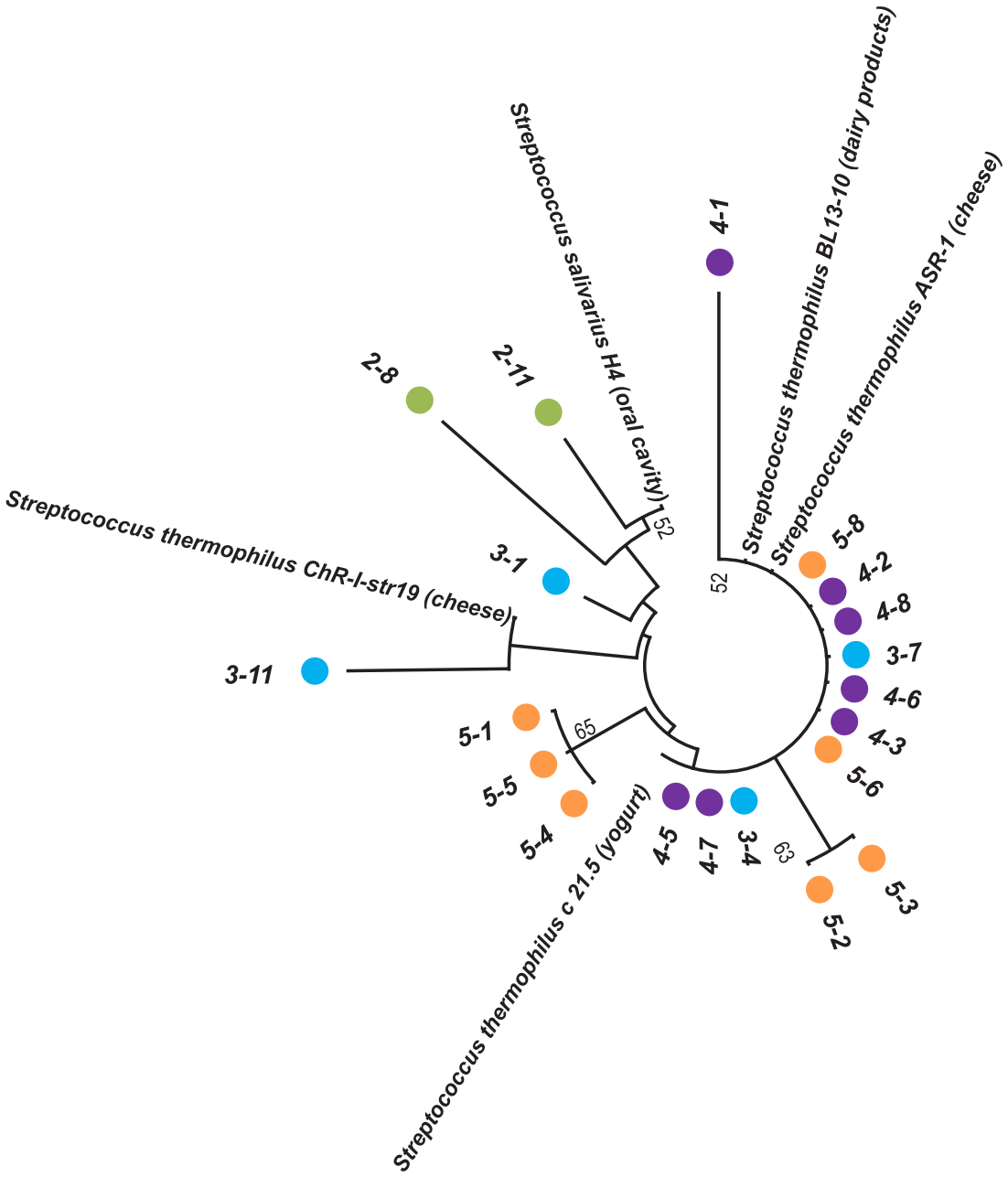


FIG. 3.
Phylogenetic tree based on V3–V8 variable fragments of the 16S rRNA gene for phylotypes assigned to the genus Streptococcus and homologous strains: the nodes show values of bootstrap support (%) above 50

CONCLUSION

In this study, the taxonomic structure and antagonistic activity of five microbial consortia with potential probiotic properties were investigated. Using molecular genetic methods, it was found that the studied microbial consortia are represented by *Enterococcus* spp. and *Streptococcus* spp. bacteria. n consortiums No. 1, No. 2, and No. 3, *Enterococcus* bacteria dominated, while in consortiums No. 4 and No. 5, *Streptococcus* dominated. Representatives of the genus *Enterococcus* were identified as *E. durans*, *E. thailandicus*, *E. faecium*; the genus *Streptococcus* was represented by the species *S. salivarius* and *S. thermophilus*.

It was shown that the effect of probiotic consortia, differing in the composition of microorganisms, can be neutral and bactericidal. The probiotic consortia studied suppressed the growth of four isolates of opportunistic bacteria, such as *Klebsiella pneumoniae* No. 493, *Enterobacter hormaechei* No. 372, *Staphylococcus aureus* No. 4 and *Pseudomonas aeruginosa* No. 25, as well as one representative of the human normobiota, *Escherichia coli* No. 495. The largest growth retardation zone was observed in *E. coli* isolate No. 495 in the presence of consortium No. 3.

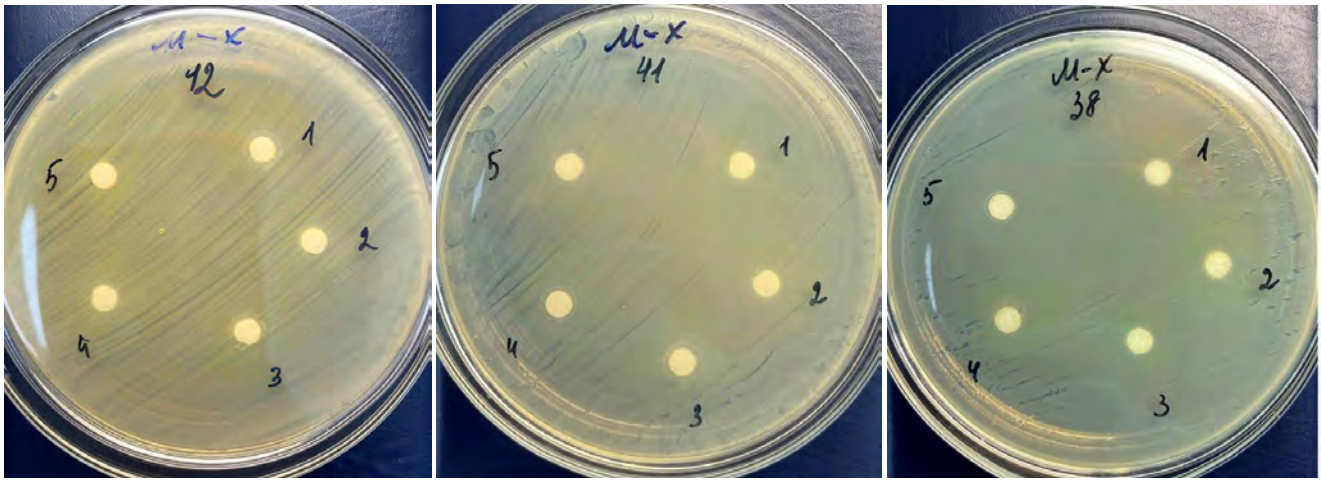
The presence of antagonistic activity in the studied microbial consortia in relation to polyresistant isolates of opportunistic bacteria may be a prospect for the crea-

TABLE 5

ANTAGONISTIC ACTIVITY OF PROBIOTIC CONSORTIA IN RELATION TO OPPORTUNISTIC BACTERIA AND HUMAN NORMOBIOTA

No.	Type of microorganism (isolate number) / characteristic [a]	Growth retardation zones, mm (M ± m)				
		No. 1	No. 2	No. 3	No. 4	No. 5
1	*Proteus mirabilis* (No. 371) / OM, ADB	0	5.5 ± 0.2	5.5 ± 0.2	0	0
2	*Enterobacter hormaechei* (No. 372) / OM, ADB	6.7 ± 0.3[b]	6.2 ± 0.2[b]	5.7 ± 0.2	6.3 ± 0.3[b]	7.0 ± 0.2[b]
3	*Klebsiella pneumoniae* (No. G) / OM, ADB	5.4 ± 0.1	5.7 ± 0.2	5.6 ± 0.2	5.4 ± 0.2	5.5 ± 0.2
4	*Enterobacter cloacae* (No. 394) / OM, ADB	5.2 ± 0.1	5.2 ± 0.1	5.2 ± 0.1	0	5.2 ± 0.1
5	*Klebsiella oxytoca* (No. 439) / OM, ADB	5.2 ± 0.1	5.2 ± 0.1	5.3 ± 0.2	5.2 ± 0.1	5.2 ± 0.1
6	*Klebsiella pneumoniae* (No. 493) / OM, ADB	6.8 ± 0.[3]b	6.4 ± 0.3[b]	6.8 ± 0.3[b]	6.9 ± 0.3[b]	6.6 ± 0.3[b]
7	*Klebsiella variicola* (No. 672) / OM, ADB	5.2 ± 0.1	5.2 ± 0.1	5.2 ± 0.1	5.2 ± 0.1	5.2 ± 0.1
8	*Staphylococcus aureus* (No. 672), / OM, ADB	5.2 ± 0.1	5.2 ± 0.1	5.2 ± 0.1	5.2 ± 0.1	5.2 ± 0.1
9	*Staphylococcus aureus* (No. 4) / OM, ADB	6.2 ± 0.3[b]	5.9 ± 0.1	6.3 ± 0.3[b]	5.2 ± 0.1	6.5 ± 0.3[b]
10	*Klebsiella pneumoniae* (No. 41HE) / OM, ADB	5.4 ± 0.1	5.6 ± 0.3	5.4 ± 0.2	5.6 ± 0.2	5.3 ± 0.1
11	*Staphylococcus aureus* (No. 846) / OM, ADB	0	5.4 ± 0.3	5.2 ± 0.1	0	5.2 ± 0.1
12	*Klebsiella pneumoniae* (No. 381) / OM, ADB	5.2 ± 0.1	5.2 ± 0.1	5.2 ± 0.1	5.4 ± 0.2	5.2 ± 0.1
13	*Pseudomonas aeruginosa* (No. 25 IMB) / OM, ADB	0	5.8 ± 0.1	6.3 ± 0.3[b]	0	5.9 ± 0.1
14	*Pseudomonas aeruginosa* (No. 54 IMB) / OM, ADB	5.2 ± 0.1	5.2 ± 0.1	5.3 ± 0.2	5.7 ± 0.2	5.2 ± 0.1
15	*Pseudomonas aeruginosa* (No. 82 IMB) / OM, ADB	5.2 ± 0.1	0	5.2 ± 0.1	5.2 ± 0.1	5.2 ± 0.1
16	*Pseudomonas aeruginosa* (No. 3 IMB) / OM, ADB	+	+	+	+	5.2 ± 0.1
17	*Pseudomonas aeruginosa* (No. 5 IMB) / OM, ADB	5.2 ± 0.1	5.2 ± 0.1	5.2 ± 0.1	5.3 ± 0.1	5.3 ± 0.2
18	*Enterobacter cloacae* (No. 25) / OM, ADB	5.2 ± 0.1	5.2 ± 0.1	5.2 ± 0.1	5.5 ± 0.2	5.2 ± 0.1
19	*Klebsiella pneumoniae* (No. 509) / OM, ADB	+	+	+	+	+
20	*Escherichia coli* (No. 473) / ADB	5.2 ± 0.2	5.2 ± 0.1	5.2 ± 0.1	5.2 ± 0.2	5.2 ± 0.1
21	*Escherichia coli* (No. 495) / ADB	7.2 ± 0.3[b]	7.9 ± 0.3[b]	8.2 ± 0.3[b]	7.4 ± 0.3[b]	7.3 ± 0.3[b]
22	*Escherichia coli* (No. 6G) / normobiota	5.2 ± 0.1	5.2 ± 0.2	5.2 ± 0.1	5.2 ± 0.1	5.2 ± 0.1
23	*Escherichia coli* (No. 133HE) / normobiota	5.2 ± 0.1	5.2 ± 0.1	5.2 ± 0.2	0	5.2 ± 0.1
24	*Escherichia coli* (ATCC 25922) / reference strain	+	+	+	+	+

Note. [a] – characteristic of the isolate; OM – opportunistic microorganism; ADB – presence of multiple antibiotic resistance; 0 – no effect; + – denser growth of the test culture around the filter; [b] – the consortium exhibited antagonism.



a ***b*** ***c***

FIG. 4.

Growth of test cultures around filters with probiotic consortiums: ***a*** *– Klebsiella pneumoniae No. 509;* ***b*** *– Escherichia coli ATCC 25922;* ***c*** *– Pseudomonas aeruginosa No. 3 IMB;* 1 *– consortium No. 1;* 2 *– consortium No. 2;* 3 *– consortium No. 3;* 4 *– consortium No. 4;* 5 *– consortium No. 5*

tion of probiotic preparations with antibacterial properties in conditions of widespread drug resistance of microorganisms.

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Conflict of interest

The authors of this article declare the absence of a conflict of interest.

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END-TO-END CONVOLUTIONAL NEURAL NETWORK FOR AUTOMATIC ENCODING FACIAL DESCRIPTOR (N-CNN) IN THE DIAGNOSIS OF INTRAUTERINE DISTRESS

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ABSTRACT

Background. *Existing methods for studying intrauterine distress, despite their prevalence, still have their limitations, so studying and assessment of fetal movements during ultrasound diagnostics can become a convenient and affordable additional tool for diagnosing this pathological condition.*

The aim of the study. *To assess the prevalence and diagnostic significance of a known set of fetal facial movements for the timely determination of intrauterine distress.*

Methods. *This prospective single-center study included 225 fetuses of a gestational age from 32 to 40 weeks. The FIGO chart was used as fitting criteria of intrauterine distress. The assessment of facial movements in all fetuses was carried out using the BabyFACS technique, where the action unit (AU) used for the assessment; its coding is carried out in strict accordance with the chart of motor descriptors (MD). Statistical data processing was carried out using SPSS Statistics 20 (IBM Corp., USA). The Mann – Whitney test was used as the main statistical parameter, where a threshold level of 0.05 was chosen to interpret the p-tests value.*

Results. *Despite the occurrence of AU1, AU2, AU3, AU4 in both groups, these MDs were recorded in the group with confirmed distress (p = 0.00001). Facial units such as AU9 and AU20 were found only in children with intrauterine distress, which, in the total amount of the MD assessment, can be considered one of the main search signs that specialists should first of all pay attention to. All motor descriptors showed high positive predictive value and diagnostic sensitivity, with the highest results registered for AU9 and AU20.*

Conclusion. *Assessment of facial units during ultrasound diagnostics can be a convenient tool as an additional diagnosis of the development of intrauterine distress and requires further study.*

Key words: *ultrasound diagnostics, neonatology, motor descriptors, facial movements, intrauterine distress, fetuses*

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СКВОЗНАЯ СВЁРТОЧНАЯ НЕЙРОНАЛЬНАЯ СЕТЬ ДЛЯ АВТОМАТИЧЕСКОГО КОДИРОВАНИЯ ЛИЦЕВЫХ ДЕСКРИПТОВ (N-CNN) В ДИАГНОСТИКЕ ВНУТРИУТРОБНОГО ДИСТРЕССА

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РЕЗЮМЕ

Обоснование. *Существующие методы исследования внутриутробного дистресса, несмотря на свою распространённость, всё ещё имеют недостатки, поэтому изучение и оценка движений плода во время проведения ультразвуковой диагностики могут стать удобным и доступным дополнительным инструментом для диагностики данного патологического состояния.*

Цель исследования. *Оценка распространённости и диагностической значимости известной совокупности лицевых движений плода для своевременного определения внутриутробного дистресса.*

Методы. *В настоящее проспективное одноцентровое исследование были включены 225 плодов с гестационным возрастом от 32 до 40 недель. В качестве критериев соответствия внутриутробного дистресса использовалась таблица FIGO. Оценка лицевых движений у всех плодов проводилась с помощью методики BabyFACS, где для оценки использовалась двигательная единица (AU, action unit), кодировка которой проводится в строгом соответствии с таблицей двигательных дескрипторов (ДД). Статистическая обработка данных проводилась с использованием SPSS Statistics 20 (IBM Corp., США). В качестве основного статистического параметра был использован критерий Манна – Уитни, где для интерпретации значения p-тестов выбран пороговый уровень 0,05.*

Результаты. *Несмотря на встречаемость AU1, AU2, AU3, AU4 в обеих группах, данные ДД регистрировались в группе с подтверждённым дистрессом (p = 0,00001). Такие лицевые единицы, как AU9 и AU20, встречались только у детей с внутриутробным дистрессом, что в общей сумме оценки ДД можно считать одними из главных поисковых знаков, на которые в первую очередь следует обратить внимание специалистам. Все двигательные дескрипторы показали высокую прогностическую ценность положительного результата и диагностическую чувствительность, где самые высокие результаты зарегистрированы для AU9 и AU20.*

Заключение. *Оценка лицевых единиц при проведении ультразвуковой диагностики может быть удобным инструментом в качестве дополнительной диагностики развития внутриутробного дистресса и требует дальнейшего изучения.*

Ключевые слова: *ультразвуковая диагностика, неонатология, двигательные дескрипторы, лицевые движения, внутриутробный дистресс, плоды*

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OBJECTIVES

To date, in numerous studies, we can see the growing interest of the scientific community in understanding individual behavioral reactions based on the assessment of facial expressions, body and head movements, or sound signals [1–4].

Facial expression can give an idea of a person's emotional state, and automatic facial expression analysis is the subject of extensive research [5], however, this method cannot be fully used in both neonatology and perinatology for several good reasons. Firstly, facial expressions of infants and fetuses include additional important units that are not present in the standard coding system [6]. Secondly, such various individual parameters as gestational age significantly affect facial features in connection with the development of the central nervous system [6]. Thirdly, the processing stage in the case of newborns or fetuses is more complex and requires the use of existing experience, since these two groups do not allow establishing communicative contact.

Having some personal experience [7, 8] in evaluating facial movements in both infants and fetuses with different gestational ages, we decided to consider the prospects for evaluating facial movements during ultrasound examination as a possible predictor of fetal distress before cardiotocography (CTG).

Fetal distress is a syndrome of respiratory and circulatory insufficiency caused by fetal intrauterine hypoxia and is closely related to changes in fetal heart rate signals [9]. Early detection and possible diagnosis of the risk of developing this condition can help prevent damage to vital organs; that is why it is so important to strengthen monitoring of the fetal condition during pregnancy in order to ensure the safety of both the fetus and the pregnant woman.

The most common method of monitoring the condition of the fetus in clinical practice is CTG monitoring [10], where the received signal consists of a curve of heart rate (HR) and a curve of uterine contraction. CTG monitoring undoubtedly helps doctors diagnose this pathological condition in time to take effective therapeutic measures to protect the health of a fetus; in addition, this examination method is absolutely safe and painless. At the moment, CTG of a fetus in the absence of any disorders in a woman is carried out starting from the 32nd week about 2 times a month, while between these examinations women often undergo ultrasound research, and then modern technologies allow real-time monitoring of facial expressions and facial movements that the fetus demonstrates [11, 12].

Thus, the study of fetal facial movements and their sequence at the time of planned ultrasound diagnostics can in the future become a convenient and affordable additional tool that helps identify predictors of fetal distress even before CTG is performed.

THE AIM OF THE STUDY

To assess the prevalence and diagnostic significance of a known set of fetal facial movements for the timely determination of intrauterine distress.

METHODS

This prospective single-center study included 225 fetuses of a gestational age from 32 to 40 weeks, whose mothers underwent examinations and inpatient treatment at the Perinatal Center of the Voronezh Regional Clinical Hospital No. 1 from 2017 till 2021. All the patients were divided into two groups: group I (n = 125) – fetuses with a CTG–confirmed diagnosis of «intrauterine distress»; group II (n = 100) – fetuses without a CTG-confirmed diagnosis of «intrauterine distress» (control group). Study entry criteria: consent of legal representatives to participate in the study; regular obstetric examinations during pregnancy; conducting the presented complex of instrumental studies on the basis of the Perinatal Center; completeness and accuracy of records of clinical observation and results of instrumental studies; absence of severe fetal dentofacial anomalies that prevent visual assessment of facial movements. The FIGO chart was used as fitting criteria of intrauterine distress, which is included in the clinical recommendations of the Russian Society of Obstetricians and Gynecologists (ROAG) 2022, where at least one criterion of the following is required for confirmation: basal heart rate less than 100 or more than 170 bpm; variability less than 5 bpm in 40 minutes or sinusoidal rhythm; lack of acceleration; variable or late deceleration. Confirmation of fetal distress was also necessarily carried out after birth, when the following diagnostic criteria were considered: Apgar score < 7 points 5 minutes after birth; assessment of the acid-base state of umbilical cord blood (pH < 7.00 and base deficiency ≥ 12 mmol/l). The assessment of facial movements in all fetuses was carried out using the BabyFACS technique, where the action unit (AU) was used for the assessment; its coding is carried out in strict accordance with the chart of motor descriptors (MD): AU1 – inner corners of eyebrows are lifted; AU2 – outer corners of eyebrows are lifted; AU3 – inner corners of eyebrows are drawn together; AU4 – eyebrows are lowered; AU9 – wrinkled nose; AU12 – lip corners are pulled up; AU14 – dimples on cheeks; AU18 – wrinkled lips; AU20 – lips are stretched; AU25 – lips are parted; AU28 – lips are sucked in. Visualization of action units was carried out using an expert-class ultrasound diagnostic device by a medical specialist with mandatory fixation of photo and video materials for subsequent evaluation and creation of a database. The assessment of action units was carried out by a certified specialist who has been trained and has a FACS (Facial Action Coding System) certificate confirming it. Statistical data processing was carried out using SPSS Statistics 20 (IMB Corp., USA), the sample size for this study

was not pre-calculated. The main outcome of the study was the determination of the prevalence of facial units and their diagnostic significance in confirmed intrauterine distress, additional research outcomes – the compilation of summary tables for each of the variables of facial units in order to determine sensitivity, specificity and prognostic value. The Mann – Whitney test was used as the main statistical parameter; the threshold level of 0.05 was chosen to interpret the *p*-tests value. This study was conducted with the written permission of the administration of the medical organization and with the approval of the local ethics committee of Voronezh State Medical University named after N.N. Burdenko of the Ministry of Health of the Russian Federation (Protocol No. 1 dated March 28, 2019).

RESULTS

When assessing the prevalence of criteria for compliance with intrauterine distress according to the FIGO chart, it can be noted that according to CTG data the prevailing criteria are monotony of rhythm and variable deceleration, which were recorded in 100 % of cases; the prevalence of other criteria for compliance with the study group is presented in Table 1.

TABLE 1

PREVALENCE OF INTRAUTERINE DISTRESS CRITERIA ACCORDING TO THE FIGO CHART IN STUDY GROUP I

Analyzed criteria	The percentage of occurrence of the criterion, %
BHR = 110–170 bpm	1.6
BHR > 170 bpm	31.2
BHR < 110 bpm	67.2
Variable monotonous rhythm	100
Early decelerations > 50	36.0
Late decelerations > 30	64.0
Variable decelerations > 50	100

Note: BHR – Basal Heart Rate.

When assessing the frequency of facial movements in each of the study groups, it was revealed which of the MDs were statistically significantly more common in the group

TABLE 2

FREQUENCY OF OCCURRENCE OF MOTOR DESCRIPTORS IN EACH OF THE STUDY GROUPS

Motor descriptors	Frequency of occurrence of MDs in group I (*n* = 125), %	Frequency of occurrence of MDs in group II (*n* = 100), %	*p*
AU1	92	5	*p* = 0.00001
AU2	95.2	13	*p* = 0.00001
AU3	94.4	13	*p* = 0.00001
AU4	91.1	8	*p* = 0.00001
AU9	92.8	0	*p* = 0.00001
AU12	4.8	75	*p* = 0.00001
AU14	4	68	*p* = 0.00001
AU18	4	49	*p* = 0.00001
AU20	92	0	*p* = 0.00001
AU25	4	49	*p* = 0.00001
AU28	4	65	*p* = 0.00001

TABLE 3

DIAGNOSTIC SIGNIFICANCE OF MOTOR DESCRIPTORS AT BASAL HEART RATE < 100 BPM OR > 170 BPM ACCORDING TO CARDIOTOCOGRAPHY

Indicators	AU1	AU2	AU3	AU4	AU9	AU20
Diagnostic sensitivity	94.16 %	89.39 %	88.54 %	91.73 %	98.27 %	100 %
Diagnostic specificity	90.47 %	94.62 %	92.55 %	89.32 %	91.74 %	92.72 %
Positive predictive value	91.86 %	95.93 %	94.30 %	90.98 %	92.68 %	93.49 %
Diagnostic effectiveness of the test	92.44 %	91.55 %	90.22 %	90.62 %	95.11 %	96.44 %

TABLE 4

DIAGNOSTIC SIGNIFICANCE OF MOTOR DESCRIPTORS IN EARLY DECELERATIONS > 50 ACCORDING TO CARDIOTOCOGRAPHY

Indicators	AU1	AU2	AU3	AU4	AU9	AU20
Diagnostic sensitivity	36.66 %	32.57 %	30.53 %	31.40 %	62.93 %	36.52 %
Diagnostic specificity	99.04 %	97.84 %	94.68 %	94.17 %	98.16 %	97.27 %
Positive predictive value	97.77 %	95.55 %	88.88 %	86.36 %	97.33 %	93.33 %
Diagnostic effectiveness of the test	65.77 %	59.55 %	57.33 %	60.26 %	80 %	66.22 %

TABLE 5

DIAGNOSTIC SIGNIFICANCE OF MOTOR DESCRIPTORS IN LATE DECELERATIONS > 30 ACCORDING TO CARDIOTOCOGRAPHY

Indicators	AU1	AU2	AU3	AU4	AU9	AU20
Diagnostic sensitivity	62.5 %	60.60 %	62.59 %	64.46 %	66.37 %	66.95 %
Diagnostic specificity	91.42 %	95.69 %	97.87 %	94.17 %	93.57 %	93.63 %
Positive predictive value	89.28 %	95.23 %	97.61 %	92.85 %	91.66 %	91.66 %
Diagnostic effectiveness of the test	76 %	75.11 %	77.33 %	78.12 %	79.55 %	80 %

TABLE 6

DIAGNOSTIC SIGNIFICANCE OF MOTOR DESCRIPTORS FOR VARIABLE DECELERATIONS > 50 ACCORDING TO CARDIOTOCOGRAPHY

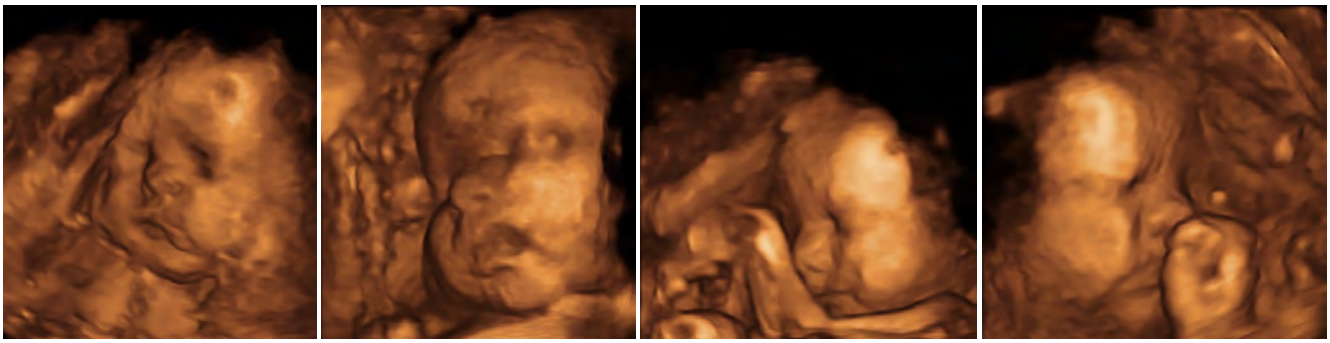
Indicators	AU1	AU2	AU3	AU4	AU9	AU20
Diagnostic sensitivity	95.83 %	90.15 %	90.07 %	93.38 %	100 %	100 %
Diagnostic specificity	90.47 %	93.54 %	92.55 %	89.32 %	91.74 %	90.90 %
Positive predictive value	92 %	95.19 %	94.39 %	91.12 %	92.80 %	92 %
Diagnostic effectiveness of the test	93.33 %	91.55 %	91.11 %	91.51 %	96 %	95.55 %

TABLE 7

ASSESSMENT OF THE COMBINED EFFECT OF ALL STUDIED PARAMETERS ON THE SEVERITY OF THE CONDITION

Groups	Means	95% CI		Standard deviation (%)	Min	Max	Q25	Median	Q75
Group I	0.38	0.36	0.40	0.10	0.07	0.47	0.33	0.33	0.47
Group II	0.20	0.19	0.20	0.02	0.00	0.20	0.20	0.20	0.20

Note. 95% CI – 95% confidence interval; Q25 – 25th percentile; Q75 – 75th percentile.



AU1 **AU2** **AU3** **AU4**

FIG. 1.

An example of ultrasound imaging of motor descriptors, which are significantly more common in fetal distress

with diagnosed intrauterine distress compared with the control group (Table 2).

Considering that the diagnosis of intrauterine distress requires confirmation by CTG, we evaluated the diagnostic sensitivity and specificity, the positive predictive value and the diagnostic effectiveness of MDs, which were statistically significantly more often recorded in the group with intrauterine distress, for each of the criteria of this pathological condition according to the FIGO chart (Tables 3–6). According to the analysis, AU9, AU20 and AU1 demonstrated the highest diagnostic sensitivity of all possible MDs.

To assess the combined effect of all the studied parameters, a total indicator of the severity of the condition was compiled, obtained by summing a proportionally weighted crossing of the threshold value: the average value in the control group is 0.2 ± 0.02, in group I – 0.38 ± 0.10 (Table 7).

DISCUSSION

Although advances in prenatal medicine allow treatment as early as the intrauterine period, the question of whether we can identify facial expression in general and, in particular, the expression of «distress» in the fetus is becoming increasingly important. Despite the fact that the experience of pain and distress is subjective and entails a psychological component, anatomical and functional development is necessarily associated with the perception of an irritant [9, 10].

Mimic «anxious» movements are important components of the development of the severity of distress in fetuses. In one of the studies, the neonatal facial coding system was used to describe in detail facial activity of premature infants from 24 to 36 weeks of pregnancy during painful procedures [11].

In our previous study, facial movements and their combinations, which we assessed as fundamental combinations of actions on the fetal face during confirmed distress, were diagnosed [12]. The basis of this study was to prove, using mathematical statistical models, that the listed facial movements are statistically significantly more common in fetuses with confirmed distress, thereby being early predictors of this condition.

When assessing the frequency of occurrence of motor descriptors, it was found that, despite the occurrence of AU1, AU2, AU3, AU4 (Fig. 1) in both groups, statistically significantly more often MD data were recorded in the group with confirmed distress ($p = 0.00001$). Facial units such as AU9 and AU20 were found only in children with intrauterine distress, which, in the total amount of the MD assessment, can be considered one of the main search signs that specialists should first of all pay attention to. All motor descriptors showed a high positive predictive value and diagnostic sensitivity, with the highest results registered for AU9 and AU20 (Fig. 2).

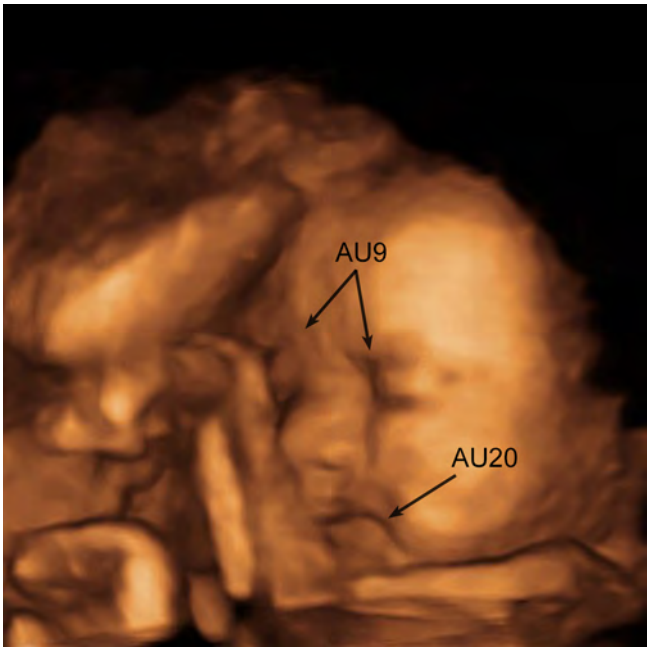


FIG. 2.
An example of ultrasound imaging of motor descriptors AU9 and AU20, which showed the highest prognostic value and diagnostic sensitivity

CONCLUSION

Assessment of facial units during ultrasound diagnostics can be a convenient tool as an additional diagnosis of the development of intrauterine distress and requires further study.

Conflict of interest

The authors of this article declare the absence of a conflict of interest.

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OBSTETRICS AND GYNAECOLOGY

AGE-RELATED DETERMINANTS OF THE METABOLIC SYNDROME IN WOMEN OF REPRODUCTIVE AGE OF THE MAIN ETHNIC GROUPS OF THE BAIKAL REGION

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ABSTRACT

The aim of the study. *To determine the age limit of the initial manifestations of the metabolic syndrome in women of reproductive age of the Caucasian and Asian ethnic groups.*

Materials and methods. *The study included women of reproductive age of the Russian and Buryat ethnic groups (n = 1231). We carried out general clinical examination, studied glycemia levels and lipid metabolism indicators. The analysis of the components of the metabolic syndrome was performed taking into account age characteristics and ethnicity. Threshold values for the age of metabolic disorders manifestation were determined.*

Results. *It was found that the main components of the metabolic syndrome in women of reproductive age of the Russian and Buryat ethnic groups were a decrease in high-density lipoprotein (HDL), an increase in waist circumference, and an increase in blood pressure. The age-related determinants of the metabolic syndrome in the population of women of reproductive age in the Baikal region were determined. It was shown that the age-related determinants of the metabolic syndrome in Caucasian and Asian women do not differ significantly and amount to 33.5 years (95% confidence interval (95% CI): 32.5; 38.5) and 36.5 years (95% CI: 27.5; 52.5), respectively. A universal critical age value was determined as 34.5 years (95% CI: 32.5; 38.5), which is associated with a significant increase in the prevalence of the metabolic syndrome in accordance with the ATP III criteria. The ranking of metabolic syndrome criteria in the studied groups revealed the following order of their occurrence: the decrease in HDL index prevailed, followed b ythe increase in waist circumference, blood pressure, glucose and triglycerides; no dependence on the ethnicity was found.*

Conclusion. *When assessing the age-related determinants of the metabolic syndrome in the mixed Caucasoid-Asian group, it is advisable to use universal approaches, taking into account the cut-off point of age, which is 34.5 years. The determination of age criteria for the correct classification of patients with respect to the manifestations of the metabolic syndrome is necessary to analyze the contribution of the age factor to the implementation of various metabolic syndrome predictors,to develop prognostic models, and to use effective age-determined markers of metabolic disorders.*

Key words: *metabolic syndrome, age, threshold values, women, ethnicity*

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ВОЗРАСТНЫЕ ДЕТЕРМИНАНТЫ МЕТАБОЛИЧЕСКОГО СИНДРОМА У ЖЕНЩИН РЕПРОДУКТИВНОГО ВОЗРАСТА ОСНОВНЫХ ЭТНИЧЕСКИХ ГРУПП ПРИБАЙКАЛЬЯ

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РЕЗЮМЕ

Цель исследования. *Определить возрастной предел начальных проявлений метаболического синдрома (МС) у женщин репродуктивного возраста европеоидной и азиатской этнических групп.*

Материалы и методы. *В исследование вошли женщины репродуктивного возраста русской и бурятской этнических групп (n = 1231); проведено общеклиническое обследование, исследование уровней гликемии и показателей липидного обмена. Выполнен анализ компонентов метаболического синдрома с учётом возрастных особенностей и этнической принадлежности. Определены пороговые значения возраста манифестации метаболических нарушений.*

Результаты. *Установлено, что основными компонентами метаболического синдрома у женщин репродуктивного возраста русской и бурятской этнических групп были снижение липопротеинов высокой плотности (ЛПВП), увеличение окружности талии, повышение артериального давления. Определены возрастные детерминанты метаболического синдрома в популяции женщин репродуктивного возраста Прибайкалья. Показано, что возрастные детерминанты метаболического синдрома у женщин-европеоидов и представительниц азиатской этнической группы существенно не отличаются и составляют 33,5 года (95%-й доверительный интервал (95% ДИ): 32,5; 38.5) и 36,5 года (95% ДИ: 27,5; 52,5) соответственно. Определено универсальное критическое значение возраста – 34,5 года (95% ДИ: 32,5; 38,5), с которым ассоциировано существенное увеличение распространённости метаболического синдрома в соответствии с критериями ATP III. Ранжирование критериев метаболического синдрома в исследуемых группах выявило следующий порядок их встречаемости: превалировало снижение показателя ЛПВП, далее – увеличение значений окружности талии, артериального давления, глюкозы и триглицеридов, отсутствовала зависимость от этнической принадлежности.*

Заключение. *При оценке возрастных детерминант метаболического синдрома в смешанной европеоидно-азиатской группе целесообразно использовать универсальные подходы с учётом точки отсечения возраста, составляющей 34,5 года. Определение возрастных критериев для правильной классификации пациенток относительно проявлений метаболического синдрома необходимо для анализа вклада возрастного фактора в реализацию различных предикторов МС, разработки прогностических моделей и применения эффективных возраст-детерминированных маркеров метаболических нарушений.*

Ключевые слова: *метаболический синдром, возраст, пороговые значения, женщины, этника*

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INTRODUCTION

Metabolic syndrome (MS) is characterized by high prevalence and is a combination of various clinical and metabolic factors, including arterial hypertension, dyslipidemia, abdominal obesity, hyperglycemia, often associated with proinflammatory and prothrombotic conditions, as well as insulin resistance [1, 2]. These factors are due to the complex interaction of the age component, the genetic component, a sedentary lifestyle, and excessive calorie intake [3]. The development of MS contributes to a decrease in the quality of life, and is also a predictor of the growth of type 2 diabetes mellitus, cardiovascular diseases, an increase in the proportion of disability and mortality of patients [4]. The multiplicity of symptoms requires an integrated approach to the problem of MS, based both on drug exposure and on the prevention of the development of this pathology, aimed at timely management of risk factors and lifestyle modification [5].

Understanding the epidemiology of MS is crucial for the treatment of this endocrine disorder. The prevalence of MS varies from 10 % to 84 % worldwide and depends on the applied diagnostic criteria, sociodemographic characteristics of the surveyed population, taking into account gender, age, ethnicity, region of residence, etc. [6]. Thus, MS is often found in Europeans, this pathology is much less common in Southeast Asian countries, while the dynamics of the prevalence of MS in this region is comparable with Western countries [4]. It is known that in the early reproductive period, the incidence of MS is slightly higher among men, and in older age, the female population with MS dominates [7].

In Russians, MS is detected in 33 % of cases aged 25–64 years, and their proportion increases with age and has regional and ethnic characteristics [5, 8]. It is known that representatives of the Buryat ethnic group are less susceptible to disorders of lipid and carbohydrate metabolism [9–11].

MS can have particularly important consequences for women's health, since it is considered as a risk factor for ovarian menstrual cycle disorders, polycystic ovary syndrome, infertility, endometrial hyperplasia, oncological processes and other pathological conditions [12, 13]. So far, there have been no studies on the development of metabolic syndrome in the reproductive age in the female population, taking into account ethnic characteristics, in the territory of the Baikal region.

One of the important issues in understanding the pathogenesis of MS is the age threshold for manifestations of metabolic changes [13, 14], since, in the clinical aspect, timely exposure to polymetabolic disorders is reversible and has a favorable prognosis [15]. Thus, it seems relevant to determine the threshold values of the age of MS manifestation in women of fertile age [16].

THE AIM OF THE STUDY

To determine the age limit of the initial manifestations of the metabolic syndrome in women of reproductive age of the Caucasian and Asian ethnic groups.

MATERIALS AND METHODS

Women of reproductive age (18–44 years old) (n = 1231) who were subject to annual preventive examination at their place of work and lived in the Irkutsk region and the Republic of Buryatia were examined. Inclusion criteria: availability of signed informed consent; willingness of the participant to comply with all research procedures; accessibility throughout the duration of the study; reproductive age – 18–44 years inclusive.

Non-inclusion/exclusion criteria: current pregnancy or lactation; ablation of the uterus and/or appendages on both sides; endometrial ablation and/or uterine artery embolization; factors that increase the risk to the subject or prevent the participant from fully fulfilling the conditions of the study or not allowing the completion of the study; unwillingness to participate or difficulties in understanding informed consent or goals and requirements research; taking insulin sensitizers for 3 months.

An anthropometric examination was performed (measurement of height, weight, waist circumference) [17]. Waist circumference (WC) was measured with a centimeter tape with an accuracy of 0.5 cm in the standing position, at the end of exhalation. The location of the tape was strictly horizontal at the level of the crista iliaca. Blood pressure (BP) was measured in the patient's sitting position, after a 5-minute rest, with an Omron automatic tonometer on the right shoulder of the subject.

Blood samples were taken from the ulnar vein in fasting state, after 12 hours of fasting. Blood serum was obtained by low-speed centrifugation.

Laboratory methods included the determination of serum lipid spectrum, high-density lipoprotein (HDL), cholesterol (CS), triglyceride (TG) and glucose levels. TG, HDL and glucose levels were determined using BioSystems diagnostic kits spectrophotometrically on a VTS-350 analyzer (BioSystems, Spain). Standardization and quality control of analyses were carried out in accordance with the requirements of the Federal System for External Quality Assessment of Clinical Laboratory Studies.

MS was determined according to the criteria of NCEP ATP III (The National Cholesterol Education Program's Adult Treatment Panel III), adopted in 2004 with changes in approaches to assessing fasting glycemia and waist circumference proposed by the International Diabetes Federation (IDF, International Diabetes Federation) in 2005. To diagnose metabolic syndrome, any three of the five criteria must be present: triglycerides ≥ 1.7 mmol/L; HDL < 1.3 mmol/L; glucose ≥ 5.6 mmol/L; blood pressure ≥ 130 mmHg or ≥ 85 mmHg; FROM ≥ 80 cm in women [3].

The implementation of data collection and storage methods was carried out using the REDcap information system.

The calculation of the sample size was performed using the interactive software PS: Power and Sample Size Calculation version 3.1.2.

Statistical methods included descriptive statistics, statistical hypothesis testing using the following criteria: t-test, Mann – Whitney U-test, Fisher's exact test, Pearson's χ^2 test, Z-test, criterion of statistical significance of the difference in *td* fractions. The level of statistical significance is $p < 0.05$.

In addition, to determine the threshold values of the age associated with the presence/absence of MS, ROC analysis was used with the calculation of the area under curve (AUC).

The implementation of statistical methods is carried out using the programming languages R 4.2.1 (R-studio; pROC library) and Python 3 (PyCharm; Pandas, Scipy libraries).

All studies were conducted in accordance with the World Medical Association Declaration of Helsinki (1964, as amended in 2013). The study was approved by the Committee on Biomedical Ethics at the Scientific Centre for Family Health and Human Reproduction Problems (extract from the minutes of the meeting No. 2.1 dated February 24, 2016).

This work was carried out within the framework of the state task «Pathophysiological mechanisms and genetic and metabolic predictors of maintaining reproductive health and longevity in various age, gender and ethnic groups» (FGMZ-2021-0002; state no. registration in the Unified State Register of Medical Sciences 121022500180-6) using the equipment of the Center for the Development of progressive personalized Health Technologies of the Scientific Centre for Family Health and Human Reproduction Problems (Irkutsk).

STUDY RESULTS

During the examination of 1,231 women of reproductive age, metabolic syndrome was detected in 297 (24.12 %) of them. The frequency of MS detection among participants of Caucasian ethnicity was 24.42 %, among Asian women – 23.4 % ($p_z = 0.37$). The average age of women with metabolic syndrome was 36.78 ± 5.7 vs. 33.59 ± 6.39 in women without MS.

The main characteristics of women of reproductive age of Caucasian and Asian ethnicity, depending on the presence and absence of metabolic syndrome, are presented in Table 1.

As can be seen in Table 1, in the subgroups with MS, both among women of Caucasian ethnicity and Asian women, the levels of TG, glucose, blood pressure and waist circumference are naturally higher with a decrease in HDL. When assessing the hierarchy of representation of various components, Caucasians with MS were more likely to have reduced HDL values (in 95.75 % (203/212)), an increase in WC (in 89.15 % (189/212)), SBP (in 68.87 % (146/212)), DBP (in 62.74 % (133/212)), glucose (in 40.09 % (85/212)) and TG (in 16.98 % (363/212)) than in the group without MS. A similar pattern was observed in the group of Asian women with MS: a high incidence of women with low HDL levels (in 95.29 % (84/85)), an increase in WC (in 91.76 % (78/85)), SBP (in 65.88 % (56/85)), DBP (in 58.82 % (50/85)), glucose was revealed (in 28.24 % (24/85)) and TG (in 15.29 % (13/85)) in comparison with the group without MS. Ethnic differences were recorded only in relation to the TG index, the frequency of increase of which was more often found in the group of Caucasians compared with Asians.

It was noted that regardless of ethnicity, the average age of women with MS was higher compared to the group without MS. Due to the importance of the age factor in the manifestation of MS, it was interesting to identify cut-off points in the population of women in the Baikal region, taking into account the ethnic factor.

At the first stage, we determined the universal value of the age associated with the manifestation of MS in the combined Caucasian-Asian population of women of reproductive age – 34.5 years (95% confidence interval (95% CI): 32.5; 38.5) (Fig. 1).

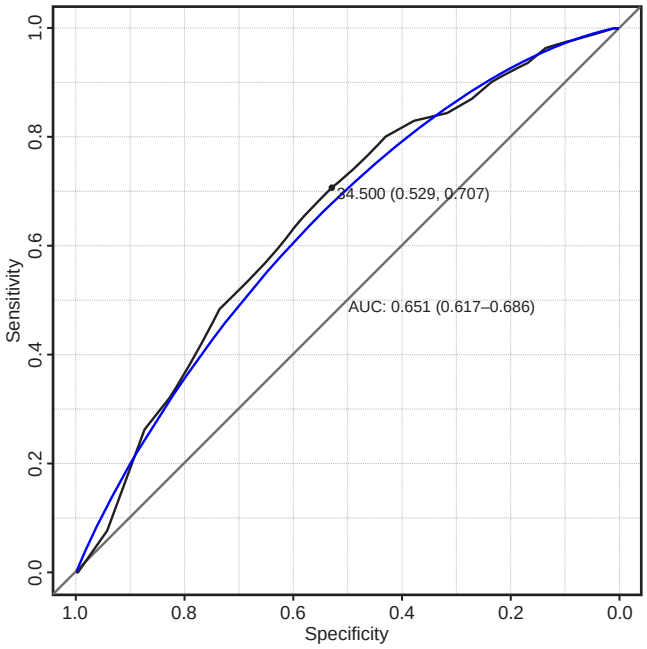

FIG. 1.

Threshold value ("cut-off") of age associated with the presence/absence of metabolic syndrome in the general population of women in the Baikal region

When analyzing the frequency of MS and its individual manifestations, taking into account the established age cut-off point, it was demonstrated that in the group of women aged ≥ 34.5 years, the frequency of MS is 17.3 % higher than in younger women (32.3 % vs. 14.9 %; $p_z = 0.0001$).

TABLE 1

CHARACTERISTICS OF WOMEN OF REPRODUCTIVE AGE OF CAUCASIAN AND ASIAN ETHNICITY WITH AND WITHOUT METABOLIC SYNDROME

Parameters	Caucasians (*N* = 868)		Asians (*N* = 363)		*p*
	MS+ (*N* = 212)	MS– (*N* = 656)	MS+ (*N* = 85)	MS– (*N* = 278)	
	1a	1b	2a	2b	
	M ± Std Me (25; 75)				
WC, cm	91.38 ± 12.08 90.00 (83.00; 100.00)	73.44 ± 9.82 73.00 (66.00; 78.00)	90.11 ± 10.91 87.00 (83.00; 95.00)	74.70 ± 10.05 73.00 (67.00; 80.00)	$p_{1a-1b} < 0.001^3$ $p_{2a-2b} < 0.001^3$ $p_{1a-2a} = 0.284^3$ $p_{1b-2b} = 0.051$
Glucose, mmol/L	5.34 ± 0.88 5.34 (4.74; 5.85)	4.84 ± 0.74 4.81 (4.30; 5.32)	5.09 ± 1.07 4.85 (4.40; 5.69)	4.62 ± 0.57 4.56 (4.18; 5.06)	$p_{1a-1b} < 0.0013$ $p_{2a-2b} < 0.0013$ $p_{1a-2a} = 0.0043$ $p_{1b-2b} < 0.0013$
HDL, mmol/L	0.95 ± 0.21 0.94 (0.84; 1.06)	1.15 ± 0.31 1.10 (0.93; 1.34)	1.01 ± 0.18 1.02 (0.88; 1.14)	1.21 ± 0.26 1.19 (1.02; 1.36)	$p_{1a-1b} < 0.0013$ $p_{2a-2b} < 0.0013$ $p_{1a-2a} = 0.0093$ $p_{1b-2b} < 0.0013$
TG, mmol/L	1.18 ± 0.49 1.05 (0.84; 1.52)	0.90 ± 0.35 0.88 (0.65; 1.13)	1.17 ± 0.41 1.10 (0.92; 1.36)	0.93 ± 0.33 0.91 (0.70; 1.16)	$p_{1a-1b} < 0.0013$ $p_{2a-2b} < 0.0013$ $p_{1a-2a} = 0.6473$ $p_{1b-2b} = 0.1603$
SBP, mmHg	135.35 ± 13.45 133.00 (128.00; 141.00)	119.04 ± 11.32 119.00 (112.00; 125.00)	135.67 ± 14.48 134.00 (126.00; 143.00)	117.65 ± 10.85 117.00 (110.00; 124.00)	$p_{1a-1b} < 0.001^3$ $p_{2a-2b} < 0.001^3$ $p_{1a-2a} = 0.958^3$ $p_{1b-2b} = 0.055^3$
DBP, mmHg	86.92 ± 9.00 87.00 (81.00; 91.00)	76.14 ± 8.60 76.00 (70.00; 81.00)	87.32 ± 11.48 87.00 (80.00; 92.00)	76.42 ± 8.13 76.00 (71.00; 81.00)	$p_{1a-1b} < 0.001^3$ $p_{2a-2b} < 0.001^3$ $p_{1a-2a} = 0.943^3$ $p_{1b-2b} = 0.595^3$
	n*/*N (%)				
WC ≥ 80 cm	189/212 (89.15 %)	125/651 (19.20 %)	78/85 (91.76 %)	72/276 (26.09 %)	$p_{1a-1b} < 0.001^2$ $p_{2a-2b} < 0.001^2$ $p_{1a-2a} = 0.670^1$ $p_{1b-2b} = 0.019^2$
Glucose ≥ 5.6 mmol/L	85/212 (40.09 %)	87/644 (12.51 %)	24/85 (28.24 %)	16/272 (5.88 %)	$p_{1a-1b} < 0.001^2$ $p_{2a-2b} < 0.001^2$ $p_{1a-2a} = 0.055^2$ $p_{1b-2b} = 0.001^2$
HDL < 1.3 mmol/L	203/212 (95.75 %)	455/644 (70.65 %)	81/85 (95.29 %)	174/272 (63.97 %)	$p_{1a-1b} < 0.001^2$ $p_{2a-2b} < 0.001^2$ $p_{1a-2a} = 0.538^1$ $p_{1b-2b} = 0.046^2$
TG ≥ 1.7 mmol/L	36/212 (16.98 %)	11/644 (1.71 %)	13/85 (15.29 %)	4/272 (1.47 %)	$p_{1a-1b} < 0.001^2$ $p_{2a-2b} < 0.001^2$ $p_{1a-2a} < 0.001^2$ $p_{1b-2b} = 0.979^2$
SBP ≥ 130 mmHg	146/212 (68.87 %)	90/656 (13.72 %)	56/85 (65.88 %)	31/278 (11.15 %)	$p_{1a-1b} < 0.001^2$ $p_{2a-2b} < 0.001^2$ $p_{1a-2a} = 0.618^2$ $p_{1b-2b} = 0.285^2$
DBP ≥ 85 mmHg.	133/212 (62.74 %)	95/656 (14.48 %)	50/85 (58.82 %)	38/278 (13.67 %)	$p_{1a-1b} < 0.001^2$ $p_{2a-2b} < 0.001^2$ $p_{1a-2a} = 0.531^2$ $p_{1b-2b} = 0.745^2$

Note. MS+ – presence of metabolic syndrome; MS– – absence of metabolic syndrome; SBP – systolic blood pressure; DBP – diastolic blood pressure; p^1 – Fisher's exact test; p^2 – Pearson's χ^2 test; p^3 – Mann – Whitney U-test

The most significant increase is the frequency of MS components in the older age group: it was registered to increase by 24.17 % (49.3 % vs. 25.1 %; p_z = 0.0001), SBP – by 21 % (36.1 % vs. 15.1 %; p_z = 0.0001), DBP – by 15.3 % (32.9 % vs. 17.5 %; p_z = 0.0001).

The rates of HDL decrease and glucose increase did not differ statistically significantly in the age groups. At the same time, some statistically significant increase in the proportion of women with increased TG was revealed – 5.8 % versus 4.6 % (p_z = 0.00003) – in the older age group.

As a result of the analysis of ROC curves for representatives of the Caucasian race, the cut-off point for age was 33.5 years (95% CI: 32.5; 38.5) (Fig. 2), for Asian women – 36.5 years (95% CI: 27.5; 52.5) (Fig. 3).

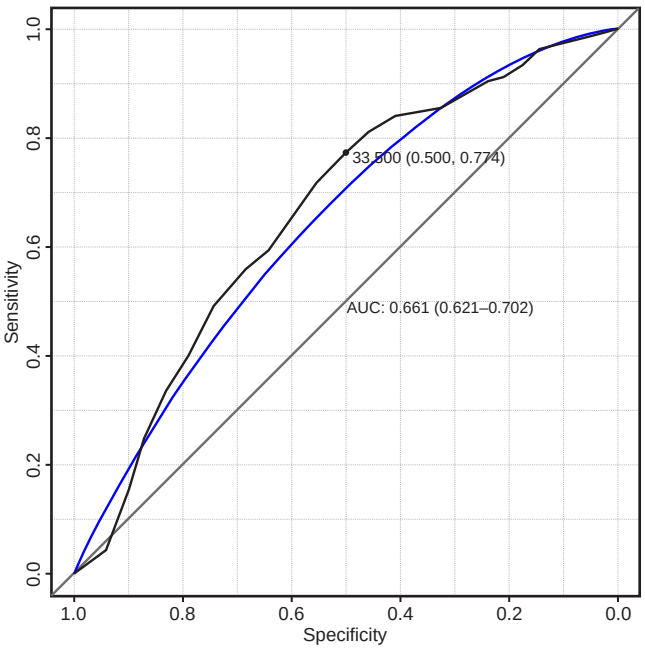

FIG. 2.

Threshold value ("cut-off") of age associated with the presence/absence of metabolic syndrome in Caucasoid women of the Baikal region

The threshold values («cut-offs») were compared using the 95% CI intersection estimation technique, while the absence of statistically significant differences in age cut-off points in the group of Caucasians and Asians was shown.

As can be seen from table 2, the use of ethnically determined criteria for determining age cut-off points is not accompanied by significant changes in the frequency of detection of MS and its individual components in older and younger women. The above confirms the expediency of applying a universal age criterion associated with the manifestation of MS in women of reproductive age.

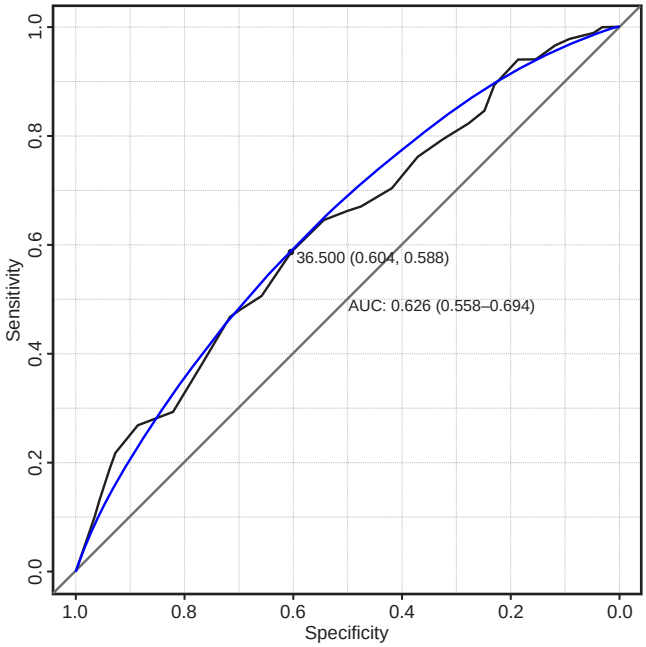

FIG. 3.

Threshold value ("cut-off") of age associated with the presence/absence of metabolic syndrome in Asian women of the Baikal region

DISCUSSION

Currently, MS has acquired the status of one of the most significant public health problems [9-11]. In patients with metabolic syndrome, compared with those without it, the probability of the onset of cardiovascular diseases over the next 5–10 years is 2 times higher, and throughout life it is many times higher. Moreover, MS is associated with a significant, namely a 5-fold increase in the risk of diabetes mellitus [18], although the syndrome itself does not include many factors reflecting the absolute risk, such as age, gender, smoking and the level of low-density lipoprotein cholesterol.

In Russia, few epidemiological studies have been conducted in various ethnic groups to study the prevalence of risk factors for cardiovascular diseases, as well as MS and its components. The results indicate some specificity of MS manifestations in women of Caucasian and Asian ethnicity.

Thus, in a study on the prevalence of arterial hypertension (AH) in different ethnic groups (Buryats, Russians) conducted by Z.H. Malakshinova in Ulan-Ude [19], Buryats registered hypertension more often than Russians, mainly due to DBP. Levels of cholesterol, LDL cholesterol and TG increased in both groups, and statistically significant ethnic differences in cholesterol levels were revealed only in the 30–39 age group.

In the Kemerovo simultaneous epidemiological study (Gornaya Shoriya) conducted in 1999 and included 550 Shorians and 665 non–Shorians over the age of 18, a higher incidence of MS was detected in women of the alien population (ethnic group – Russians), whereas

TABLE 2

COMPARATIVE ANALYSIS OF THE FREQUENCY OF METABOLIC SYNDROME AND ITS CHARACTERISTICS IN WOMEN OF CAUCASIAN AND ASIAN ETHNICITY IN AGE GROUPS DEFINED USING UNIVERSAL AND ETHNICALLY DETERMINED THRESHOLD VALUES OF THE AGE

Parameters	Caucasians (*N* = 868) < 34.5 years (1a) (*N* = 420)	Caucasians (*N* = 868) ≥ 34.5 years (2a) (*N* = 448)	Difference 2a–1a (%)	Caucasians (*N* = 868) < 33.5 years (1b) (*N* = 376)	Caucasians (*N* = 868) ≥ 33.5 years (2b) (*N* = 492)	Difference 2b–1b (%)	Comparison 1a–1b (p_z)	Comparison 2a–2b (p_z)
	n/*N* (%)			*n*/*N* (%)				
WC ≥ 80 cm	98/417 (23.50 %)	216/446 (48.43 %)	24.93	80/374 (21.39 %)*	234/489 (47.85 %)	26.46	0.48	0.86
HDL decrease < 1.3 mmol/L	321/418 (76.79 %)	337/438 (76.94 %)	0.15	288/374 (77.01 %)	370/482 (76.76 %)	0.24	0.94	0.95
Glucose ≥ 5.6 mmol/L	78/418 (18.66 %)	94/438 (21.46 %)	2.80	68/374 (18.18 %)	104/482 (21.58 %)	3.39	0.86	0.97
TG ≥ 1.7 mmol/L	19/418 (4.55 %)	28/438 (6.39 %)	1.85	17/374 (4.55 %)	30/482 (6.22 %)	1.68	1.0	0.92
SBP ≥ 130 mmHg	67/420 (15.95 %)	169/448 (37.72 %)	21.77	59/376 (15.69 %)*	177/492 (35.98 %)	20.28	0.92	0.58
DBP ≥ 85 mmHg.	77/420 (18.33 %)	151/448 (33.71 %)	15.37	67/376 (17.82 %*)	161/492 (32.72 %)	14.90	0.85	0.75
Presence of MS	59/420 (14.05 %)	153/448 (34.15 %)	20.10	48/376 (12.77 %)*	164/492 (33.33 %)	20.57	0.60	0.79
Parameters	**Asians (*N* = 363) < 34.5 years (1a) (*N* = 161)**	**Asians (*N* = 363) ≥ 34.5 years (2a) (*N* = 202)**	**Difference 2a–1a (%)**	**Asians (*N* = 363) < 33.5 years (1b) (*N* = 204)**	**Asians (*N* = 363) ≥ 33.5 years (2b) (*N* = 159)**	**Difference 2b–1b (%)**	**Comparison 1a–1b (p_z)**	**Comparison 2a–2b (p_z)**
	n/*N* (%)			*n*/*N* (%)				
WC ≥ 80 cm	47/160 (29.38 %)	103/201 (51.24 %)	21.87	65/202 (32.18 %)	85/159 (53.46 %)	21.28	0.57	0.68
HDL decrease < 1.3 mmol/L	105/158 (66.46 %)	150/199 (75.38 %)	8.92	136/200 (68.00 %)	119/157 (75.80 %)	7.80	0.76	0.93
Glucose ≥ 5.6 mmol/L	20/158 (12.66 %)	20/199 (10.05 %)	2.61	23/200 (11.50 %)	17/157 (10.83 %)	0.67	0.74	0.81
TG ≥ 1.7 mmol/L	8/158 (5.06 %)	9/199 (4.52 %)	0.54	10/200 (5.00 %)	7/157 (4.46 %)	0.54	0.98	0.98
SBP ≥ 130 mmHg	21/161 (13.04 %)	66/202 (32.67 %)	19.63	32/204 (15.69 %)	55/159 (34.59 %)	18.90	0.48	0.70
DBP ≥ 85 mmHg.	25/161 (15.53 %)	63/202 (31.19 %)	15.66	33/204 (16.18 %)	55/159 (34.59 %)	18.41	0.87	0.49
Presence of MS	28/161 (17.39 %)	57/202 (28.22 %)	10.83	35/204 (17.16 %)	50/159 (31.45 %)	14.29	0.95	0.50

the frequency and severity of hypertension was more pronounced in Shorians [20].

A study on the characteristics of MS was also conducted among the indigenous small-numbered peoples of the Republic of Sakha (Yakutia) (96 Dolgans and 90 Evenks) of working age, from 30 to 59 years old. The association of arterial hypertension with MS was more pronounced in women compared to men.

According to our data, the frequency of MS and the hierarchy of its manifestations do not depend on ethnicity, however, ethnospecific differences in triglyceride levels were revealed.

This fact is linked to a generally accepted understanding of the nature of dyslipidemia. According to a number of studies, hypertriglyceridemia is often caused by an alimentary factor, and the European type of diet contributes to an excessive intake of fatty and carbohydrate-containing foods, which in turn has a high association with the development of coronary heart disease, nonfatal myocardial infarction, even after eliminating other risk factors. At the same time, it is believed that in certain cases hypertriglyceridemia does not affect atherosclerotic manifestations in the walls of blood vessels, and it depends on which of the five types of lipoproteins they are packed into.

It is known that the incidence of MS is associated with the age of the patient. As the total life expectancy of people increases, the number of MS patients increases; this fact determines the social significance of the problem [11, 21].

According to the results of our study, the average age in the group with MS was statistically significantly higher than in women without MS. When determining the threshold value of the age associated with a higher probability of MS, we determined that in the combined Caucasian-Asian population of women of the age category from 34.5 years, the number of women with various manifestations of metabolic syndrome increases significantly.

At the same time, an attempt to determine ethnically differentiated threshold values of the age revealed the lack of advantages of using this approach.

CONCLUSION

As a result of the study, it was found that when assessing the age determinants of MS in a mixed Caucasoid-Asian population, it is advisable to use universal approaches taking into account the age cut-off point of 34.5 years. The determination of age criteria for the correct classification of patients with respect to the manifestations of the metabolic syndrome is necessary to analyze the contribution of the age factor to the implementation of various metabolic syndrome predictors, to develop prognostic models, and effective age-determined markers of metabolic disorders.

Conflict of interest

The authors of this article declare the absence of a conflict of interest.

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ASSESSMENT OF THE COURSE OF THE GESTATIONAL PROCESS USING SURVEY METHOD AND DEPENDING ON THE LABORATORY CONFIRMED PRENATAL ALCOHOL USE (CROSS-SECTION STUDY)

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ABSTRACT

Background. *Studying the problem of alcohol consumption by pregnant women using modern laboratory research methods has valuable theoretical and practical significance.*
The aim of the study. *To determine the characteristics of the pregnancy course in women consuming alcohol in the prenatal period confirmed by survey and laboratory tests, depending on the phosphatidylethanol levels.*
Materials and methods. *We examined 863 women under observation at the Irkutsk Regional Perinatal Center for the period from 2014 to 2021 To confirm the fact of alcohol consumption in the prenatal period, 545 women were surveyed, 318 women were examined using laboratory analysis. The diagnostic biomarker for alcohol was PEth:16:0/18:1. To assign pregnant women to the control group, a PEth concentration of ≤ 8 ng/ml was taken. If the PEth concentration was> 8 ng/ml, pregnant women were classified as heavy drinkers. Clinical and laboratory indicators of the course of pregnancy and childbirth were carried out in comparative groups.*
Results. *It has been established that every second woman of reproductive age took alcohol before pregnancy. 24.2 % of women did not stop consuming alcohol in the prenatal period. At the same time, the risk of congenital malformations was high, since 20.4 % of women consumed alcohol in the first trimester of pregnancy. Based on the results of the survey, it was revealed that in women who consumed alcohol in the prenatal period, the following pathological conditions are statistically significantly more common: anemia, congenital heart defects in fetuses, prematurity of gestational age, labor anomalies, uterus subinvolution. Based on the results of laboratory confirmation of alcohol consumption, it was established that parity of birth, intrauterine growth retardation, and premature birth were statistically significantly more often in pregnant women who drink.*
Conclusion. *Thus, in order to obtain the most meaningful and high-quality results, it is necessary to conduct larger studies. In addition, maternal blood biomarkers should be used to confirm levels of alcohol consumption throughout all trimesters of pregnancy.*

Key words: *alcohol, pregnancy, phosphatidylethanol (PEth), fetus*

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ОЦЕНКА ТЕЧЕНИЯ ГЕСТАЦИОННОГО ПРОЦЕССА У ЖЕНЩИН МЕТОДОМ ОПРОСА И В ЗАВИСИМОСТИ ОТ ЛАБОРАТОРНО ПОДТВЕРЖДЁННОГО ФАКТА УПОТРЕБЛЕНИЯ АЛКОГОЛЯ В ПРЕНАТАЛЬНОМ ПЕРИОДЕ (КРОСС-СЕКЦИОННОЕ ИССЛЕДОВАНИЕ)

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РЕЗЮМЕ

Обоснование. *Изучение проблемы употребление алкоголя беременными посредством современных лабораторных методов исследования несёт в себе ценное теоретическое и практическое значение.*
Цель исследования. *Определение особенностей течения беременности у женщин, принимающих алкогольные напитки в пренатальном периоде, подтверждённое методом опроса и лабораторно, в зависимости от уровней фосфатидилэтанола.*
Материалы и методы. *Обследовано 863 женщины, находящиеся под наблюдением в Областном перинатальном центре г. Иркутска за период с 2014 по 2021 г. Для подтверждения факта употребления алкоголя в пренатальном периоде методом опроса обследованы 545 женщин, методом лабораторно подтверждённого анализа – 318. Диагностическим биомаркером алкоголя был PEth:16:0/18:1. Для отнесения беременных в группу контролям была взята концентрация PEth ≤ 8 нг/мл. В случае значения PEth > 8 нг/мл беременные были отнесены к группе пьющих женщин. Клинико-лабораторные показатели течения беременности и родов проводилась в сравнительных группах.*
Результаты. *Установлено, что каждая вторая женщина репродуктивного возраста принимала алкоголь до беременности. 24,2 % не прекращали потреблять алкогольные напитки в пренатальном периоде. При этом риск возникновения врождённых пороков развития был высоким, так как в первом триместре беременности 20,4 % женщин употребляли спиртные напитки. По результатам анкетирования выявлено, что у женщин, употребивших алкоголь в пренатальном периоде, статистически значимо чаще встречаются следующие патологические состояния: анемия, врождённые пороки сердца у плодов, недоношенность гестационного возраста, аномалии родовой деятельности, субинволюция матки. По результатам лабораторного подтверждения употребления алкоголя установлено, что паритет родов, задержка внутриутробного развития плода, преждевременные роды статистически значимо чаще определялись у пьющих беременных.*
Заключение. *Таким образом, с целью получения наиболее содержательного и качественного результата необходимо провести более масштабные исследования. Кроме того, для подтверждения уровня потребления алкоголя на протяжении всех триместров беременности следует применить биомаркеры материнской крови.*

Ключевые слова: *алкоголь, беременность, фосфатидилэтанол (PEth), плод*

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INTRODUCTION

All over the world, including Russia, one of the ancient destructive habits in world history is excessive alcohol abuse and related consequences [1].

Throughout the nine months of pregnancy, every woman should know that alcohol is toxic to the growing fetus. An expectant mother who resorts to drinking alcohol in anticipation of the birth of a child does not even imagine how easily ethanol enters directly to the fetus, through the entire circulatory system. According to the results of numerous studies, most women continue to drink alcohol during the first month of pregnancy, not knowing that they are pregnant. It should be noted that it is in the first trimester of pregnancy that there are critical periods of development, during which a continuous epithelial cover of the villi is formed – the future placental barrier, which at this stage does not protect the embryo from any external influences [2–5]. At the same time, there is a high risk of complications of pregnancy and the birth of children with fetal alcohol spectrum disorders (FASD). This condition includes disorders that will affect children's health for life and are characterized by a combination of congenital physical and mental defects [6–8]. The diagnosis of FASD requires accurate knowledge of the amount and frequency of alcohol consumed. For this purpose, a survey of women is conducted using various questionnaires [9–11]: T-ACE, TWEAK, AUDIT. The information obtained through the survey is prone to subjectivism, and a person can often indicate deliberately false data. Consequently, biomarkers such as phosphatidylethanol are used to obtain an objective picture of a woman's alcohol consumption [12], the definition of which allows us to establish both the fact and the amount of alcohol consumed.

Based on the results of numerous studies and as practice demonstrates, after the fact of pregnancy is established, the percentage of women who drink alcohol decreases, but does not reach the required level. According to T.N. Balashova et al. [13], after pregnancy diagnosis, about 20 % of women report continued alcohol consumption, which leads to adverse pregnancy outcomes. It has been shown that there is no safe dose of alcohol during pregnancy. The pathophysiological effects of both moderate and low doses of alcohol on the gestational process and the fetus have been proven [14-19].

In order to determine the level of alcohol consumption during pregnancy for the purpose of earlier diagnosis and treatment of fetal lesions, as well as prevention, in addition to existing questionnaires, it is also necessary to pay attention to the study of maternal blood markers. As is known, there are direct and indirect groups of blood biomarkers, which are determined by the laboratory method.

In practice, direct markers of alcohol consumption are practically not used today, since the period of their content in biological fluids varies from 8-12 hours to no more than 7 days, and it is also impossible to differentiate a single alcohol intake from chronic alcoholism. Direct markers include ethyl esters of fatty acids, ethylglucuronide, and ethyl sulfate. On the contrary, the group of indirect biomarkers, such as aspartate aminotransferases, alanine aminotransferases, and gamma-glutamyltransferases, can vary quite widely [20, 21].

To date, there are many studies devoted to the study of another direct biomarker of ethanol, phosphatidylethanol (PEth), in order to obtain an objective picture of alcohol consumption. Due to the long elimination half-life of alcohol, it accumulates in the blood. In this case, it becomes possible to detect it within 28 days after the last alcohol intake [21].

Thus, as a result of the analysis of domestic and foreign literature, it was found that the problem of alcohol consumption by women in the prenatal period is relevant and promising. Therefore, the study of this problem and the conduct of research through modern laboratory research methods will be of great theoretical and practical importance.

In this regard, **the aim of our study** was to determine the characteristics of the course of the gestational process in women who consumed alcoholic beverages in the prenatal period, confirmed by a survey and laboratory, depending on the levels of phosphatidylethanol.

MATERIALS AND METHODS

A total of 863 women who were under observation at the Irkutsk Regional Perinatal Center for the period from 2014 to 2021 were interviewed.

For the purpose of a more convenient perception, the groups in which the survey was conducted are indicated by Arabic numerals (1st and 2nd), and the groups with laboratory confirmation of blood alcohol are indicated by Roman numerals (I and II).

545 women were examined by the survey method, using validated questionnaires, to identify the fact of alcohol consumption in the prenatal period.

Group 1 (n = 261) included women who had never consumed alcoholic beverages during pregnancy (control group).

The 2nd group (n = 284) included women who consumed alcoholic beverages in the prenatal period.

The average age of pregnant women in group 1 was 29.64 ± 5.9 years, and group 2 was 28.75 ± 6.1 years. No statistically significant differences were found when comparing the age indicators ($p > 0.05$).

To determine the PEth biomarker in the blood, that is, for laboratory confirmation of alcohol consumption, only 318 women were examined. Out of 318 pregnant women, 194 women never consumed alcoholic beverages throughout pregnancy (group I – control), 121 women consumed alcoholic beverages in the prenatal period (group II). Clinical and laboratory parameters of the gestational process and childbirth were evaluated in comparative groups.

The main group includes women who meet the following criteria: alcohol consumption during gestation;

belonging to the European race; absence of severe somatic pathology; signing of informed consent; accessibility throughout the duration of the study; current pregnancy; willingness of the participant to comply with all research procedures.

The control group included women with the following criteria: non-use of alcohol and nicotine during pregnancy; belonging to the European race; absence of severe somatic pathology; willingness of the participant to comply with all research procedures; signing informed consent; current pregnancy; accessibility throughout the duration of the study.

Excluded from the study were women who had severe somatic pathology; exacerbation of chronic diseases; presence of exacerbations of infectious diseases (bacterial and viral); presence of sexually transmitted infections, including HIV infection; woman's use of nicotine and narcotic drugs; chronic alcohol dependence, as well as the studied women who changed their place residents and those who refused further supervision.

In working with pregnant women, the ethical principles set forth in the World Medical Association Declaration of Helsinki (1964; last revised in October 2013) were observed. All pregnant women have received informed consent to conduct the study. The conduct of this study was approved by the Ethics Committee of the Scientific Centre for Family Health and Human Reproduction Problems (Protocol No. 2 dated March 4, 2021).

So, in the study groups, which were formed on the basis of the fact of alcohol consumption during pregnancy, diagnosed by the survey method, questionnaires (screening tools) [8-10] T-ACE, TWEAK, AUDIT were used. At the same time, the information is subject to subjectivity, since patients can often make deliberately false data. As described above, biomarkers are used to obtain an objective picture.

In this regard, for laboratory confirmation of alcohol consumption, the presence of such ethanol biomarkers in the blood of pregnant women as PEth:16:0/16:0, PEth:16:0/18:1, PEth:18:1/18:1 was determined. Moreover, the presence of each of the three PEth and their combinations was determined separately in the blood (Fig. 1).

Blood sampling in pregnant women was performed in fasting state from the ulnar vein at 38–40 weeks of gestation. The determination of phosphatidylethanol biomarkers was carried out on the basis of the Laboratory of Personalized Medicine of the Scientific Centre for Family Health and Human Reproduction Problems on a mass spectrometer. The analytical complex is based on an ultra-high-performance liquid chromatograph Shimadzu Nexera X2 (Kyoto, Japan) with an auto-injector and a pumping unit for creating a gradient on the high-pressure side and a tandem three-quadrupole mass-selective detector Shimadzu LCMS 060 (Kyoto, Japan) with a hybrid ion source DUIS.

During the observation of the course of the gestational process and the development of the fetus, a gravidogram was filled in, which indicated the initial body weight, the height of the woman, an increase in the circumference of the abdomen and the height of the uterine fundus. Blood pressure and basic biochemical and hemodynamic parameters were determined in dynamics. According to the generally accepted standard, all pregnant women were examined by a therapist,

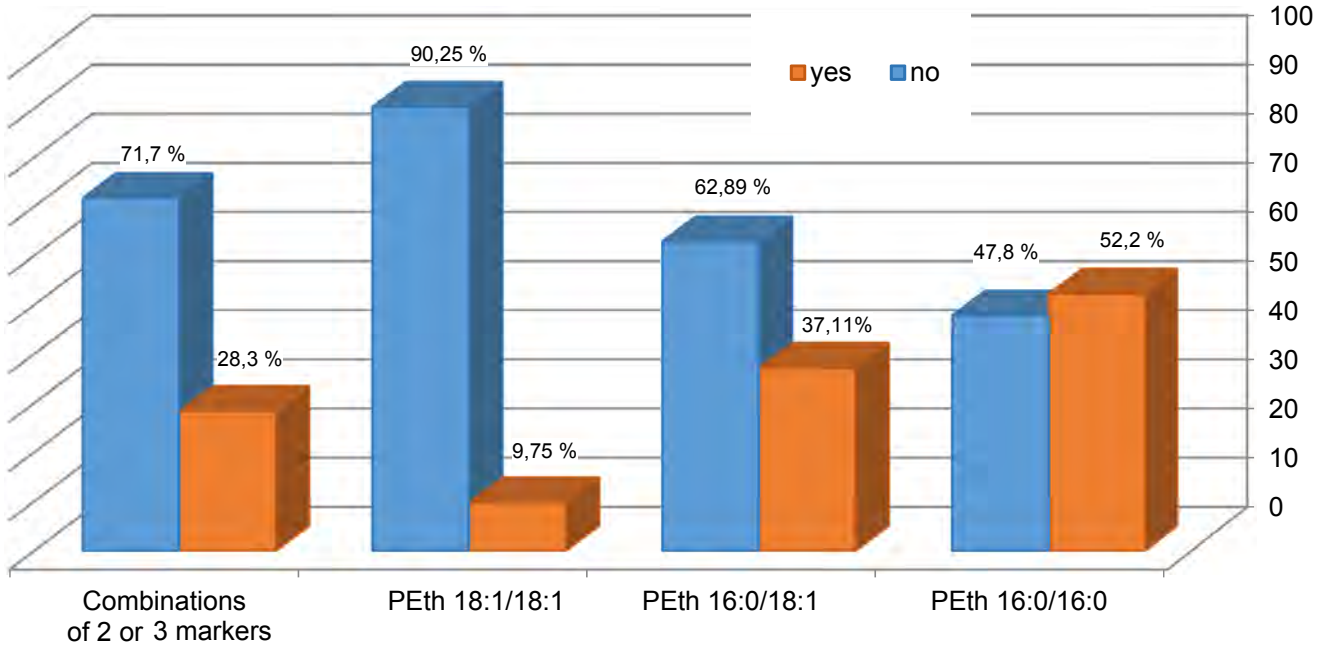

FIG. 1.
Laboratory confirmed fact of alcohol consumption in the prenatal period

neurologist and electrocardiography. The examination of other narrow specialists was carried out only according to indications.

According to the generally accepted standard, ultrasound with measurement of uteroplacental and fetal blood flow was used on Voluson E8 (GE Healthcare, USA), Philips 22, 11 (Philips, Netherlands) devices operating in real time to assess the fetal condition of the fetus. Using cardiotocography on Sonicaid Team devices (Oxford Medical, UK), Advanced Fetal Monitoring System BFM-800 (BIONICS Co. Ltd., South Korea), fetal cardiac activity in the antenatal period was determined.

We also analyzed the available medical documentation (exchange cards and birth histories).

A frequency analysis was performed, comparing the results of PEth:16:0/16:0, PEth:18:1/18:1 with a diagnostic biomarker of alcohol consumption (PEth:16:0/18:1). In the process of analyzing the literature and obtained data, it was found that PEth:16:0/18:1 was the most informative marker in comparison with PEth:16:0/16:0 and PEth:18:1/18:1 [22].

According to the literature [23], a limit of ≤ 8 ng/ml was taken for PEth:16:0/18:1 in order to classify pregnant women into the control group (the group of non-drinking women). The group of pregnant women who drink alcoholic beverages, depending on the concentration of PEth:16:0/18:1 (> 8 ng/ml), was divided into categories, and the dose of alcohol consumed was determined (Table 1).

The statistical analysis of the obtained data was carried out using the Statistica 10 statistical and application software package (StatSoft Inc., USA; the license holder is the Scientific Centre for Family Health and Human Reproduction Problems). Depending on the type of data distribution, various statistical analysis algorithms were used. Descriptive statistics were used to represent quantitative data. In the statistical analysis of the data, the differences in the compared indicators were considered significant at $p < 0.05$.

THE RESULTS OF A STUDY OF WOMEN BEFORE AND DURING PREGNANCY BASED ON A SURVEY

According to the analysis of the data obtained, no statistically significant differences in family status, education, and socio-demographic indicators were revealed ($p > 0.05$). In both groups of pregnant women, extragenital pathology (kidney, liver, bronchial asthma, diabetes mellitus, including gestational, thyroid diseases, heart defects, hypertension, epilepsy, etc.) occurred with the same frequency ($p > 0.05$).

The study revealed that every second woman of reproductive age consumed alcohol before pregnancy, and 24.2 % of them continued to drink alcoholic beverages during the present pregnancy ($p < 0.05$) (Fig. 2).

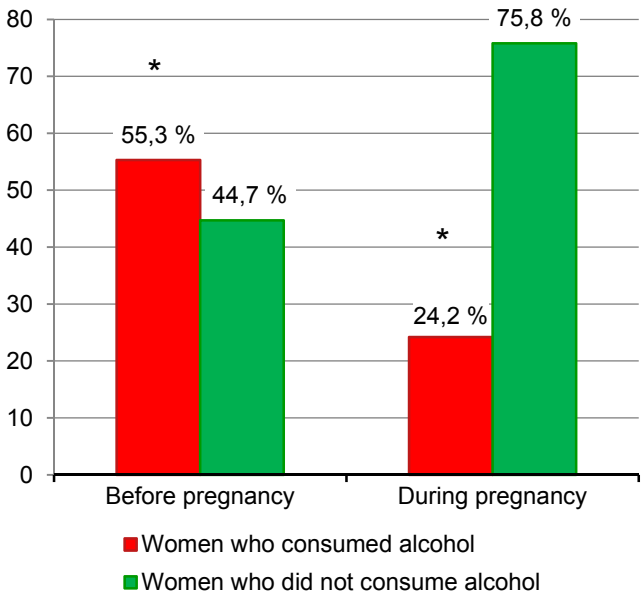

FIG. 2.
*Alcohol consumption before and during pregnancy based on a survey data: * – p < 0.05*

TABLE 1

GROUP OF PREGNANT WOMEN WHO HAD LABORATORY CONFIRMED BLOOD LEVELS OF PETH:16:0/18:1

PEth concentration:16:0/18:1 in the blood of pregnant women, ng/ml	Number of pregnant women ($N = 121$)	Doses of alcohol consumed
8 < PEth:16:0/18:1 < 45	52 % ($n = 63$)	< 1
45 ≤ PEth:16:0/18:1 < 127	31 % ($n = 38$)	≥ 1
PEth:16:0/18:1 ≥ 127	17 % ($n = 20$)	≥ 1

The data obtained show that of all women who consumed alcohol during pregnancy, 20.4 % did not stop drinking alcoholic beverages in the first trimester of pregnancy, which leads to a high risk of congenital malformations, including fetal alcohol syndrome and fetal alcohol spectrum disorders. As a result of studying the qualitative characteristics of alcohol consumption by women before and during pregnancy, it was found that before pregnancy 35.5 % of women consumed beer, 40.6 % – wine, 3.8 % – vodka. In the prenatal period, 20.7 % of pregnant women preferred wine, 13.4 % beer, and 1.2 % vodka from alcoholic beverages.

The results of the study show that almost every second pregnant woman was diagnosed with anemia, but statistically significantly more in the group of pregnant drinkers. So, in the control group, this indicator was 40.23 %, and in the group of women who consumed alcoholic beverages during pregnancy – 48.24 % ($p < 0.05$).

Of the congenital malformations in the fetus, it was found that congenital heart disease (CHD) of the fetus turned out to be significant. In the third trimester of pregnancy, according to the results of screening ultrasound examination, the diagnosis of fetal CHD was diagnosed in 9.96 % of pregnant women of the 2nd group. In the control group, this figure was 1.41 %. Thus, in fetuses of pregnant women who consumed alcoholic beverages in the prenatal period, CHD was diagnosed more often than in the control group ($p < 0.05$).

It was found that of the complications of labor, labor anomalies (LA) (discoordinated contractions of the myometrial muscle) They were statistically significantly more common ($p < 0.05$) in the group of women drinkers: in the group of non–drinkers, the frequency of LA was 3.07 %, in the group of drinkers – 6.34 %.

It was revealed that, according to the results of the analysis of the indicators of complications of the postpartum period, a statistically significant sign was uterine subinvolution. In the control group, its frequency was 11.6 %, and in the 2nd group – 19.54 % ($p < 0.05$).

It was determined that prematurity of gestational age was noted in 4.78 % of newborns in the control group and in 10.95 % of children born to drinking women ($p < 0.05$). The influence of alcohol on the degree of maturity and development of the fetus has been proven, as statistically significant differences were obtained when comparing the indicators of the 1st and 2nd groups.

THE RESULTS OF A STUDY OF WOMEN DURING PREGNANCY BASED ON LABORATORY-CONFIRMED ALCOHOL CONSUMPTION IN THE PRENATAL PERIOD

As a result of the analysis of the obstetric history, it was revealed that the parity of childbirth (the presence of childbirth in the anamnesis) it was statistically significantly more common in pregnant drinkers ($p < 0.05$). There were no statistically significant differences in other indicators ($p > 0.05$) (Table 2).

Table 3 shows the parameters characterizing the course of a real pregnancy in the study groups. As shown in the ta-

TABLE 2

OBSTETRIC HISTORY OF PREGNANT WOMEN WITH LABORATORY CONFIRMED ALCOHOL CONSUMPTION IN THE PRENATAL PERIOD

Parameters (M ± SD)	The entire sample (*N* = 318)	Group I (control): PEth:16:0/18:1 ≤ 8ng/ml (*N* = 194)	Group II (comparison): PEth:16:0/18:1 > 8 ng/ml (*N* = 121)	*p*
Delivery parity	–	2.83 ± 1.96	3.42 ± 2.35	**0.024***
	Outcome of previous pregnancies (M ± SD)			
The birth of a living child	1.21 ± 1.30	1.14 ± 1.27	1.34 ± 1.35	0.175
The birth of a stillborn child	0.02 ± 0.14	0.01 ± 0.12	0.02 ± 0.16	0.890
Spontaneous miscarriage	0.26 ± 0.55	0.22 ± 0.53	0.34 ± 0.58	0.141
Medical abortion	0.51 ± 1.08	0.42 ± 0.85	0.68 ± 1.37	0.196
Ectopic pregnancy	0.02 ± 0.18	0.01 ± 0.14	0.03 ± 0.22	0.771

Note. * – $p < 0,05$.

TABLE 3

CHARACTERISTICS OF THE PARAMETERS OF THE PRESENT PREGNANCY COURSE IN THE STUDY GROUPS

Parameters	The total number of women in the study groups, *n*/*N* (%)	Group I (control): PEth:16:0/18:1 ≤ 8ng/ml (*n* = 194), *n*/*N* (%)	Group II (comparison): PEth:16:0/18:1 > 8 ng/ml (*n* = 121), *n*/*N* (%)	*p*
Nausea, vomiting, early toxicosis	173/315 (54.92)	107/194 (55.15)	66/121 (54.55)	0.447
Anemia	177/315 (56.19)	116/194 (59.79)	61/121 (50.41)	0.103
Vaginal bleeding	32/315 (10.16)	21/194 (10.82)	11/121 (9.09)	0.642
UTS diseases	55/314 (17.52)	34/193 (17.62)	21/121 (17.36)	0.728
Increased BP	43/315 (13.65)	32/194 (16.49)	11/121 (9.09)	0.063
Fever or t > 37.8 °C	40/314 (12.74)	29/194 (14.95)	11/120 (9.17)	0.234
Diabetes mellitus	52/315 (16.51)	30/194 (15.46)	22/121 (18.18)	0.606
Swelling of the lower extremities	94/313 (30.03)	57/193 (29.53)	37/120 (30.83)	0.912
Swelling of the upper extremities	74/315 (23.49)	42/194 (21.65)	32/121 (26.45)	0.347
Candidiasis vaginitis	70/313 (22.36)	39/193 (20.21)	31/120 (25.83)	0.245

ble, in the studied groups, when comparing the indicators of pregnancy (early toxicosis, anemia, vaginal bleeding, diseases of the urinary track system (UTS), increased blood pressure (BP), fever or t > 37.8 °C, diabetes mellitus, edema of the upper and lower extremities, candidiasis vaginitis). There were no statistically significant differences ($p > 0.05$).

When analyzing the state of fetal intrauterine development, it was found that fetal intrauterine growth retardation (IUGR) was statistically significantly more common in women who drank compared with non-drinkers, which amounted to 2.6 % and 0.6 %, respectively ($p < 0.05$).

Among the complications of labor, it was revealed that preterm delivery was statistically significantly more common in the group of women who consumed alcoholic beverages in the prenatal period, which was confirmed by laboratory method, compared with the control group – 8.0 % and 4.8 %, respectively ($p < 0.05$).

Summing up the results of this study, it can be concluded that every second woman of reproductive age took alcohol before pregnancy, and 24.2 % of them continued to drink alcoholic beverages during the present pregnancy ($p < 0.05$). It was found that in the first trimester, 20.4 % of pregnant women consumed alcoholic beverages, which increases the risk of congenital malformations, including fetal alcohol syndrome and fetal alcohol spectrum disorders.

The results of the study obtained on the basis of the survey show that the following pathological conditions are statistically significantly more common in women who consumed alcohol in the prenatal period: anemia, fetal CHD, prematurity of gestational age, labor anomalies, uterine subinvolution ($p < 0.05$).

In our study, the diagnostic biomarker of alcohol consumption was PEth:16:0/18:1, which was determined in 37.11 % of pregnant women. The results of the study, based on laboratory confirmation of alcohol consumption, indicate that the parity of childbirth (the presence of childbirth in the anamnesis), fetal sound, premature birth, were statistically significantly more often determined in pregnant women with confirmed alcohol consumption in the prenatal period ($p < 0.05$).

ADVANTAGES AND LIMITATIONS OF THE STUDY

The advantage of the study is that for the first time in the Irkutsk region, a laboratory analysis method was used, in particular, the determination of alcohol bio-

markers (PEth:16:0/16:0, PEth:18:1/18:1, PEth:16:0/18:1), to confirm the fact of alcohol consumption in the prenatal period.

This study also had a number of limitations. Firstly, the study was partially based on the results of the survey, which reduces the diagnostic value of the data obtained. As mentioned above, information obtained in this way is subject to subjectivity. Based on this, biomarkers (PEth) are used to obtain an objective picture, which determine both the fact and the amount of alcohol consumed.

Secondly, for laboratory confirmation of alcohol, only a single blood sample was taken throughout pregnancy – at 38–40 weeks of gestation, which also reduces the diagnostic value of the data obtained. Due to the long half-life of alcohol consumption, PEth accumulates in the blood and can only be detected within 28 days after the last intake. Therefore, it is necessary that further studies be based on the determination of PEth at the screening time of the examination of pregnant women (in each trimester and at the end of pregnancy).

Despite the above, the conducted research has great theoretical and practical significance and will allow the use of a laboratory method to confirm the fact and amount of alcohol consumption in the practice of doctors. This will help in determining one of the most important risk factors (alcohol) during pregnancy and reduce the risk of complications of the gestational process and fetal complications.

CONCLUSION

An analysis of domestic and foreign literature, as well as the results of our study, show that the problem of alcohol consumption by women at reproductive age and during pregnancy is relevant and promising. There is a need for pregravid training, abstinence from alcohol consumption by women. It is extremely important to conduct the most extensive studies using biomarkers of maternal blood to determine the level of alcohol consumption during pregnancy throughout the gestation period, which will allow us to obtain more informative and high-quality results and further introduce this method into the practical activities of doctors, in particular obstetricians and gynecologists. From the point of view of the medical and social significance of the current problem, further studies of the mechanisms of alcohol's effect on the fetus and effective preventive measures should be developed.

Every pregnant woman should realize that alcohol is toxic to a growing fetus throughout the entire 40 weeks of pregnancy and that there is no safe dose of alcohol during pregnancy!

Conflict of interest

The authors of this article declare the absence of a conflict of interest.

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PREVALENCE OF POLYCYSTIC OVARY SYNDROME IN A POPULATION OF WOMEN OF REPRODUCTIVE AGE USING ROTTERDAM 2003 CRITERIA (LITERATURE REVIEW)

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ABSTRACT

Polycystic ovary syndrome (PCOS) is considered a common endocrine disorder among women of reproductive age, and the associated health risks persist throughout life. At the same time, there is a wide range of variations in the incidence of the syndrome (4–21 %), which in turn is explained by the influence of the study population characteristics, including ethnicity and race, as well as the applied diagnostic criteria.

The aim of the study. *To systematize the available data on the prevalence of polycystic ovary syndrome using the Rotterdam 2003 criteria in a population of women of reproductive age.*

Materials and methods. *The search for information was carried out using Internet resources (PubMed, EMBASE, Google Scholar, eLibrary). Literature sources for the period 1990–2023 were analyzed. As a result, the article presents current data on the prevalence of PCOS using the Rotterdam 2003 definitions, the features of the PCOS incidence in hospital and non-selective (medically unbiased) populations, as well as in various ethnic groups. The review also discusses current guidelines for conducting studies on the PCOS prevalence.*

Conclusion. *The latest guidelines on the diagnosis and management of patients with PCOS, published in 2018, propose to consider the provisions adopted in Rotterdam as the basis for the diagnosis of the syndrome; at the same time, the need to take into account racial and age characteristics is noted.*

Key words: *PCOS, prevalence, ethnicity, population, Rotterdam criteria, women of reproductive age*

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РАСПРОСТРАНЁННОСТЬ СИНДРОМА ПОЛИКИСТОЗНЫХ ЯИЧНИКОВ В ПОПУЛЯЦИИ ЖЕНЩИН РЕПРОДУКТИВНОГО ВОЗРАСТА ПРИ ИСПОЛЬЗОВАНИИ КРИТЕРИЕВ ROTTERDAM 2003 (ОБЗОР ЛИТЕРАТУРЫ)

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РЕЗЮМЕ

Синдром поликистозных яичников (СПКЯ) считается распространённым эндокринным заболеванием среди женщин репродуктивного возраста, а связанные с ним риски для здоровья сохраняются в течение жизни. В то же время отмечается широкий диапазон вариаций частоты встречаемости синдрома (4–21 %), что в свою очередь объясняется влиянием характеристик исследуемой популяции, в том числе этнической и расовой принадлежностью, а также применяемыми диагностическими критериями.

Цель исследования. *Систематизировать имеющиеся данные о распространённости синдрома поликистозных яичников при использовании критериев Rotterdam 2003 в популяции женщин репродуктивного возраста.*

Материалы и методы. *Поиск информации проводился с использованием интернет-ресурсов (PubMed, EMBASE, Google Scholar, eLibrary). Проанализированы литературные источники за период 1990–2023 гг. В результате в рукописи представлены современные данные о распространённости СПКЯ при применении дефиниций Rotterdam 2003, особенности частоты встречаемости синдрома в госпитальных и неселективных (медицински непредвзятых) популяциях, а также в различных этнических группах. В обзоре также обсуждаются современные рекомендации по проведению исследований по распространённости СПКЯ.*

Заключение. *В последнем руководстве по диагностике и ведению пациентов с СПКЯ, опубликованном в 2018 г., предлагается рассматривать положения, принятые в Роттердаме, как базовые относительно диагностики синдрома; при этом отмечается необходимость учитывать расовые и возрастные особенности.*

Ключевые слова: *СПКЯ, распространённость, этника, популяция, критерии Rotterdam, женщины репродуктивного возраста*

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INTRODUCTION

Polycystic ovary syndrome (PCOS) is considered a common endocrine disorder among women of reproductive age, and the associated health risks persist throughout life. Even postmenopausal women with PCOS may exhibit hyperandrogenism and insulin resistance [1–3]. In recent years, the prevalence of PCOS has been increasing [4], which may be due to improved methods of diagnosing the disease. At the same time, there is a wide range of variations in the incidence of the syndrome (4–21 %) [5–7], which in turn is explained by the influence of the study population characteristics, including ethnicity and race, as well as the applied diagnostic criteria. Nevertheless, it is estimated that up to 105 million women of childbearing age suffer from PCOS worldwide [8].

The criteria presented by the European Society of Human Reproduction and Embryology (ESHRE) in 2003 are the most frequently used in research and clinical practice. In Rotterdam (Rotterdam 2003) [8], according to which the presence of at least two of the following three signs is required to establish the diagnosis of PCOS: clinical or biochemical hyperandrogenism (HA); oligo/amenorrhea (OA); polycystic ovarian morphology (PCOM) according to ultrasound, – after the exclusion of PCOS-simulating states. These conditions include, first of all, congenital adrenal hyperplasia (CAH), thyroid dysfunction, hyperprolactinemia [1, 9]. A large contribution to the development of diagnostic criteria was made by a group of experts from the National Institutes of Health (NIH) in 2012 [10]. The improvement consisted in maintaining the broad inclusive diagnostic criteria of Rotterdam 2003 while differentiating the syndrome into phenotypes. The proposed phenotypes included the following manifestations: 1) hyperandrogenism + ovulatory dysfunction; 2) hyperandrogenism + polycystic ovarian structure by ultrasound; 3) ovulatory dysfunction + polycystic ovarian structure by ultrasound; 4) androgen excess + ovulatory dysfunction + polycystic ovarian structure by ultrasound [11].

In 2018, there was another revision of the International Recommendations for the Diagnosis and Treatment of PCOS [1], where new provisions were proposed. The main change was the possibility to take into account elevated levels of androstenedione (A4) and dehydroepiandrosterone sulfate (DHEA-S) in the diagnostic process if the values of free or total testosterone are within the reference values. In addition, a new "cut-off point" for the number of follicles per ovary was determined due to the fact that the progress of equipment has increased the sensitivity of ultrasound [1] in relation to the diagnosis of PCOS.

THE AIM

To systematize the available data on the prevalence of polycystic ovary syndrome using the Rotterdam 2003 criteria in a population of women of reproductive age.

MATERIALS AND METHODS

The search for information was carried out using Internet resources (PubMed, EMBASE, Google Scholar, eLibrary). Literature sources for the period 1990–2023 were analyzed. As a result, the article presents current data on the prevalence of PCOS using the Rotterdam 2003 definitions, the features of the PCOS incidence in hospital and non-selective (medically unbiased) populations, as well as in various ethnic groups.

There are 6 published systematic reviews and meta-analyses summarizing comparative studies of PCOS prevalence using the Rotterdam 2003 definitions [8].

For example, a meta-analysis involving a total of 19,226 Iranian women aged 10 to 45 years showed that, according to the Rotterdam 2003 criteria, PCOS was diagnosed in 19.5 % of cases [12]. In another study, 24 articles were analyzed, which presented the overall prevalence of the syndrome – 10 % [5, 7] in accordance with the Rotterdam 2003 definitions. In addition, the study revealed a high incidence of isolated hirsutism, HA, OA and PCOM – 13 %, 11 %, 15 % and 28 % respectively. The authors focus on the heterogeneity of research, and therefore, consider it necessary to strengthen the standardization of methods to improve the comparability of the prevalence of PCOS worldwide.

In 2018, M.A. Skiba et al. analyzed 21 studies of the incidence of PCOS over the period from 1990 to 2018 [13]. In this review, the authors presented a unified definition of PCOS prevalence based on the NIH and Rotterdam 2003 criteria and confirmed the statistical significance of the differences when using these criteria ($p < 0.0001$). On the contrary, when comparing the data obtained using the diagnostic classifications Rott and AES (Androgen Excess Society), there were no differences in the incidence of the syndrome ($p = 0.201$). The authors suggested that the higher prevalence of PCOS reported in studies using the latest diagnostic approaches was due to the inclusion of the ultrasound diagnostic criterion. Moreover, differences in estimates of PCOS prevalence could be explained by the lack of standardization of criterion values, the diversity of clinical phenotypes and the study groups.

In 2021, Chinese scientists [14] assessed the prevalence of polycystic ovary syndrome in Chinese women based on an analysis of 69 studies. A total of 154,599 participants were included, of which 12,845 women were diagnosed with PCOS. The prevalence of PCOS was 10.01 % (95 % confidence interval (95% CI): 8.31–11.89 %). The authors note a lower incidence of PCOS among Chinese women compared, for example, with the prevalence of the disease among women in the Middle East (16 %) [15] and associate such variability with both racial characteristics and optimization of approaches to diagnosing the syndrome. In addition, the heterogeneity of the occurrence of PCOS in China was noted, depending on the affiliation of the surveyed audience to a certain economic zone. The analysis of the subgroups showed that the incidence of the syndrome in different regions was as follows: 13.35 % in the West, 7.82 % in the East, 14.24 % in the cen-

ter and 8.68 % in the North-East. At the same time, a meta-analysis of Indian scientists in 2022, including 11 studies, found the overall prevalence of PCOS among Indian women at 11.33 %.

A meta-analysis of F. Chiaffarino et al. (2022) noted the same prevalence of PCOS in European countries and the United States using the same criteria for diagnosing the syndrome [16]. As a result, the overall prevalence of the disease, according to the Rotterdam 2003 definitions, was about 19.5 % in the absence of significant heterogeneity by geographical region [16]. However, differences in the prevalence of PCOS phenotypes were noted: the incidence of phenotype A was higher, and phenotype C is lower in the USA compared to European countries (Table 1).

A. Yasmin et al. in their systematic review of 2022, after analyzing 118 studies, they noted the presence of variations in the clinical manifestations of PCOS depending on geographical regions among different ethnic groups [18]. So, in one of the largest studies of the prevalence of PCOS among the American population living in different geographical territories, the prevalence of the syndrome in the southern regions was 47.5 % higher than in the rest of the country [19].

It is believed that the prevalence of PCOS differs significantly in non-selective and hospital populations. Recent studies show that PCOS is usually characteristic of patients with acne, hirsutism, oligoanulation, obesity and infertility. So, 12 % of PCOS prevalence was reported among women with hirsutism [13], 82 % in patients with clinically pronounced androgen excess [20]. It is important to note that a significant proportion of women with clinical hyperandrogenism had the classic phenotype of the syndrome [21].

S.E. Allen et al. analyzed the frequency of hyperandrogenism and PCOS among women with oligoanulation [20]. The authors demonstrated that the prevalence of PCOS in the group of patients with a long history of oligoovulation reached 38 % vs. 5 % in the cohort of women with episodes of ovulatory dysfunction. In general, up to 40 % of nulliparous women with a menstrual cycle of 45 days or more were identified as patients with PCOS [20–23]. The Australian authors noted that the incidence of polycystic ovarian structure according to ultrasound data among 100 female partners of infertile men reached 23 %, while 12 % of women were diagnosed with three of the three criteria of the disease in accordance with Rotterdam 2003 [24]. Moreover, in a cross-sectional study conducted at the University infertility Clinic, PCOS was identified in 46 % of infertile women as one of the main causes of infertility [25]. There is some evidence that the prevalence of the syndrome in infertile women depends on race. For example, it is significantly higher among South Asians compared to Caucasians (44.2 % vs. 11.5 %; relative risk (RR) – 6.1; 95 % CI: 2.2–16.7) [26]. Similar results were demonstrated by Russian researchers when studying the causes of infertility in women of Caucasian and Asian ethnicity living in Eastern Siberia. The peculiarity of the group of women in the Caucasian population was also the high incidence of PCOS compared to the Asian population (33 (22.92 %) cases vs. 9 (8.65 %) cases) [27–31].

A clear link has been established between PCOS and obesity [32]. B.O. Yildiz et al. conducted a study of Turkish data on two population-based studies of the prevalence of PCOS and the hospital database of all patients with PCOS who had not received treatment before. In this study, in women with underweight, normal, overweight and obese in Turkey, the prevalence of the syndrome was 8.2 %, 9.8 %, 9.9 % and 9.0 %, respectively. The highest proportion of patients with PCOS (12.4 and 11.5 %) was found in women with a BMI of 35–40 kg/m^2 and more than 40 kg/m^2, respectively [33]. The incidence of PCOS in 421 obese Chinese patients was quite high (67 %), but it did not correlate with the presence of metabolic syndrome [34].

Databases of public health authorities and resources of health insurance systems were also used to study the prevalence of PCOS. In a cross-sectional study conducted by L. Gabrielli et al., the medical records of 859 Bra-

TABLE 1

SYSTEMATIC REVIEWS AND META-ANALYSES OF THE PCOS PREVALENCE USING THE ROTTERDAM 2003 DIAGNOSTIC CRITERIA

Authors	Year	Design (as suggested by the authors)	Rotterdam 2003, % [95% CI]
Jalilian A. et al. [12]	2015	Meta-analysis	19.5 [2.24–8.14]
Bozdag G. et al. [5]	2016	Systematic review and meta-analysis	10 [8–13]
Skiba M.A. et al. [13]	2018	Systematic review and meta-analysis	12 [10–15]
Wu Q. et al. [14]	2021	Meta-analysis	10.01 [8.3–11.8]
Bharali M.D. et al. [17]	2022	Systematic review and meta-analysis	11.33 [7.69–15.59]
Chiaffarino F. et al. [16]	2022	Systematic review and meta-analysis	19.5 [17.3–21.6]

zilian women undergoing cervical cancer screening in primary health care facilities were analyzed [35]. It was found that according to the Rotterdam 2003 criteria, the prevalence of PCOS is 8.5 %.

Limited epidemiological data were obtained from secondary analysis of databases and registries of non-PCOS studies. In 2010, C. Moran et al. reported a lower estimate of the prevalence of PCOS in Mexican female volunteers – 6.6 % (Rotterdam 2003) [36]. Among 827 women participating in a cross-sectional study of relatives of patients with cardiovascular diseases in the Dallas study (2000–2002), PCOS, according to the Rotterdam 2003 criteria [8], was diagnosed in 19.6 % of the examined [37].

Undoubtedly, the above studies are important and valuable. Nevertheless, the results of PCOS assessment in specialized medical institutions are definitely at risk of bias due to the characteristics of the sample of participants [38]. Therefore, non-selective (medically objective) studies are more representative and therefore preferable for epidemiological studies. These cases, identified in preclinical conditions, allow scientists to establish population "control" and determine the prevalence of PCOS.

Population-based research is the "gold standard" for estimating prevalence, but the method has its limitations. When conducting such experiments, different approaches are used to recruit participants. For example, they use a random sample from families, communities, and age groups. In Sri Lanka in 2008, V. Kumarapeli et al. conducted a cross-population study to identify the prevalence of PCOS and its phenotypes [39]. The authors compiled a questionnaire and offered it to the interviewers to fill out in order to identify probable cases of PCOS, and then sent the probable cases to experts for further analysis. With previously identified cases, the overall prevalence according to the Rotterdam 2003 diagnostic criteria was 6.3 % [39]. Later, in a retrospective study of a certain age group conducted in 2010, W.A. March et al. demonstrated that the prevalence rates of PCOS in accordance with the Rotterdam 2003 definitions and AES were twice as high as those obtained using the NIH criteria. Significantly 68–69 % of PCOS patients identified in this study had not been diagnosed with polycystic fibrosis before.

In a population-based study involving women of reproductive age living in randomly selected areas of Iran, the prevalence of PCOS, depending on diagnostic criteria, was 7.1–14.6 % [40]. These figures are consistent with the previously announced frequency of PCOS detection in Iranian women referred for mandatory premarital screening: the indicator was 7–15.2 % with various diagnostic criteria [41]. In 2014, in Iran, H. Rashidi et al. conducted an epidemiological study estimating the prevalence of PCOS at 14.1 % in accordance with the Rotterdam approach [42]. However, in 2013 in China, a large-scale study among ethnic communities showed a much lower prevalence of PCOS in accordance with the Rotterdam 2003 criteria, which was 5.6 % [43]. At the same time in 2014, J. Zhuang et al. noted that the prevalence of PCOS in Chinese women aged 12 to 44 years varied from 7.1 to 11.2 %, depending on the diagnostic criteria used [44].

The population model identifies PCOS in non-selective groups of the population who need medical examination for non-medical reasons: annual medical examination at work, before applying for a job, etc. For example, the prevalence of PCOS among employees of a public institution in Turkey, according to the Rotterdam 2003 criteria, reached 19.9 % [45].

Another model of objective epidemiological research uses population groups undergoing medical examination for non-productive medical reasons. In 2008. X. Chen et al. analyzed 915 Chinese women of reproductive age during the annual survey. This representative epidemiological study revealed a 2.4 % prevalence of PCOS in accordance with the Rotterdam 2003 criteria [46].

An analysis of healthy volunteers and medical staff may be useful, but its quality is lower due to a systematic selection error. Among women of reproductive age working at the University of Copenhagen Hospital, the overall prevalence of PCOS was 16.6 % according to the Rotterdam 2003 criteria. However, the researchers noted that the frequency of PCOS detection decreased significantly when the subjects were divided by age categories: from 33.3 % in women under 30 years of age to 10.2 % in women over 35 years of age ($p < 0.001$). The authors suggest that the studied population (healthcare practitioners) and the exclusion of women taking hormonal contraceptives (HC) could have caused a systematic selection error [47].

Obviously, we can argue that ethnicity and race influence the heterogeneity of the prevalence and clinical manifestations of PCOS. Thus, PCOS is less common in East Asians than in Caucasians, so the Asian phenotype of PCOS attracts the attention of researchers [52]. The incidence of PCOS (according to NIH 1990 criteria) in black and white women is comparable and amounts to 8.0 and 4.8 %, respectively [53]. T. Ding et al. (2017) [15] using various criteria analyzed the prevalence of PCOS by ethnicity in a systematic review and meta-analysis in 13 studies. They found the lowest prevalence of PCOS (5.6 % (95% CI: 4.4–7.3 %) according to Rotterdam 2003 criteria) among the Chinese group. This review showed how important it is to develop ethnicity-sensitive recommendations to prevent under- or overdiagnosis of PCOS [15]. Relatively recently, H.J. Kim et al. reported the impact of race and ethnicity on the standardization of PCOS diagnosis [15, 54].

To improve the quality and comparability of PCOS prevalence studies, AES has announced the release of practical recommendations for the development and conduct of epidemiological and phenotypic studies of PCOS [55]. The published document describes the main recommendations for the study plan, it also provides some recommendations on the selection of the study population, diagnostic criteria, type of observational study, as well as primary and secondary endpoints. According to the recommendations, it is important to use generalized population groups, broad diagnostic criteria and high sensitivity methods in assessing the individual characteristics of PCOS in the study of its prevalence. It is also important and strongly recommended to give a precise definition of what is "normal" for the study population. It is noteworthy that the rec-

TABLE 2

PREVALENCE OF PCOS ACCORDING TO ROTTERDAM 2003 DEFINITIONS IN NON-SELECTIVE POPULATIONS

Authors (year)	Country	Study design*	Population	Prevalence,% [95% CI] (if available)
Chen X. et al. (2008) [46]	China	Observational study	915 women aged 20–45 years living in Guangzhou, examined during the annual medical examination	2.4 %
Kumarapeli V. et al. (2008) [39]	Sri Lanka	Cross-sectional study of certain communities	A random sample of 2,915 women aged 15–39 years permanently residing in the Gampaha area	6.3 % [5.9–6.8]
March W.A. et al. (2010) [48]	Australia	A retrospective study of a certain age group	728 women born in 1973–1975 in one maternity hospital in Adelaide, examined in adulthood, 27–34 years old	11.9 %
Moran C. et al. (2010) [36]	Mexico	A prospective cross-sectional study	150 female volunteers from Mexico, 20–45 years old, employee of the Hospital of Obstetrics and Gynecology of the Mexican Institute of Social Welfare (Mexico City)	6.6 % [2.3–10.9]
Mehrabian F. et al. (2011) [49]	Iran	Cross-sectional study	820 women aged 17–34 years selected during mandatory premarital medical examination in Isfahan	15.2 %
Tehrani F.R. et al. (2011) [40]	Iran	The study of certain communities	1126 women aged 18–45 years, randomly selected from the population of various geographical regions of Iran	14.6 % [12.3–16.9]
Gabrielli L. et al. (2012) [35]	Brazil	Observational	859 women subject to cervical screening	8.5 %
Yildiz B.O. et al. (2012) [45]	Turkey	Cross-sectional study	392 women aged 18–45 years, employees of the State Institute in Ankara	19.9 %
Li R. et al. (2013) [43]	China	The study of certain communities	15,924 women aged 19–45 years from 152 cities and 112 villages in 10 provinces and municipalities of China	5.6 %
Lauritsen M.P. et al. (2014) [47]	Denmark	A prospective cross-sectional study	447 women (20–40 years old), employees of the University Hospital of Copenhagen.	16.6 %
Rashidi H. et al. (2014) [42]	Iran	The study of certain communities	646 women aged 18–45 years living in urban areas of three randomly selected cities in Khuzestan province	14.1 %
Zhuang J. et al. (2014) [44]	China	Cross-sectional study of certain communities	1,645 Chengdu residents aged 12–44 years	11.2 %
Deswal R. et al. (2019) [50]	India	Cross-sectional study, stratification sampling method	2,248 women aged 16–45 years, urban and rural residents	4.21 % 5,4 %
Ganie M.A. et al. (2020) [51]	Kashmir, India	Cross-sectional study	962 women aged 15–45 years from educational institutions in Kashmir	35.3 %

Note. * – by definition of the authors.

ommendations provide researchers around the world with tools for conducting very high-quality reliable epidemiological studies of PCOS [55].

The latest guidelines on the diagnosis and management of patients with PCOS, published in 2018 [1], propose to consider the provisions adopted in Rotterdam as the basis for the diagnosis of the syndrome [1]; at the same time, the need to take into account racial and age characteristics is noted [1].

CONCLUSION

Based on the results of the analysis, it can be concluded that the prevalence of PCOS according to the criteria of Rotterdam 2003 in the USA, Spain, Brazil, Mexico, Iran and Asia ranges from 6 % to 19.5 %. According to the Rotterdam 2003 definitions, the prevalence of PCOS in Indian women was the highest (35.3 %), while in Chinese women it was the lowest. The presence of the influence of race or ethnicity on the prevalence of the disease is also confirmed. The differences are often very small, which may be due to the variety of study designs, sampling characteristics, as well as the limitations of classical epidemiological studies of the prevalence of PCOS in a non-selective population.

Further epidemiological studies are needed to better understand PCOS and finalize its diagnostic criteria. The data currently available are insufficient to make definitive conclusions about the exact prevalence of the disease. The known facts about PCOS and its incidence in different geographical regions are not convincing enough to confirm significant differences in the prevalence of the syndrome in different ethnic groups.

Conflict of interest

The author of this article declares that there is no conflict of interest.

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COMPARATIVE MORPHOLOGICAL CHARACTERISTICS OF THE UTEROPLACENTAL AREA IN ABNORMAL PLACENTATION

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ABSTRACT

The aim. *To carry out a comparative morphological characteristic of the uteroplacental area with abnormal placentation – pl. accreta, pl. increta, pl. percreta.*
Materials and methods. *The study included 47 patients with atypical placentation; the comparison group included 10 healthy pregnant women with uterine scar after a previous caesarean section. A histological study of uteroplacental area samples was performed with hematoxylin and eosin, methylene blue staining. An immunohistochemical study with primary antibodies to cytokeratin 7 (CK7), Hif2a, vascular endothelial growth factor, α-SMA was carried out. The differences between the compared values were considered to be statistically significant at $p < 0.05$.*
The results of the study. *Pl. accreta was determined in 12 (25.5 %), pl. increta – in 30 (63.9 %), pl. percreta – in 5 (10.6 %) patients. In all patients of the main group, the decidua was completely or partially absent in the area of abnormal placentation or was replaced by an uneven layer of fetal fibrinoid. Cases when placental villi unevenly penetrated into the thickness of myometrium in the form of "tongues" or "coves" bordered by fetal fibrinoid and often located intermuscularly were defined as pl. increta (n = 26). Cases with the placental villi ingrowth to the serous membrane were considered as pl. percreta (n = 5). In cases with deep variants of ingrowth (pl. increta and pl. percreta) (n = 31), the villi were visualized in the lumen of the vessels and the thinning of the lower uterine segment with the presence of stretched muscle bundles was revealed. Aseptic necrosis of the myometrium was found: in 2 (16.7 %) of 12 women with pl. accreta, in 26 (86.7 %) of 30 women with pl. increta and in 5 (100 %) women with pl. percreta. There were no areas of necrosis in the myometrium of the women of comparison group.*
Conclusion. *The appearance and increase of myometrial necrosis zones in response to an increase in the depth of placental villus ingrowth were detected. Myometrial necrosis zones could be the cause of activation of angiogenic factors and an important stimulus for the development of abnormal vascularization in placenta accreta spectrum.*

Key words: *angiogenesis, aseptic necrosis, placenta accreta spectrum, invasion in the myometrium, trophoblast, fetal fibrinoid*

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СРАВНИТЕЛЬНАЯ МОРФОЛОГИЧЕСКАЯ ХАРАКТЕРИСТИКА МАТОЧНО-ПЛАЦЕНТАРНОЙ ОБЛАСТИ ПРИ АНОМАЛЬНОМ ПРИКРЕПЛЕНИИ ПЛАЦЕНТЫ

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РЕЗЮМЕ

Цель исследования. *Провести сравнительную морфологическую характеристику маточно-плацентарной области при аномальном прикреплении плаценты – плотном прикреплении, врастании и прорастании плаценты.*
Материалы и методы. *В исследование включено 47 пациенток с атипичной плацентацией; группа сравнения – 10 здоровых беременных женщин с рубцом на матке после предыдущего кесарева сечения. Выполнено гистологическое исследование образцов маточно-плацентарной области с окрашиванием гематоксилином и эозином, метиленовым синим, а также проведено иммуногистохимическое исследование с первичными антителами к цитокератину-7, фактору роста эндотелия сосудов, гладкомышечному актину-альфа. Различия между сравниваемыми величинами признавались статистически значимыми при $p < 0,05$.*
Результаты исследования. *Плотное прикрепление плаценты (pl. accreta) было определено у 12 (25,5 %), врастание плаценты (pl. increta) – у 30 (63,9 %), прорастание плаценты (pl. percreta) – у 5 (10,6 %) пациенток. У всех пациенток основной группы децидуальная оболочка полностью или частично отсутствовала в зоне аномальной плацентации или была замещена неравномерным слоем плодного фибриноида. При pl. increta (n = 26) ворсины плаценты проникали в толщу миометрия неравномерно, в виде «языков» или «бухт», окаймлённых плодным фибриноидом, и часто располагались межмышечно. Случаи с прорастанием ворсин до серозной оболочки рассматривали как pl. percreta (n = 5). При глубоких вариантах врастания (pl. increta и pl. percreta) (n = 31) ворсины визуализировались в просвете сосудов, наблюдалось истончение нижнего маточного сегмента с присутствием растянутых мышечных пучков. Обнаружены асептические некрозы миометрия: у 2 (16,7 %) из 12 женщин с pl. accreta, у 26 (86,7 %) из 30 женщин с pl. increta и в 5 (100 %) случаях при pl. percreta. Участки некрозов в миометрии группы сравнения отсутствовали.*
Заключение. *Обнаружено появление и увеличение зон некроза миометрия в ответ на увеличение глубины врастания ворсин плаценты, которые могут являться причиной активизации ангиогенных факторов и важным стимулом развития аномальной васкуляризации.*

Ключевые слова: *ангиогенез, асептические некрозы, врастание плаценты, инвазия в миометрий, трофобласт, плодный фибриноид*

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INTRODUCTION

Placenta accreta spectrum (PAS) is a complication of pregnancy associated with its abnormal attachment to the uterine wall, in which the placenta does not separate spontaneously after childbirth, which can lead to perinatal complications, massive blood loss and maternal mortality. Worldwide, the incidence of placenta accreta varies from 1.7 to 900 per 100,000 births (an average of 189 per 100,000), which is associated with variability in the formulation of the diagnosis and its clinical confirmation [1], and in recent decades has been about 1 case per 500 births [2–5].

According to the FIGO (International Federation of Gynecology and Obstetrics) classification, which includes clinical and pathomorphological criteria, there are several degrees of placenta accreta: 1st degree (placenta accreta) – dense attachment or increment of the placenta to the muscle layer; 2nd degree (placenta increta) – villi germinate the muscle layer; 3rd degree (placenta percreta) – the placenta sprouts all layers of the uterus. Placenta percreta is divided into subtypes: 3a – invasion within the serous membrane of the uterus; 3b – invasion into the bladder; 3c – invasion into other organs/tissues of the pelvis [6].

In addition to the above FIGO classification, there is an equivalent Russian systematization of atypical placentation [7]. According to the Russian authors, the English-language terms correspond to the following Russian-language equivalents: pl. accreta – pathological growth of the placenta with complete or partial absence of the basal (main) part of the falling shell; pl. increta – placenta accreted to the myometrium and/or placenta penetrated into the myometrium; pl. percreta – placenta fused to the perimeter either with the uterus and organs lying next to it/the placenta penetrated into the perimetrium or with penetration into adjacent structures [7].

An increase in the frequency of placenta accretion in recent decades has been associated with an increase in indications for cesarean section (CS). However, placental overgrowth is also found in women without a scar on the uterus, but who have undergone other manipulations that lead to a violation of the integrity of the endometrium (surgical abortions, diagnostic or therapeutic curettage) [8–11]. Studying the morphofunctional features of the uteroplacental region during placenta accreta and identifying the mechanisms of formation of this complication will allow us to better understand its nature and further develop prevention and treatment tactics. Due to this, the aim of the study was to conduct a comparative morphological characterization of the uteroplacental region in abnormal placental attachment – dense attachment, ingrowth and germination of the placenta.

MATERIALS AND METHODS

The present study included 57 women, of whom 47 patients made up the main group (gestation period – 36.3 (35; 38) weeks) with histologically confirmed placental ingrowth. The comparison group consisted of 10 women at 38.5 (38; 39) gestation weeks with the presence of a scar on the uterus after a previous cesarean section, but without signs of placenta accretion. The work is based on the morphological analysis of samples of the myometrium and placenta. The study did not include patients with severe fetal pathology, multiple pregnancies, and fetus birth defects.

The study was performed in accordance with the ethical principles of the World Medical Association Declaration of Helsinki [12] and the Rules of Clinical Practice in the Russian Federation (Order of the Ministry of Health of the Russian Federation dated June 19, 2003 No. 266) [13]. The study was approved by the Bioethical Commission of the Avtsyn Research Institute of Human Morphology (Protocol No. 35 (11) dated March 23, 2022).

The FIGO classification was used to assess the depth of placental ingrowth [6, 14].

The material for the study was obtained during surgical delivery. Fragments of the myometrium with areas of dense attachment and ingrowth of placental villi were excised according to the operational tactics developed at Vidnovsky Perinatal Center [15].

For histological examination, the obtained pieces were fixed in a 10 % solution of neutral formalin, pH = 7.4 (Biovitrum, Russia) for 24 hours, then enclosed in paraffin, according to the standard procedure. Serial paraffin sections with a thickness of 4 microns were dewaxed and stained with hematoxylin and eosin. To prepare semifine sections with a thickness of 1 μm, samples of the uteroplacental region in the zone of dense attachment and ingrowth were fixed in a solution of 2.5 % glutaraldehyde and 1 % paraformaldehyde in a 0.1 M phosphate buffer (pH = 7.4); then additional fixation was carried out in a 1.5 % OsO_4 solution, dehydration and pouring to araldit. The average area of the semifine section was 0.97 ± 0.3 mm^2. The semifine sections were stained using the PAS method and finished with methylene blue.

Immunohistochemical staining of micropreparations was performed on a closed-type immunostainer (Ventana; Roche, UK) using primary antibodies to cytokeratin-7 (SK-7, cytokeratin-7; Thermo Fisher, USA; dilution 1:300; cat. No. PA5-82291) – for trophoblast visualization; to hypoxia-induced factor Hif2a (Abcam, UK; dilution 1:250, cat. No. ab109616) – to assess the severity of local hypoxia; to smooth muscle actin-alpha (α-SMA, α smooth-muscle actin; Cell Mark, Sweden; without dilution, cat. No. 202M) – to characterize the myometrium and vascular wall; to endothelial vascular growth factor (VEGF, vascular endothelial growth factor; Spring Bioscience, USA; without breeding, cat. No. E2611) – for visualization of vascular endothelium. The product of the immunohistochemical reaction was determined in the form of brown staining in the membrane and/or cytoplasm of cells. The negative and positive controls were set in accordance with the manufacturer's recommendations.

Statistical analysis of the research results was performed using MS Excel 2010 application software packages (Microsoft Corp., USA) and Statistica 10 software (StatSoft Inc.,

USA). The descriptive characteristics of quantitative indicators are presented in the form of median (Me), 25 % and 75 % percentiles (P25 and P75). The Mann – Whitney non-parametric test of the statistical significance of was used to assess the representative sampling of the compared groups. For indicators characterizing qualitative characteristics, the absolute value and the relative value were indicated as a percentage. The differences were considered statistically significant when the significance criterion was less than 0.05.

STUDY RESULTS

When analyzing the comparability of the comparison groups, there were no intergroup differences ($p \geq 0.05$) in age, patient body mass index, birth parity and the number of previous cesarean sections (CS) (Table 1).

Macro- and microscopic characteristics of the placenta and myometrium

The areas of ingrowth looked intraoperatively like a hernia with thinning of the uterine wall (Fig. 1a). In addition, in some cases (n = 33), areas of dense yellowish consistency with sizes from 0.5 to 3 cm^2 in diameter were visualized in the myometrium, which, during histological examination, represented areas of myometrial necrosis. Sometimes (n = 31) a network of large-diameter blood vessels was present in the myometrium in the uteroplacental region (Fig. 1b).

In the main group (n = 47), 22 (46.8 %) pregnant women had a medical history of one CS; 17 (36.2 %) had two CS; 4 (8.5 %) had three CS; 2 (4.25 %) had four CS, and 2 (4.25 %). In women, ingrowth was diagnosed in the absence of a scar on the uterus after cesarean section.

As a result of histological examination, it was revealed that 12 (25.5 %) patients showed dense attachment of the placenta (pl. accreta), 30 (63.9 %) – pl. increta, 5 (10.6 %) – pl. percreta, of which 2 patients had involvement of the bladder wall (3b), and in 3 patients the villi of the placenta germinated myometrium, located up to the serous layer of the uterus (3a).

In all patients of the main group, the decidual membrane was completely or partially absent in the zone of abnormal placentation or was replaced by an uneven layer of eosinophilic homogeneous substance visually corresponding to the deposits of fetal fibrinoid (FF). Cases when placental villi penetrated the thickness of the myometrium unevenly in the form of "tongues" or "bays" (Fig. 1c), bordered by fetal fibrinoid (Fig. 1d), and often located intermuscularly, were defined as pl. increta (n = 26). Cases with the placental villi ingrowth to the serous membrane were considered as pl. percreta (n = 5). In deep variants of ingrowth (pl. increta and pl. percreta) (n = 31), villi were visualized in the lumen of the vessels (Fig. 1e), and there was also thinning of the lower uterine segment with the presence of stretched muscle bundles (Fig. 1e), (Fig. 1b–f).

In 2 (16.7 %) in cases out of 12 with pl. accreta, in 26 (86.7 %) out of 30 with pl. increta and in all cases with pl. percreta, the presence of necrosis zones in the myometrium was noted (Table 2), representing varying degrees of eosinophilic, with an uneven mesh-granular structure, areas in the absence of nuclear basophilia (Fig. 1g).

In some women, there was a slight focal and diffuse inflammatory infiltration in the uteroplacental region (detected in 1–2 out of 10 visual fields at magnification ×400), represented mainly by macrophages and lymphocytes with an admixture of single neutrophils ($p > 0.05$). Due to the weak inflammatory infiltration, these areas of necrosis can be called aseptic. Along with this, FF deposits were present in the uteroplacental region. In the area of necrosis, villi embedded in FF with dystrophic changes, the so-called "shadow villi" (Fig. 1g), took place.

No necrosis zones were found in the myometrium of the comparison group. In the main group, areas of necrosis were observed in all forms of ingrowth, and the frequency of their occurrence in the group increased with the depth of ingrowth of villi into the uterine wall (Table 2).

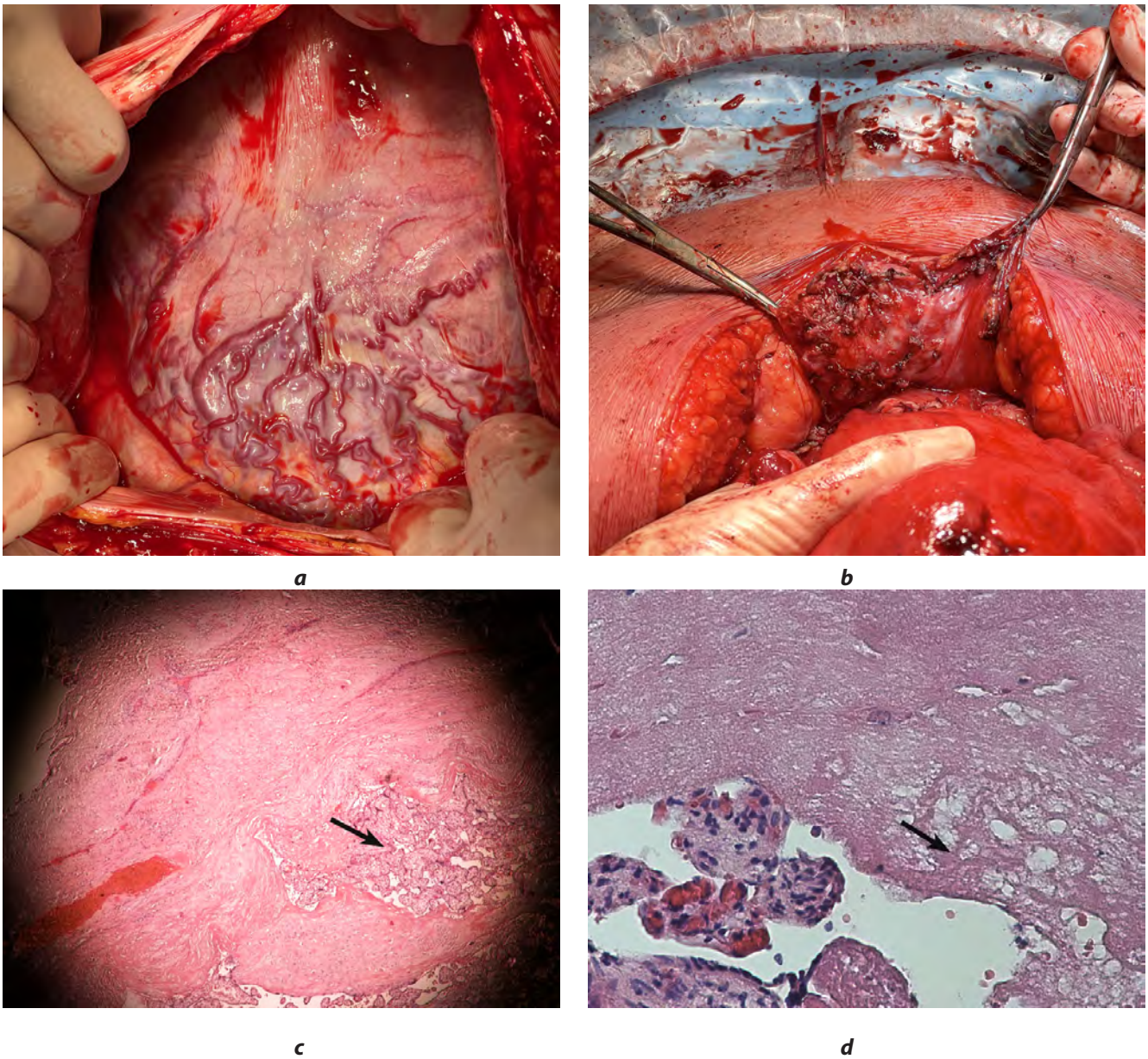
TABLE 1

COMPARABILITY OF COMPARISON GROUPS (MANN – WHITNEY U-TEST, $p < 0.05$)

The studied indicators, Me (P25; P75)	Patient groups		The level of statistical significance, Mann – Whitney U-test ($p < 0.05$)
	Main group (n = 47)	Comparison group (n = 10)	
Age, years	35 (32.5; 36.5)	34 (29; 36)	0.32
BMI, kg/m^2	27.7 (25.5; 30.7)	29.4 (26.8; 31.6)	0.33
Delivery parity, n	2 (1; 2)	1 (1; 2)	0.23
The number of cesarean sections in the anamnesis	2 (2; 3)	3 (2; 4)	0.1

When analyzing the maturity of the villous tree in the comparison group, the villi corresponded to the gestation period with a balance of mature intermediate (20–30 %) and terminal (up to 60–70 %) villi (Fig. 1h). In patients with placental ingrowth, the predominance of mature intermediate villi was noted; the proportion of fully capillarized terminal villi reached 10–20 %, single immature intermediate villi were found in small limited areas.

Trophoblast cells were poorly visualized when stained with hematoxylin and eosin (Fig. 1c–j), however, they were well detected when stained with methylene blue (Fig. 2a–c), including in the area of aseptic necrosis of the uterine wall (Fig. 2d). Trophoblast cells were also intensively stained during immunohistochemical examination with primary antibodies to cytokeratin-7 (Fig. 2f–h). The trophoblast in myometrium was represented both as single cells (Fig. 2h) and as groups of cells



a ***b*** ***c*** ***d***

FIG. 1.

Morphological features in placenta accreta spectrum. ***a*** *– uterine aneurysm with pronounced vascular collaterals on the anterior surface of the uterus (pl. increta).* ***b*** *– coagulated vessels between the posterior wall of the bladder and the anterior wall of the uterus (pl. percreta) (intraoperative picture).* ***c*** *– invasion in the form of "tongues" or "bays"; hematoxylin and eosin staining (marked by arrow), magnification ×20.* ***d*** *– an increased amount of fibrinoid against the background of the absence of decidual cells, contributed to the adhesion of the villus and does not prevent the penetration of invasive trophoblast into the myometrium thickness; hematoxylin and eosin staining, magnification ×100. e – thinning of the uterine wall to the serous membrane (marked by arrow), a lack of a decidual plate and accumulation of fibrinoid deposits; magnification ×20. f – visualization of placental villi in the lumen of blood vessels (marked by arrow); hematoxylin and eosin staining, magnification ×100.*

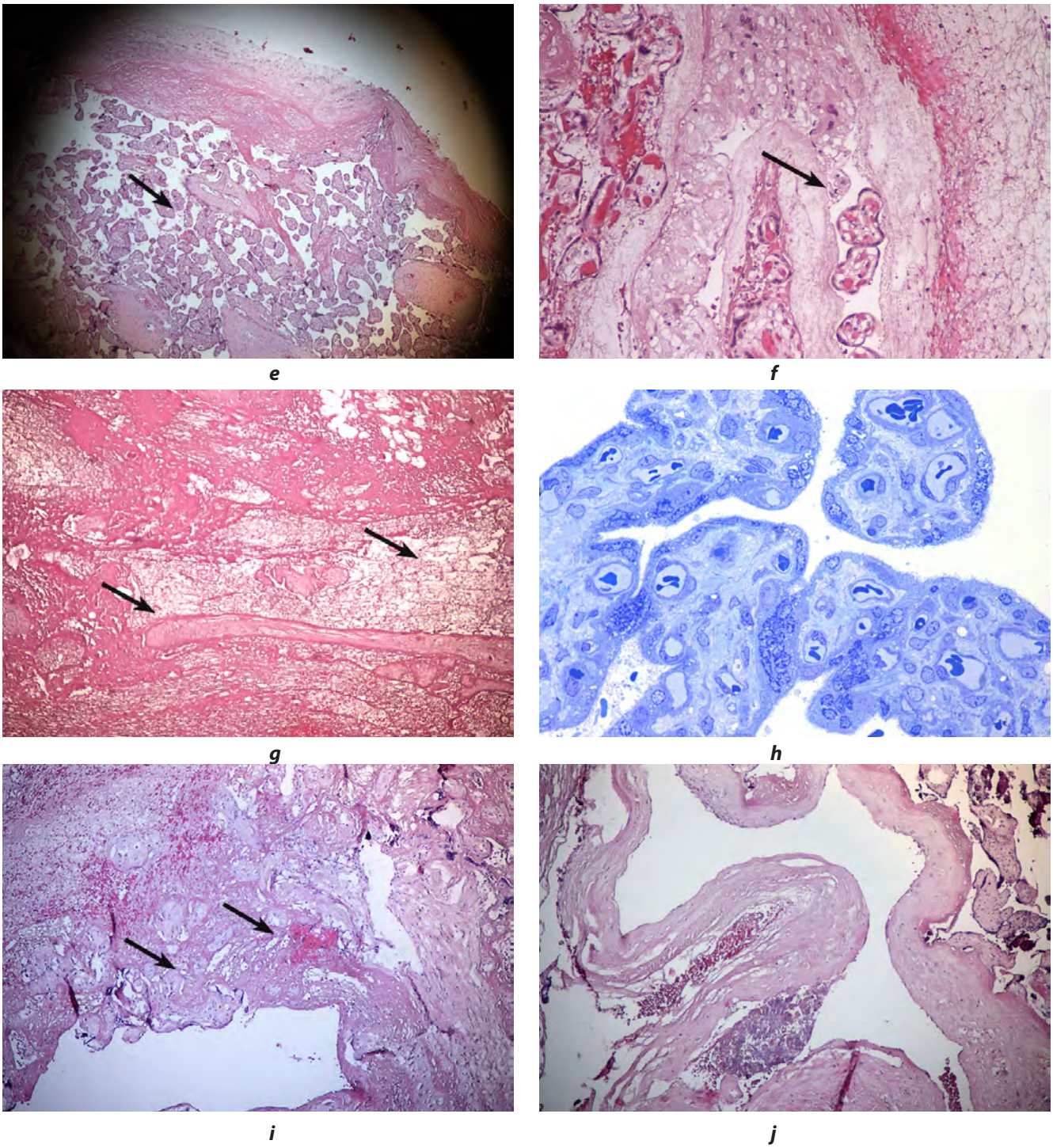


FIG. 1. (continued)

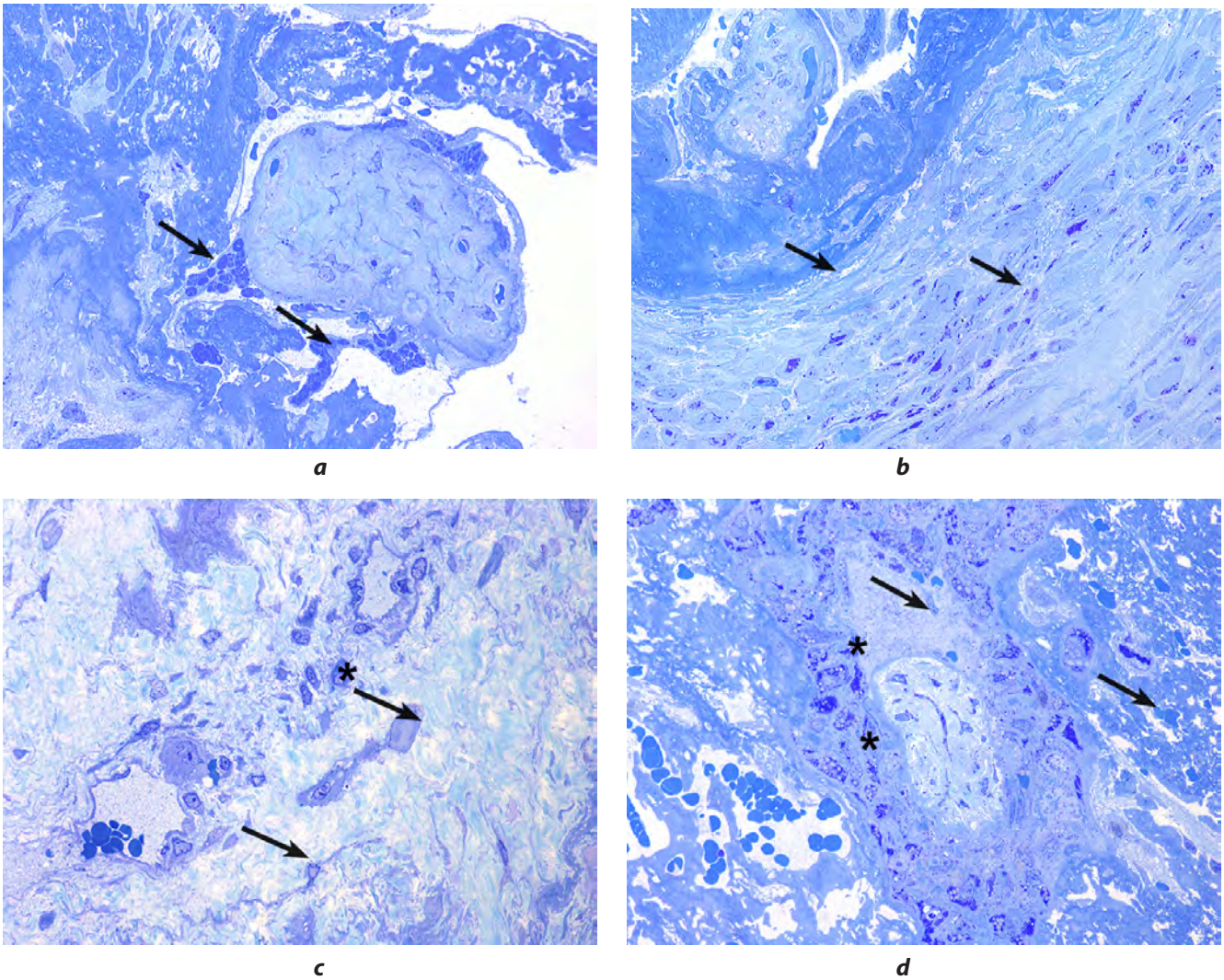
Morphological features in placenta accreta spectrum. ***e*** *– thinning of the uterine wall to the serous membrane (marked by arrow), a lack of a decidual plate and accumulation of fibrinoid deposits; magnification ×20.* ***f*** *– visualization of placental villi in the lumen of blood vessels (marked by arrow); hematoxylin and eosin staining, magnification ×100.* ***g*** *– aseptic necrosis of the myometrium is represented by monomorphic eosinophilic fields with local hemorrhages; there are "ghost villi" with the absence of nuclear basophilia and dystrophic changes; no inflammatory infiltration; aseptic necroses are associated with deep placenta accreta spectrum; hematoxylin and eosin staining, magnification ×100.* ***h*** *– villous tree in pl. increta (syncytiotrophoblast without any signs of damage) in its structure corresponds to the physiological course of pregnancy; methylene blue staining, magnification ×600.* ***i*** *– placental villi ingrowth into the lumen of the vessel; the vessel wall is replaced by fetal fibrinoid (marked by arrow); hematoxylin and eosin staining, magnification ×40.* ***j*** *– vessel in the ingrowth area with a thinned wall; hematoxylin and eosin staining, magnification ×100*

TABLE 2

REVEALING OF MYOMETRIUM ASEPTIC NECROSES DEPENDING ON THE DEPTH OF INVASION

The depth of invasion	The presence of necrosis zones in the myometrium	The proportion of women with necrosis zones in the myometrium of UPA
Healthy with CS	0/10	0 %
Placenta accreta	2/12	17 %
Placenta increta	26/30	87 %
Placenta percreta	5/5	100 %

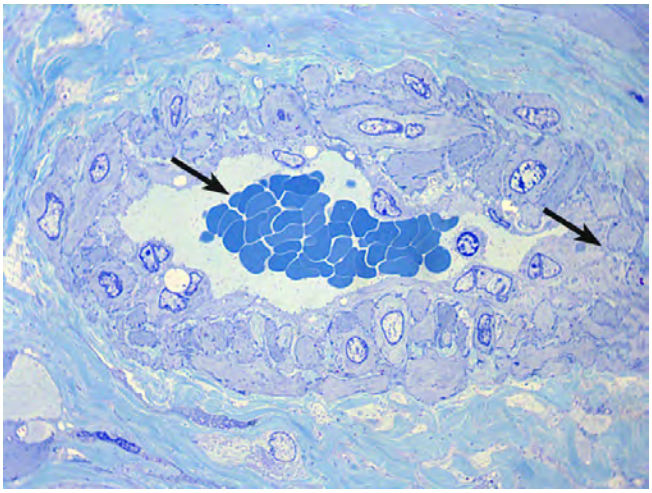
Note. CS – cesarean section; UPA – uteroplacental area.



a ***b*** ***c*** ***d***

FIG. 2.

Morphological features of the placenta and myometrium in placenta accreta spectrum. ***a*** *–invasive trophoblast cells are separated from the villus in the zones of the syncytial sprouts and penetrate into myometrium in the form of a beam (group with placenta accreta spectrum); fetal fibrinoid does not prevent trophoblast invasion (marked with arrows); methylene blue staining, magnification ×1200.* ***b*** *– increased deposits of fetal fibrinoid against the background of the destruction of the decidual plate; fetal fibrinoid does not limit trophoblast invasion (marked with arrows); in the myometrium, the dystrophic changes in the cells are visible, which is confirmed by the presence of pink granules (marked with asterisks); methylene blue staining, magnification ×600.* ***c*** *– abnormal invasion into the myometrium during placenta ingrowth; methylene blue staining (trophoblast cells are marked with arrows), magnification ×400.* ***d*** *– "ghost villus" in the zone of myometrial necrosis (homogeneous bluish masses), trophoblast is visualized around the villus (marked with arrows); magnification ×600.*



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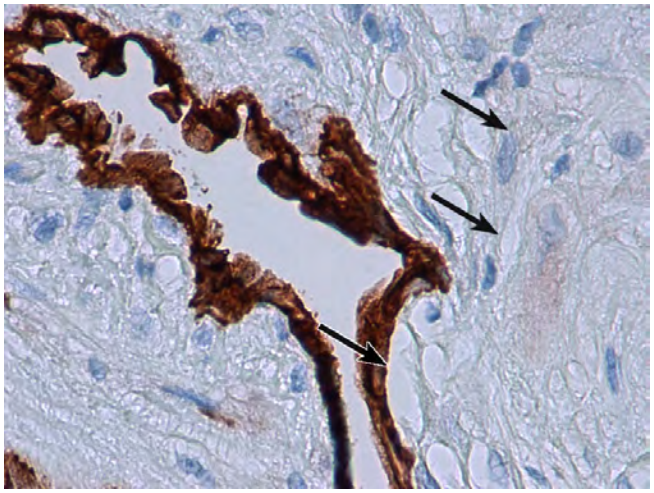

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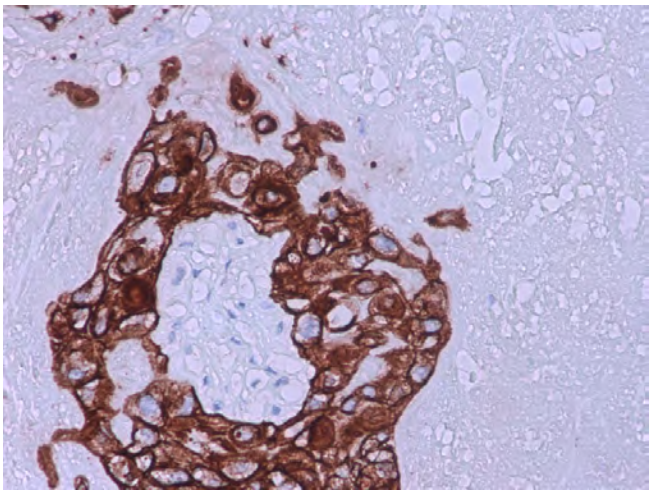

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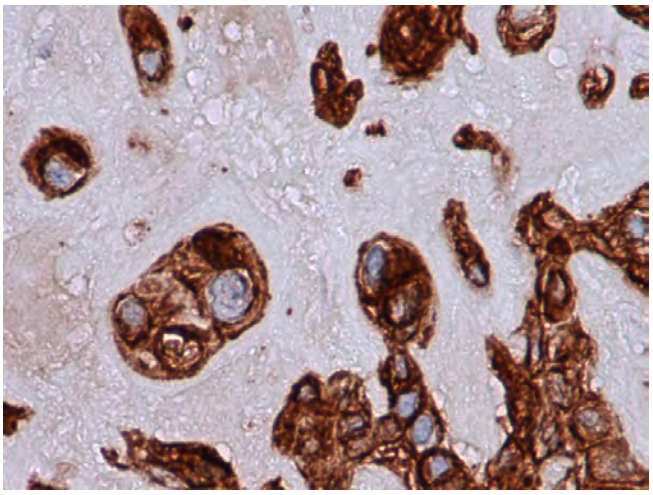

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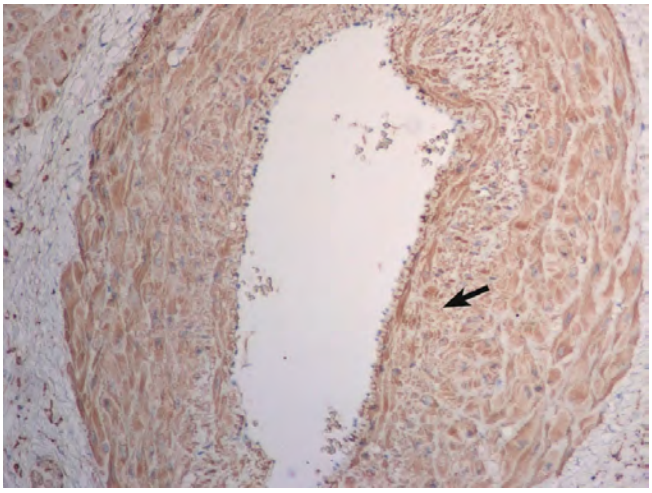

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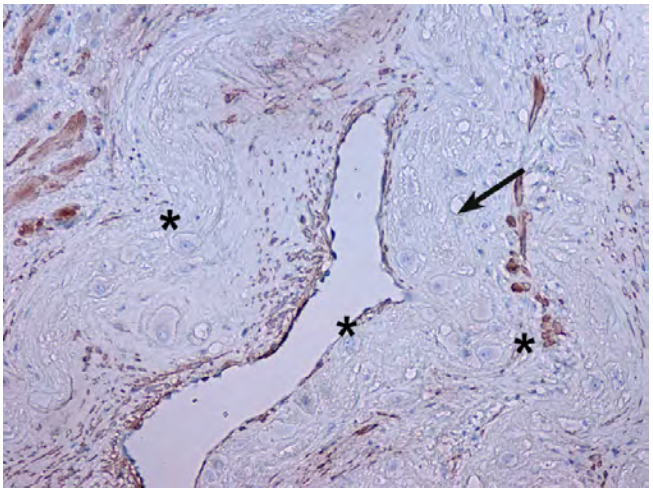

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FIG. 2. (continued)

Morphological features of the placenta and myometrium in placenta accreta spectrum. ***e*** *– replacement of the endothelium of myometrial vessel with trophoblast cells (marked by arrows) during physiological pregnancy; methylene blue staining, magnification ×1000.* ***f*** *– replacement of the vascular endothelium during uncomplicated pregnancy with trophoblast cells; trophoblast cells are stained brown with primary CK7 antibodies (marked by arrows), magnification ×400.* ***g***, ***h*** *– trophoblast cells in the area of myometrium aseptic necrosis are stained with primary CK7 antibodies; magnification ×200 (****g****), ×400 (****h****).* ***i***, ***j*** *– immunohistochemical study with primary α-SMA antibodies, positive staining of the vessel wall outside the area of ingrowth (marked by arrows) (****f****) and negative staining of the vessel wall in the area of the placental bed (****g****) (trophoblast cells are marked by asterisks); magnification ×200 (****i****), ×400 (****j****))*

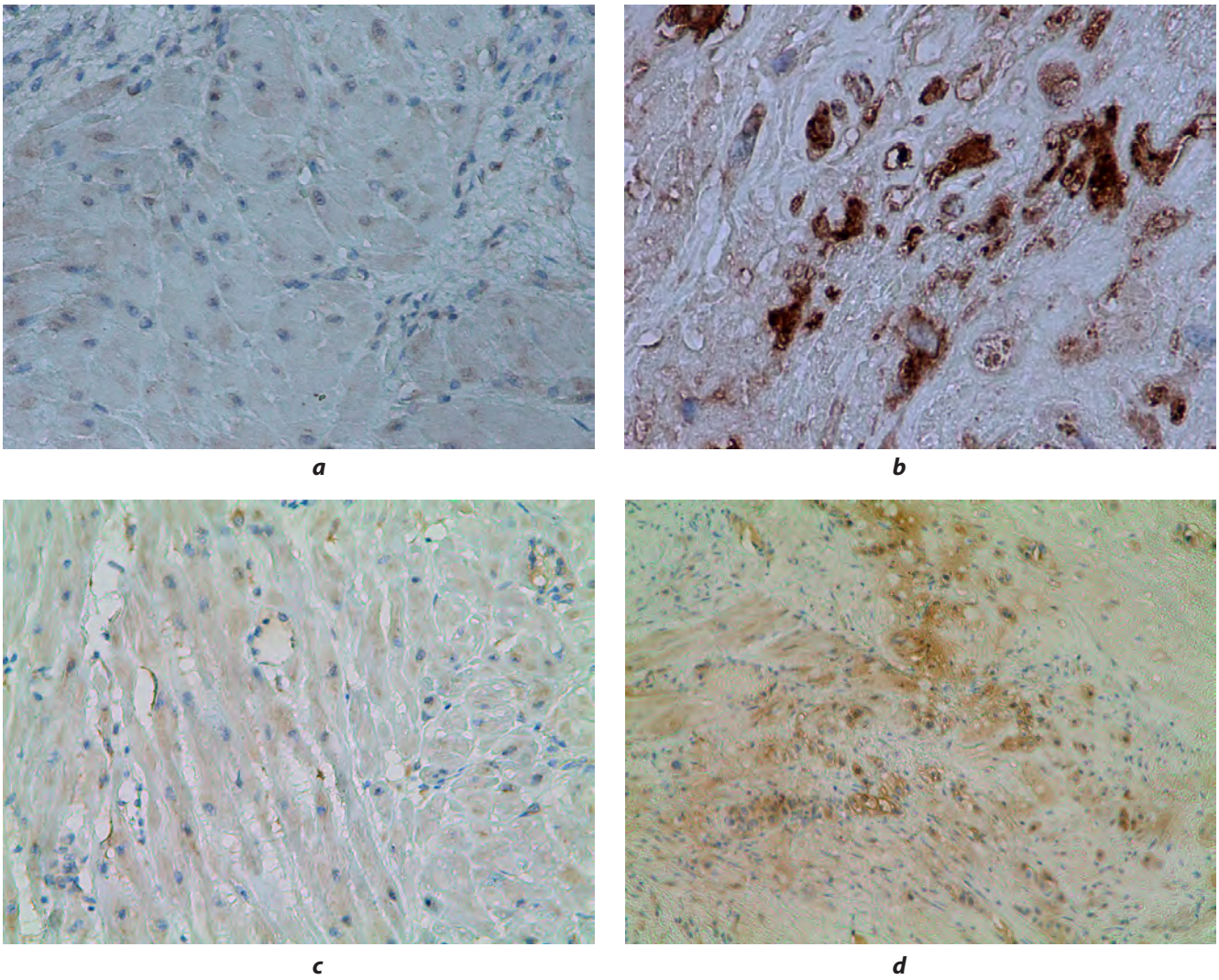

FIG. 3.

*Expression of hypoxia, and angiogenesis markers in placenta accreta spectrum: **a** –immunohistochemical study of myometrium with primary Hif2a antibodies during uncomplicated pregnancy, magnification ×200; **b** – aseptic myometrium necrosis with the presence of stained cells(Hif2a+) of invasive trophoblast, magnification ×400; **c**, **d** – immunohistochemical study with primary VEGF antibodies during uncomplicated pregnancy (a weak staining of the myometrium), moderately stained cells of invasive trophoblast (VEGF+) in the zone of aseptic myometrium necrosis in the area of ingrowth, magnification ×200 (**c**), ×100 (**d**)*

(Fig. 2f–h). If in the comparison group during physiological pregnancy, the transformation of uterine vessels occurred by replacing the endothelium and smooth muscle membrane of the vessel with an invasive trophoblast (Fig. 2e, f), then when the placenta grew, a violation of the topography of trophoblast invasion was observed and trophoblast cells were found not only in the vessels of the myometrium and among muscle fibers, but also in the scar tissue of the myometrium (Fig. 2b, c).

Smooth muscle cells were detected in the wall of non-modeled vessels (Fig. 3i) and were absent in the remodeled vessels of the placental bed area (Fig. 3j).

There were no villi in the myometrium of the uteroplacental region of the comparison group, and the hypoxia index Hif2a was practically not detected histochemically (Fig. 3a), as well as VEGF (Fig. 3c). In women with atypical placentation, there was a marked increase in Hif2a expression in trophoblast-like cells in areas near necrosis zones (Fig. 3b). In addition, an increase in VEGF expression was noted in these areas (Fig.3d)

DISCUSSION

The main factor contributing to abnormal attachment of placental villi and further invasion of the myometrium is considered to be previous uterine surgery, which leads to pathological decidualization in the scar area [6, 9, 10]. In our study, we conducted a comparative characterization of the uterine scar zone in women with normal pregnancy and women with placental villi ingrowth. As in the works of other authors, we have revealed the ingrowth of villi in the area of scar tissue. We found that damage to the uterine wall was accompanied by the formation of necrotic zones, which were absent in cases of scars without ingrowth. Moreover, the proportion of cases

with necrosis zones increased with the depth of ingrowth (Table 2), possibly reflecting the outcome of the process of myocyte damage as a result of abnormal trophoblast invasion with a violation of the architectonics of the uterine wall and vascular network (Fig. 2b, c), which most likely leads to impaired blood supply and ischemia of the adjacent myometrium.

Significantly, during physiological pregnancy, trophoblast invasion is strictly programmed and stops at the level of the spiral arteries, leading to an expansion of their diameter without affecting the radial and arcuate arteries located closer to the surface of the uterus [16–18]. When the placenta grows, trophoblast invasion becomes unregulated and often goes beyond the boundaries of scar tissue, affecting the adjacent layers of the myometrium [19] and the intravascular space, as shown in our study (Fig. 1c–g).

To confirm the hypothesis about the leading role of impaired blood supply to the uterine wall during the ingrowth of placental villi and subsequent ischemia in the appearance of necrotized areas of the myometrium, we studied the content of hypoxia factor Hif2a and vascular endothelial growth factor in the uteroplacental region. Previously, the important role of Hif2a in the first trimester in chorionic invasion was established [20], which is to some extent due to hypoxic conditions. All the women included in our study were in the third trimester of pregnancy, and we did not find data on the content of Hif2a in the uteroplacental region at this time in the literature. We found a more pronounced expression of Hif2a in trophoblast cells in the myometrial necrosis zones of the main group (Fig. 3b) compared with the myometrium of the comparison group (Fig. 3a). The content of VEGF in the myometrium in the main group during this gestation period was higher compared to the comparison group, which was consistent with the findings of other researchers [21, 22]. Surprisingly, a living trophoblast was found in the necrotized zones, actively expressing hypoxia factors, which could probably stimulate an increase in VEGF content in surrounding living tissues and promote enhanced angiogenesis directed to these zones; the presence of fetal fibrinoid in these zones may also contribute to its survival [23, 24] and be a manifestation of one of the programmed types of cell death – programmed necrosis – necroptosis [25].

As a result, necrosis zones with the expression factor of hypoxia Hif2a trophoblast may be one of the main causes of the abnormal vascular network observed in PAS (Fig. 1a, b).

The analysis of the maturity of the villous tree indicated that the maturation period of the villous tree was ahead of 2–3 weeks, given that the delivery period of patients with placenta accreta corresponded to 36–37 weeks [10].

CONCLUSION

Morphological examination of the uteroplacental region in PAS revealed the appearance and increase of myometrial necrosis zones in response to an increase in the depth of ingrowth of placental villi. During normal pregnancy, foci of uterine wall necrosis were absent. Necrosis zones may be the cause of activation of angiogenic factors and an important stimulus for the development of abnormal vascularization in PAS. In view of this, there is an increasing need for careful excision of areas of altered myometrium, especially areas of aseptic necrosis, to ensure the quality of subsequent metroplasty [15].

Conflict of interest

The authors of this article declare the absence of a conflict of interest.

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BIOLOGY AND MEDICAL BIOLOGY

DATA ON THE DISTRIBUTION OF THE *HAEMAPHYSALIS CONCINNA* TICK IN THE IRKUTSK REGION AND THE REPUBLIC OF BURYATIA

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ABSTRACT

The ixodid tick Haemaphysalis concinna (Koch, 1844) is a carrier of pathogens of vector-borne diseases of viral, bacterial and protozoal etiology. This tick was previously considered an adventive relict species in the Baikal region, but has recently shown a tendency to increase its numbers and expand its habitat.

The aim of the study. *To generalize the available and newly received information on the distribution of the H. concinna tick in the Irkutsk region and the Republic of Buryatia; to carry out the comparative analysis of the results in order to identify the dynamics of the development of populations of this species of ixodid ticks in the Baikal region.*

Materials and methods. *The coordinates of H. concinna detection points in our studies were obtained using a GPS navigator directly in the field. Ticks were caught during the period of their maximum activity from plants using a flannel flag. Approximate geographic coordinates of H. concinna tick detection points were established when analyzing the maps published by other researchers.*

Results. *As a result of the generalization of our own data and data from literary sources, 52 georeferenced detection points of H. concinna were obtained on the territory of the Baikal region. A map showing the distribution of H. concinna in the territory of the Irkutsk region and the Republic of Buryatia is presented. It is shown that in a number of surveyed areas there are stable populations of this species of tick, which tend to increase in their numbers and expand their range.*

Conclusions. *Considering these data, as well as the fact that H. concinna ticks are actively involved in the circulation of pathogens of natural focal diseases in humans and animals, we can conclude that it is necessary to systematically monitor the populations of this vector species in the Baikal region.*

Key words: *Haemaphysalis concinna, population, geographical distribution, vector-borne infections, natural foci*

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ДАННЫЕ О РАСПРОСТРАНЕНИИ КЛЕЩА *HAEMAPHYSALIS CONCINNA* НА ТЕРРИТОРИИ ИРКУТСКОЙ ОБЛАСТИ И РЕСПУБЛИКИ БУРЯТИЯ

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РЕЗЮМЕ

Иксодовый клещ Haemaphysalis concinna (Koch, 1844) является переносчиком возбудителей трансмиссивных заболеваний вирусной, бактериальной и протозойной этиологии. Этот клещ, ранее считавшийся в Прибайкалье заносным реликтовым видом, в последнее время демонстрирует тенденцию к увеличению своей численности и расширению территории обитания.

Цель исследования. *Обобщение имеющейся и вновь полученной информации о распространении клеща H. concinna на территории Иркутской области и Республики Бурятия; сравнительный анализ результатов для выявления динамики развития популяций этого вида иксодовых клещей в Байкальском регионе.*

Материалы и методы. *Координаты мест обнаружения H. concinna в наших исследованиях получены с использованием GPS-навигатора непосредственно в полевых условиях. Клещей отлавливали в период их максимальной активности с растительности с помощью фланелевого флага. Приблизительные географические координаты точек обнаружения клещей H. concinna другими исследователями были установлены нами в ходе анализа опубликованных ими карт.*

Результаты. *В результате обобщения собственных данных и данных из литературных источников было получено 52 геопривязанных точки обнаружения H. concinna на территории Байкальского региона. Представлена карта, отражающая распространение H. concinna на территории Иркутской области и Республики Бурятия. Показано, что в ряде обследованных районов присутствуют стабильные популяции клеща этого вида, которые имеют тенденцию к увеличению своей численности и расширению ареала.*

Выводы. *Учитывая эти данные, а также тот факт, что клещи H. concinna активно участвуют в циркуляции возбудителей природно-очаговых заболеваний человека и животных, можно сделать вывод о необходимости систематических наблюдений за популяциями этого вида переносчика на территории Байкальского региона.*

Ключевые слова: *Haemaphysalis concinna, популяция, географическое распространение, трансмиссивные инфекции, природные очаги*

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INTRODUCTION

The ixodid tick *Haemaphysalis concinna* (Koch, 1844) (class Acari, family Ixodidae, genus *Haemaphysalis* Koch.) is the type species for the genus *Haemaphysalis*, is an ancient relict species that evolved in the humid and warm climate of the Tertiary period. Currently, these ticks have survived only in those biotopes where the combination of hydrothermal factor is favourable for their existence [1]. It is the northernmost representative of the ticks of the genus *Haemaphysalis*, the majority of species of which are inhabited in the humid tropics and subtropics. It colonises deciduous and mixed forests in the warm temperate climate zone of Eurasia, especially humid habitats such as the shores of lakes and rivers. *H. concinna* is an exophilic tick with three hosts, uses a pasture type of lurking. During the warm season, ticks in various developmental phases may be active simultaneously on vegetation and feeders. The development period of one generation is 3–5 years [2–4]. Among the hosts of *H. concinna*, 60 species of wild mammals and 77 species of birds have been recorded. The species composition of the hosts that supply blood to ticks is different in each region. The greatest species diversity of *H. concinna* hosts has been found in the Russia's Far East. These ticks also frequently feed on livestock as well as domestic animals [1].

The duration of the *H. concinna* activity season largely depends on the region of their habitat. Thus, in the south of the Russia's Far East, in the foothills of the Crimea and the Caucasus, the adults are active from March-April to September-October. The peak of their activity is observed in June-July. In the foothills of the Altai and Sayan mountains, adult ticks appear in late April, but disappear as early as mid-August. The maximum of tick activity in this area can be observed at the end of May – beginning of June. The maximum number of larvae and nymphs is found everywhere at approximately the same time – in June and July [1].

H. concinna is a reliably confirmed vector of various tick-borne pathogens that cause human and animal diseases, and is endemic to vast territories of Europe and Asia [5].

In a study by F. Rubel et al. (2018) an analysis of scientific literature data was undertaken, as a result of which 656 geo-linked locations of *H. concinna* in Eurasia were obtained, modern maps reflecting the geographical distribution and climatic adaptation of this tick species were presented. According to the authors, the distribution area of *H. concinna* extends from the Spanish Atlantic coast in the west to the Russian Kamchatka in the east and is divided into a large number of clusters or individual habitats. *H. concinna* is the second most common species of ticks taken from birds, after *Ixodes ricinus* and the third most common species of ticks collected from vegetation in Central Europe [5].

The south-to-north distribution of *H. concinna* ranges from about 28–64° North latitude. The northern limit of *H. concinna* distribution can be reasonably determined by the southern regions of the Republic of Yakutia, where the tick can be observed up to 63.8° north latitude [6]. In Central China, the tick *H. concinna* was predominantly observed up to 28° north latitude. However, the three locations in China south of 28° north latitude, including the southernmost distribution at 21.93° north latitude/101.29° east longitude, described by R.-X. Sun et al. (2017) [7], should be interpreted with caution. These data have not been confirmed by other publications. The larvae and nymphs of *H. concinna* are also being known to be migrated long distances by birds, and described tick findings have been found in the path of East Asian/Australasian migratory birds [5]. The same may be also true for the finding of ticks of this species far to the north of the Russian Federation in the area of 87.71° east longitude/68.06° north latitude on the Taimyr Peninsula and in the vicinity of Yakutsk, where ticks are probably carried in birds [1]. In connection with these findings, F. Rubel et al. (2018) considered the above-mentioned locations as outliers and not as part of the distribution range of *H. concinna* [5].

In the European part of Eurasia, this type of tick has been found in Spain, France, Germany, Austria, Hungary, the Czech Republic, Slovakia, Italy, Croatia, Bosnia and Herzegovina, Serbia, Poland, Romania, Greece, Turkey, Iran. In eastern Eurasia, *H. concinna* has been found in China, Mongolia, Vietnam, Japan, North Korea, and South Korea [1, 5]. A significant part of the *H. concinna* range can be found in territories that were formerly belonged to the Soviet Union. In the former USSR republics, *H. concinna* was found in Belarus, Moldavia, Georgia, Armenia, Azerbaijan, Kazakhstan, Kyrgyzstan, Turkmenistan, and Uzbekistan [1, 3].

The most extensive habitats of *H. concinna* in Russia (formerly in the Soviet Union) are usually located in the foothills of large mountain systems in the south of the country (Caucasus, Pamir and Tien Shan, Altai and Sayan Mountains) and in the Russian Far East. In the European part of Russia, *H. concinna* inhabits the Crimean region, Krasnodar and Stavropol territories, as well as the northern lowland areas of the Chechen Republic and the Republic of Dagestan, as well as the Republic of Ingushetia. The only finding of a tick of this species in the northern part of Rostov region was most likely due to being carried by birds. In the south of the Urals, the tick *H. concinna* can be found in a strip along the valley of the Ural River in the Orenburg and Chelyabinsk Regions and the Republic of Bashkortostan [1].

The largest parts of the *H. concinna* range are located in the Asian part of the Russian Federation. Extensive habitats are found in the Omsk, Novosibirsk, Tomsk regions, in the foothills of the Altai Mountains (Kemerovo Region, Altai and Krasnoyarsk Territories) [1, 8, 9] and Sayan Mountains (south of the Irkutsk Region, Republic of Buryatia) [10–13], in Yakutia [6] and in the Russian Far East (Zabaikalsky Territory (formerly – Chita region), Amur region, Khabarovsk and Primorsky Territories) [1, 14]. *H. concinna* tick was delivered to Petropavlovsk-Kamchatsky together with rats (a single larva was found). Ticks of this species are periodically imported to Sakhalin Island with livestock from Primorsky Territory, but they do not adapt to the island [1].

In the Crimean mountains, the habitat altitude of this tick reaches 1200 m, in the Caucasus – 2000 m, in the Tien Shan – 2500 m, in Sikhote–Alin – 700–800 m [1, 2].

The identification of the peculiarities of *H. concinna* distribution has become of scientific interest both from the point of view of studying the evolution and zoogeography of ixodid ticks belonging to the genus *Haemaphysalis*, as well as in view of their possible role in the transmission of some vector-borne diseases. This species is a vector for the transmission of tick-borne encephalitis virus. The natural infection of adult *H. concinna* ticks and their ability to preserve and transmit pathogens of tularemia, brucellosis, listeriosis, North Asian tick-borne typhus, piroplasmosis, Western equine encephalitis, and Japanese encephalitis have been established [1, 5]. There is evidence that *H. concinna* is a vector of *Rickettsia heilongjiangensis*, the causative agent of Far Eastern spotted fever [15, 16]. In addition, nucleic acids of Buran virus (BURV) and murine gamma herpes virus 68 [17, 18], bacteria *Anaplasma* ssp. [19], *Ehrlichia* sp. [19, 20], Candidatus *Neoehrlichia mikurensis*, *Borrelia* sp., *Rickettsia* sp., *Coxiella burnetii* [5], protozoa *Babesia* sp., *Theileria* sp. were isolated from ticks of this species [5, 21]. The problem of the possible role of *H. concinna* in the circulation of the above-mentioned pathogens in a certain area is beyond the reach of a clear understanding about the distribution peculiarities of this species.

THE AIM OF THE STUDY

Comparison of the results of our own long-term observations conducted in the Irkutsk Oblast and the Republic of Buryatia with the data of other authors since the first mention of *H. concinna* finding here to reveal the dynamics of population development of this species of ixodid ticks in the Baikal region of the Russian Federation.

MATERIALS AND METHODS

In order to monitor habitats and further surveys for the existence of pathogens, *H. concinna* ticks were collected from vegetation on the flag. Tick sampling was undertaken from mid-April to the end of June. The coordinates of the tick collection points were determined using the GPSMAP 78s multifunction navigator (Garmin, Taiwan). 21 out of 33 districts of the Irkutsk region and 9 out of 21 districts of the Republic of Buryatia were surveyed.

The map published by F. Rubel et al. was used as a basis for visual graphical presentation of information about the distribution of the *H. concinna* tick in the Baikal region. The points marked on it were digitized and supplemented with the results obtained during our own long-term observations. The authorship of the points of *H. concinna* tick findings previously indicated on the map was determined using text and graphical data published by N.N. Lebedeva et al. (1981) [1], G.A. Danchinova et al. (2012) [22]. In selecting tick collection sites, we relied on previously published data of our colleagues from the Irkutsk Research Institute of Epidemiology and Microbiology [10, 22, 23] and the Irkutsk Research Anti-Plague Institute of Siberia and the Russia's Far East [11, 12].

RESULTS AND DISCUSSION

One of the cluster regions where a concentration of *H. concinna* habitats is observed is the Baikal region, a mountainous area in the south of Eastern Siberia, adjacent to Lake Baikal from the west and east in the Irkutsk region and the Republic of Buryatia.

At the first stage of our study, we analysed data published in the scientific literature concerning the distribution of *H. concinna* in the Baikal region from the time of the first findings to the beginning of our research, which allowed us to identify points for monitoring. So far, only a few maps describing the distribution of this tick species in Eurasia have been published [1, 2, 14]. In the study by F. Rubel et al. (2018), all previously obtained data were summarised, analysed using a digital map of the world according to the Köppen – Geiger climate classification and presented in the form of modern maps reflecting the geographical distribution and climatic adaptation of *H. concinna* in Eurasia. One of these maps we have taken as a basis for a visual geographical presentation of the material. Table 1 presents the data that have been collected by the authors of the article since 1990 to the present and data previously published by other authors.

Until the end of the 70s of the twentieth century, only two isolated finds of *H. concinna* ticks were recorded in the Baikal region. This species was considered to be a non-native species and only occasionally found on vegetation and feeders [4]. The first report of finding *N. concinna* near Irkutsk dates back to 1950. [24]. Later, the nymph of *H. concinna* was met on a grouse in Usolsky district [25]. This fact casts doubt on the assumption that *H. concinna* was brought to the Baikal region by birds, since the grouse is not a migratory bird [23]. Since the late 1970s *N. concinna* has been consistently found on the territory of Usolskiy District in an average of one to five specimens, which also excludes bringing it by birds as a cause of its presence here. In June 1983, 57 individuals of this species were collected in this area, and its abundance in one of the sites amounted to 38 specimens per 1 flag/km. The tick was found on the above-floodplain terraces in birch and mixed mixed-grass forests on the gentle slopes of hills in cuttings. But more often than in other places, it was found on the sides of old logging roads [4, 23]. The existence of stable local populations of this tick has been shown in the lower reaches of the Belaya River, in the valley of the Haita River, in the vicinity of the Aransahoy settlement. In 2008, we collected 5 specimens of *H. concinna* ticks per flag in the vicinity of this settlement. During the 2019–2022 monitoring, a survey of the vicinity of Aransakhoy village and the floodplain of the Haita river was undertaken. It should be noted that tick collection points and route length were similar in these years. The number of *H. concinna* ticks captured from vegetation per flag is shown in Table 1. 88 specimens

were collected in 2019, 108 specimens in 2020, 141 specimens in 2021, and 167 specimens in 2022.

During monitoring studies in natural foci of tick-borne infections in suburban areas of Irkutsk, single specimens of *H. concinna* were found along the main highways (Table 1). In 1992, we collected 2 specimens (43 km) from vegetation on the road adjacent to the Baikalsky Tract. In 2020, 1 specimen was found on the 53 km of this tract. *H. concinna*. In 2014, 1 specimen was collected on the 7–9 km road to Mel'nichnaya Pad. *H. concinna*. In 2021 and 2022, we found 1 specimen of tick in the vicinity of Dobrolet village (Goloustnensky Tract). In June 2011, a male tick of this species was found on vegetation in the Angarsk region [26].

Since the early 1980s, *H. concinna* has been regularly found in single specimens in other areas of the Baikal region. Thus, in 1970 ticks of this species were observed in the Alarsky district [13], in 1987 – in Bayandaevsky [23], in 2007 – in Nukutsky [22]. 22 years later, we managed to collect 2 specimens of *H. concinna* ticks in the vicinity of the village of Turgenevka in Bayandaevsky district. Tick habitats were observed in the territories of the Ust-Ordynsky Buryat district. The thesis work of O.V. Melnikova (2018) reported the capture of 163 specimens of *H. concinna* on the Kachugsky Tract and the territory of the Ust-Ordynsky Buryat district in the period from 2005 to 2015. In 2008, we captured 51 specimens of *H. concinna* per flag in this area on the side of the road Yelovka – Krasny Yar village; 1 specimen of this tick was collected in 2009, in 1 km from Ust-Orda village. E.A. Vershinin et al. reported the detection of a sustainable tick population of this species inhabiting a swampy area of an abandoned forest road between spruce-green-moss forest and ploughed farmland in the vicinity of the Yelovka village. The number of *H. concinna* in this area reached 14 specimens per flag-hour [27]. Between 2009 and 2012, we collected 169 specimens of *H. concinna* from vegetation per flag in the Ekhirit-Bulagatsky district. *H. concinna*. During the 2013–2022 monitoring period, we captured a total of 388 specimens of *H. concinna*. The ticks were collected from vegetation in a mixed forest, in birch forest outliers, along paths and roads, in shrubby thickets and on the territory of a swampy meadow. We noted a slight decrease in the number of *H. concinna* ticks since 2016, which may be associated with the drying up of bogs, as well as with the relocation of livestock grazing to other pastures. Only 6 specimens of *H. concinna* were possible to collect at these locations in 2021, which was caused by flooding of the monitoring sites due to the extremely snowy winter and abundant rainfall in early spring of this year.

The first mention of *H. concinna* ticks being found in the Cheremkhovsky district dates back to 1970 [13]. We found ticks of this species in the vicinity of Talniki village in 1992 (2 specimens) and near Nizhnyaya Iret village in 2020 (17 specimens). There is evidence in the literature about the presence of *H. concinna* ticks in the Ziminsky, Kachugsky and Slyudyansky districts of the Irkutsk region [22, 27].

The *H. concinna* tick is much less likely to attack humans and is of less epidemiological importance in the transmission of infectious agents than the taiga tick. This is confirmed by the data of the Center for the Diagnosis and Prevention of tick-borne infections of the Scientific Centre for Family Health and Human Reproduction Problems. During the period 2007–2011, only 7 cases of human bites by ticks of this species were registered (4 females and 3 males). The attacks occurred throughout the spring-summer period, from April to August. The geography of *H. concinna* tick bites once again proves their widespread distribution in the Pre-Baikal region: these are the Ekhirit-Bulagatsky district (Gakhany, 2007), the vicinity of Shelekhov (2008) and Irkutsk (2008) cities, Kachugsky district (2010), Usolsky district (2011), Oyok and Revyakino villages of Irkutsk district [26]. These data were also considered in preparing a map of the distribution of the *H. concinna* tick in the Irkutsk region.

H. concinna tick in the Republic of Buryatia is characterized by sporadic occurrence and mosaic distribution over the territory. There are references in the literature about single finds of ticks of this species in Barguzinsky, Pribaikalsky [10, 11], Ivolginsky, Selenginsky [4, 12, 14], Kabansky and Tunkinsky districts [4, 10]. We discovered *H. concinna* ticks in 1992 in the vicinity of the Mishikha River (Kabansky district, 1992), the floodplain of the Irkut River (Tunkinsky district, 1992, 2019), and the vicinity of Gusinoozersk (Selenginsky district, 2019). In 2021, we observed ticks of this species on the road leading to Ust-Barguzin village (1 specimen) and on the Holy Nose peninsula (2 specimens) (Barguzin district, 2021).

Within the territory of the Baikal region there are zones of sympatry of ticks of different species, while in forest and taiga landscapes, as a rule, the *I. persulcatus* tick dominates, and in steppe dominates tick of *Dermacentor* genus. These tick species differ significantly both in territorial distribution and in activity peaks, so it is rarely possible to collect representatives of all three genera in one natural habitat at the same time.

During monitoring studies (2018-2022) in the Usolsky, Ekhirit-Bulagatsky and Cheremkhovsky districts, we collected ticks of *I. persulcatus* and *Dermacentor* species along with *H. concinna*. At the same time, *H. concinna* accounted for from 51.4 to 95.4 % of the total collection of captured ticks in the Usolsky district, and from 11.3 to 28.9 % in the Ekhirit-Bulagatsky district. One should note that the number of ticks of different species was compared when they were captured simultaneously at the same monitoring point. In 2020, ticks of three species were simultaneously collected from vegetation in the Cheremkhovsky district in the vicinity of Nizhnyaya Iret village, and *H.* concinna was the most abundant of them (46 %).

According to our observations, in the Usolsky district, we can assume the displacement of the *I. persulcatus* tick by the *H. concinna* tick. While in the 80s only single specimens were found in this area [27], the current percentage of *H. concinna* from the total number of ticks caught here is 51–95 % (Table 2). In the Ekhirit-Bulagatsky district, the abundance of *H. concinna* varies significantly over the years. At the same time, the share of ticks of this species from the total number of ticks in the collection is 11–30 %. These data indicate the presence of stable populations of the *H. concinna* tick in these three areas.

TABLE 1

LOCATIONS OF *HAEMAPHYSALIS CONCINNA* TICKS IN THE SURVEYED AREAS OF IRKUTSK REGION AND THE REPUBLIC OF BURYATIA

Area where H. concinna ticks have been found	Year of collection	Coordinates of the area	Qty. of findings	Ref.
Irkutsk region				
Irkutsk district				
Recreational area of Irkutsk*	1950	–	1	[24]
Surroundings of Irkutsk*	2008	52.292° N, 104.238° E	1	[26]
Mill pad, 7-9 km of road 25N-218	2014	52.1749° N, 104.3145° E	1	
Baikal Tract, 43 km	1992	52.0345° N, 104.6454° E	2	
Bolshaya Rechka village, 53 km of the Baikal Tract	2020	51.957° N, 104.755° E	1	
Goloustnensky Tract, floodplain of the Ushakovka river*	–	52.330° N., 104.810° E	–	[13]
Goloustnensky Tract, Dobrolet village	2021 2022	52.2517° N, 104.8402° E	1 1	
Alexandrovsky Tract, floodplain of the river*	2006	52.887° N, 103.797° E	–	[22]
The surroundings of Ust-Baley* village	2006	52.648° N, 103.984° E	–	[22]
The surroundings of Moskovshchyna village	2022	52.547° N, 104.116° E	2	
Kachugsky Tract, Oyok* village	2011	52.576° N., 104.470° E	1	[26]
Revyakina* village	2011	52.582° N, 104.623° E	1	[26]
Angarsk district*	2011	52.406° N, 103.965° E	1	[26]
Shelekhovsky district, the surroundings of Shelekhov*	2008	52.209° N, 104.133° E	1	[26]
Slyudyansky district				
Surroundings of Slyudyanka* city	2011	51.712° N, 103.583° E	1	[27]
Highway R-258, near the Murino* village	2011	51.465° N, 104.414° E	–	[27]
Usolsky district				
The floodplain of Haita river*	1965	–	1 nymph	[25]
The floodplain of Haita river*	1970	52.646° N, 103.350° E	–	[13]
The floodplain of Haita river, the road to Aransakhoy village	2019 2020 2021 2022	52.694° N, 103.286° E 52.702° N, 103.280° E 52.701° N, 103.276° E 52.694° N, 103.282° E	45 56 79 70	

TABLE 1 (*continued*)

1.7 km southwest of Aransakhoy village, floodplain of Haita river	2019 2020 2021 2022	52.624° N, 103.234° E 52.622° N, 103.235° E 52.622° N, 103.232° E 52.624° N, 103.230° E	43 52 62 97	
Surroundings of Aransakhoy village	2008	52.636° N, 103.221° E	5	
In the lower course of Belaya river*	1987	–	2-57	[23]
In the valley of Haita river*	1987	–		[23]
Alarsky district*	1970	53.209° N, 103.191° E	–	[13]
Nukutsky district*	2007	53.77° N, 103.12° E	–	[22]
Bayandaevsky district				
Highway 25N-013 between Olzony village and Lura village*	1987	52.932° N, 105.370° E	–	[23]
Turgenevka village	2009	53.016° N, 105.656° E	2	
Ekhirit-Bulagatsky district				
Floodplain of Murin river*	2006	52.720° N, 104.875° E	–	[22]
Near Gakhana village*	2007	53.048°N, 104.893° E	1	[26]
3-4 km from Yelovka village on the way to Krasny Yar village (roadside)	2008	52.593° N, 104.835° E 52.585° N, 104.845° E	32	
6-7 km from Yelovka village on the way to Krasny Yar village (roadside)	2008	52.575° N, 104.868° E 52.569° N, 104.880° E	19	
4.5 km from Yelovka village, (mixed forest, birch forest outliers, shrubby thickets, slightly swampy meadow)	2009–2012 2013 2014 2015 2016 2017 2018 2019 2020 2021 2022	52.584° N, 104.849° E The territory within the coordinates: 52.578°N, 104.877° E 52.568° N, 104.860° E 52.591° N, 104.852° E 52.579° N, 104.832° E	169 84 80 59 21 15 27 21 31 6 44	
1 km from Ust-Orda	2009	52.822° N, 104.810° E	1	
Cheremkhovsky district				
The road to Nizhnyaya Iret village*	1970	52.825° N, 102.496° E	–	[13]
Surroundings of Talniki village	1992	52.784° N, 102.440° E	2	
Surroundings of Nizhnyaya Iret village	2020	52.969° N, 102.501° E	17	
Ziminsky district, the bank of Kimiltey river*	2007	54.185° N, 102.017° E	–	[22]
Kachugsky district				

TABLE 1 (*continued*)

The floodplain of Manzurka river, highway 25N-013*	2006	53.700°N, 105.978° E	–	[22]
The floodplain of Kulenga river*	2006	53.774° N, 105.361° E	–	[22]
The surroundings of Kachug village*	2010	–	1	[26]
The Republic of Buryatia				
Barguzinsky district				
The floodplain of Barguzin river*	1970	53.867° N, 109.953° E	1	[10]
The road to Ust-Barguzin	2021	53.2914° N, 108.8439° E	1	
Svyatoy Nos Peninsula	2021	53.6805° N, 108.9885° E	2	
Pribaikalsky district				
The floodplain of Irkilik river, the R438 road*	1962	52.154° N, 107.753° E	–	[11]
The floodplain of Khaim river*	1970	52.688° N, 108.509° E	–	[10]
Ivolginsky district				
The section along the A-340 highway*	1974	51.687° N, 107.162° E	–	[14]
The coast of Selenga river along the R-258 highway*	2006	51.539° N, 107.346° E	–	[22]
Selenginsky district				
Surroundings of Gusinoozersk	2019	51.234° N, 106.586° E	6	
The floodplain of Selenga river along the A-340 highway*	2006	51.033° N, 106.653° E	–	[22]
Kabansky district				
Mishikha river	1992	51.629° N, 105.539° E	1	
The floodplain of the Bolshaya Kultushnaya river*	1970	51.883° N, 106.135° E	–	[10]
The floodplain of Bolshaya Rechka river*	1970	51.743° N, 106.463° E	–	[10]
The floodplain of Selenga river near Nikolsk village*	1966	52.061° N, 106.864° E	–	[12]
Surroundings of Oymurskoye village*	2006	52.338° N, 106.851° E	–	[22]
Tunkinsky district				
Highway A-333, the floodplain of Irkut river*	1970	51.696° N, 102.041° E	–	[10]
The surroundings of Tunka village, Akhalik river*	1970	51.770° N, 102.604° E	–	[10]
The floodplain of Irkut river	1992	–	27	
The river bank away from the A-333 highway	2019	51.6179° N, 102.7160° E	1	

Note. * – data are obtained from literary sources; "–" – no data available.

CONCLUSION

Thus, in the course of this study we have summarised and substantially supplemented the available data about the distribution of the *H.* concinna tick throughout the territory of the Baikal region. This tick was found in 12 out of 21 surveyed districts of the Irkutsk region and in 6 out of 9 studied districts of the Republic of Buryatia. The results obtained indicate the sporadic occurrence and mosaic distribution of the tick of this species in the territory of the Baikal region. The tendency to expand the range of thermophilic ticks *H. concinna* has been observed in recent decades as a result of favourable changes in the thermal regime. It is increasingly being observed on old logging roads, cuttings, in secondary mixed forests, drying bogs in the Irkutsk region, where it has not been found before.

The existence of stable local populations of *H. concinna* at the territory of Ekhirit-Bulagatsky, Usolsky, and, apparently, Cheremkhovsky districts of the Irkutsk region has been confirmed (Table 2). We can assume, based on our observations of the ixodid tick population in Usolsky district, that in certain natural habitats there is a displacement of the *I. persulcatus* tick by the *H. concinna* tick. In the Irkutsk region, *H. concinna* is regularly found on roads adjacent to major highways, but in single specimen.

Figure 1 shows all the habitats of *H. concinna* in the territory of the Baikal region, which we have succeeded in obtaining so far. The habitat of this tick species can be assumed to be confined to the southern regions of Irkutsk region. The distribution of *H. concinna* has been established in the range from 51.465° to 54.185° N. Among the surveyed areas in which this tick was not observed, all are north of these boundaries. However, this may be explained by the greater remoteness of these territories from Irkutsk city, and hence by their less frequent monitoring. The northernmost areas were not surveyed during this study. Neither did we find in the literature any reports by other authors about findings of this tick in more northern territories of the Irkutsk region.

Several new *H. concinna* tick detection points have been added in the Republic of Buryatia. These are two geo-linked points in the Barguzinsky district and one point each in Kabansky, Selenginsky and Tunka districts (Table 1). The others have been determined by analysing publications of other authors [10, 13, 14] and distribution maps of this tick species that they submitted [1, 5, 22]. Similar to the Irkutsk region, the areas adjacent to Lake Baikal and on the way of the main highways turned out to be more surveyed (Table 1, Fig. 1). The distribution of *H. concinna* has been established in the range from 51.033° to 53.867° N. We can, however, assume that the probability of finding *H. concinna* in the territory of other districts of the Republic of Buryatia that are located to the south of Lake Baikal is quite significant, since this tick has been found in the southern districts of the Trans-Baikal Territory (formerly Chita region) [1, 14] and in the northern districts of Mongolia [4].

Despite the fact that *H. concinna* is not the dominant species of ixodid ticks in the territory of the Baikal region, it can play a certain epidemiological role as a vector of a number of vector-borne diseases. It is important to consider that some natural foci at the territory of the Baikal region are zones of sympatry of ticks of the genera *Ixodes*, *Dermacentor*, *Haemaphysalis*. These closely related ticks may be able to preserve and transmit the same pathogens by feeding on the same feeders, which facilitates a wider spread of pathogens across different biotopes. In turn, the transfer of pathogens of vector-borne diseases from one vector to another through the same reservoir hosts can contribute to their more intense genetic variability and thus accelerate the evolution of pathogens.

The present data collection and analysis can be considered only as a first step towards a more complete determination of the distribution of *H. concinna* and subsequent identification of its involvement in the persistence and transmission of vector-borne pathogens of human and animal diseases in natural foci throughout the territories adjacent to Lake Baikal.

TABLE 2

PERCENTAGE OF TICKS OF DIFFERENT SPECIES IN THREE DISTRICTS OF THE IRKUTSK REGION

District of Irkutsk region	Type of tick	Number of collected specimens / percentage by year				
		2018	2019	2020	2021	2022
Ekhirit-Bulagatsky	*I. persulcatus*	197 / 86.4	69 / 76.7	139 / 81.8	47 / 88.7	108 / 71.1
	H. concinna	27 / **11.8**	21 / **23.3**	31 / **18.2**	6 / **11.3**	44 / **28.9**
	Dermacentor sp.	4 / 1.8	–	–	–	–
Usolsky	*I. persulcatus*	–	6 / 6.4	102 / 48.6	25 / 25.1	8 / 4.6
	H. concinna	–	88 **/ 93.6**	108 / **51.4**	141 / **84.9**	167 / **95.4**
Cheremkhovsky	*I. persulcatus*	–	–	13 / 35.1	–	–
	H. concinna	–	–	17 / **46.0**	–	–
	Dermacentor sp.	–	–	7 / 18.9	–	–

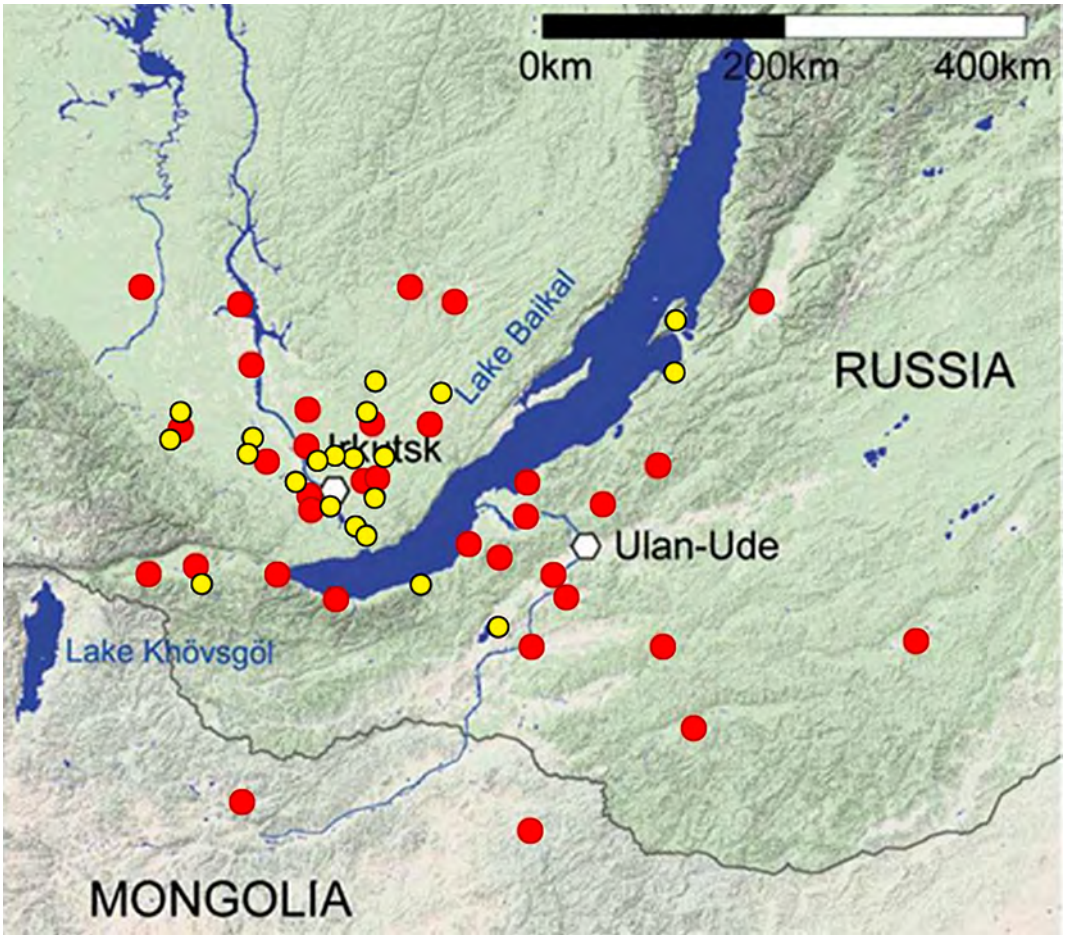

FIG. 1.

Points of H. concinna tick detection in the districts of the Irkutsk region and the Republic of Buryatia: yellow colour indicates the points identified in our study and superimposed on the map published earlier (cited in [5]).]

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Conflict of interest

The authors of this article declare the absence of a conflict of interest.

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GENETICS, PROTEOMICS AND METABOLOMICS

SEQUENCING OF A FRAGMENT OF THE LEPTIN GENE IN ADOLESCENTS WITH DIFFERENT WEIGHT STATUS

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ABSTRACT

Background. *Obesity is a significant social problem among the population of the world. The leptin gene (LEP) is currently considered as a potential candidate gene influencing metabolic disorders associated with predisposition to overweight and obesity. Leptin plays an important role in body weight homeostasis by influencing food intake and energy expenditure and maintaining constant energy stores. A defect in the leptin gene may be one of the causes of obesity and, as a result, of various obesity-associated pathologies.*

The aim of the study. *To search for single-nucleotide polymorphisms (SNP) of the leptin gene in adolescents with different weight status.*

Methods. *The study involved 20 adolescents aged 11–17 years with normal body weight and overweight/obesity. Research methods: assessment of clinical status with anthropometry; Sanger sequencing of the leptin gene fragment localized in the intron of this gene – (5'-AGCCTTGTTTTCATCATCTGGA, 3'-TGGGAGGAATCGCTCTCAGA). We also carried out bioinformatic processing of sequencing results.*

Results. *As a result of the study, the optimal conditions for amplification of the 891 bps leptin gene region were selected for the above mentioned primer pair of the LEP gene (s16_L891, s16_R891). Based on the results of sequencing, 45 single nucleotide substitutions of the LEP gene were identified, including 23 single nucleotide substitutions which were not previously registered in GenBank. In the group of adolescents with overweight and obesity, 14 unregistered single nucleotide substitutions of the LEP gene and 13 registered SNPs were identified in the GenBank database. In the group of adolescents with normal body weight, these SNPs were not found.*

Key words: *leptin gene, sequencing, overweight, obesity, leptin*

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СЕКВЕНИРОВАНИЕ ФРАГМЕНТА ГЕНА ЛЕПТИНА У ПОДРОСТКОВ С РАЗНЫМ СТАТУСОМ ВЕСА

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РЕЗЮМЕ

Ожирение – значимая социальная проблема среди населения всего мира. В настоящее время ген лептина (LEP) рассматривается как потенциальный ген-кандидат, влияющий на метаболические нарушения, ассоциированные с предрасположенностью к избыточной массе тела и ожирению. Лептин играет важную роль в гомеостазе массы тела, влияя на потребление пищи и расход энергии и поддерживая постоянные запасы энергии. Дефект гена лептина может быть одной из причин ожирения и, как следствие, различных патологий, связанных с ожирением.

***Цель исследования**. Поиск однонуклеотидных полиморфизмов (SNP, single-nucleotide polymorphism) гена лептина у подростков с разным статусом веса.*

***Методы.** В исследовании приняли участие 20 подростков 11–17 лет с нормальной массой тела и избыточной массой тела/ожирением. Методы исследования: оценка клинического статуса с антропометрией; секвенирование по методу Сенгера фрагмента гена лептина, локализованного в интроне данного гена – (5'-AGCCTTGTTTTCATCATCTGGA, 3'-TGGGAGGAATCGCTCTCAGA). Также проведена биоинформационная обработка результатов секвенирования.*

***Результаты.** В результате исследования проведён подбор оптимальных условий амплификации участка гена лептина размером 891 п. н. для вышеуказанной пары праймеров гена LEP (s16_L891, s16_R891). По результатам секвенирования идентифицировано 45 однонуклеотидных замен гена LEP, в том числе 23 однонуклеотидные замены ранее не зарегистрированные в GenBank. В группе подростков с избыточной массой тела и ожирением идентифицировано 14 незарегистрированных однонуклеотидных замен гена LEP и 13 зарегистрированных SNP в базе данных GenBank. В группе подростков с нормальной массой тела данные SNP не обнаружены.*

***Ключевые слова:** ген лептина, секвенирование, избыточная масса тела, ожирение, лептин*

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INTRODUCTION

Childhood obesity is a major public health problem. Currently, there is a significant increase in the number of overweight and obese children and adolescents, with a majority of them being obese in adulthood as well [1]. The risk of obesity and its consequences in adulthood is higher if the problem begins in childhood. Obesity is the cause of type 2 diabetes mellitus, non-alcoholic fatty liver disease, cardiovascular and other diseases [2–9].

Body weight regulation is a complex process of neurohumoral regulation involving various neurotransmitters, including leptin. Leptin is a polypeptide hormone secreted by adipose tissue and provides regulation of energy, neuroendocrine and metabolic processes in the body. Leptin has an impact on the processes of energy intake and consumption, fat and carbohydrate metabolism, and the production of hypothalamic and pituitary hormones [10].

The leptin gene (*LEP*) is located on chromosome 7 and consists of three exons separated by two introns [11, 12]. According to the National Center for Biotechnology Information (NCBI), 4,239 single nucleotide substitutions have been described for this gene [13]. Today, 2 polymorphisms (*rs2167270, rs7799039*) are of the greatest interest, the contribution of which to the formation of metabolic disorders in obese patients has been proven [14].

The search for other, including new, polymorphisms of the leptin gene, their contribution to the formation of metabolic disorders in patients of different races, populations, and different age groups is still relevant.

THE AIM OF THE STUDY

To search for single-nucleotide polymorphisms (SNP) of the leptin gene in adolescents with different weight status.

METHODS

The study included 20 adolescents aged 11 to 17 years (14.8 ± 0.45 years), including 14 girls and 6 boys, with different weight status: 12 overweight and obese adolescents (standard deviation score (SDS) of body mass index (BMI) > 1), 8 adolescents with normal body weight (SDS BMI < 1). All teenagers are representatives of European ethnicity (at the example of Russians). The characteristics of the groups are presented in Table 1.

Thus, the groups are comparable in gender and age. The mean values of anthropometric indices are statistically significantly different between groups with different weight status – normal and overweight.

In working with adolescents, we observed the ethical principles required by the World Medical Association Declaration of Helsinki (World Medical Association Declaration of Helsinki, 1964, as revised in 2013; amended at the 64th General Assembly of the World Medical Association, Brazil) and paragraph 5 of Article 24, "Rights of Minors" Fundamentals of Legislation of the Russian Federation about the Protection of Citizens' Health of 22.07.1993 No. 5487-1 (as amended on 20.12.1999). All participants have informed parental consent (if the age of the subject is less than 14 years old) or informed consent of the surveyed sample (if the age of the subject is 14 years or older).

At study inclusion, adolescents had their linear height and body mass (BM) measured, then body mass index (BMI; kg/m^2) was calculated. The trial subjects were weighed with an accuracy of 0.1 kg in standard light clothing and without shoes on a platform hand scale. Height was measured with a stationary height meter with an accuracy of 0.1 cm. BMI was calculated as a person's weight in kg divided by his height in m^2. The height and weight parameters of adolescents were evaluated using WHO reference values using the AnthroPlus calculator [15]. Standard deviation values from population mean values were determined for BMI – SDS was calculated using the Auxology 1.0 b17 computer software application (Pfizer, USA). BW was con-

TABLE 1

CHARACTERISTICS OF STUDIED GROUPS OF ADOLESCENTS

Indicators	Control group (n = 8), Me [Q_1; Q_3]	Overweight and obese group (n = 12), Me [Q_1; Q_3]	p
Age (years)	16.5 [14.75; 17]	14.5 [13.5; 16]	0.97
Height (cm)	167.2 [161.825; 183.1]	158.5 [155.25; 163.125]	0.031*
Weight (kg)	63 [57.95; 66.25]	74.8 [72; 82.975]	0.001*
BMI (kg/m^2)	20.4 [19.625; 21.575]	30.05 [27.80; 33.15]	0.000016*
SDS BMI	0.04 [–0.49; 0.9225]	2.845 [2.335; 3.065]	0.000016*

Note. * – the noted criteria are statistically significant at the level of $p < 0.05$, Mann – Whitney U test was used in the comparison.

sidered excessive at a BMI in the range of 25.0–29.9 kg/m^2, obesity – at a BMI = 30 kg/m^2 [16].

Blood samples were collected into 5 ml vacuum tubes containing K_3-ethylenediaminetetetraacetic acid (K_3EDTA) in order to analyse DNA. DNA isolation was carried out by the sorbent method using the DNA sorb-B kit (Federal Budget Institution of Science Central Research Institute of Epidemiology of Rospotrebnadzor, Russia).

The primers were selected using the Primer-BLAST software [17] (Fig. 1). Oligonucleotides were synthesized in Eurogen CJSC.

One of the primer pairs was matched to an 891 bps fragment of the leptin gene localised in the intronic part of this gene:

1) s16_L891 – 5'-AGCCTTGTTTTCATCATCTGGA (absolute number of nucleotides – 22, molecular weight 6670 (Mw)g/mol);

2) s16_R891 – 3'-TGGGAGGAATCGCTCTCAGA 9 (absolute number of nucleotides – 20, molecular weight 6163 (Mw)g/mol).

Optimal amplification conditions were selected for this pair.

Stock solutions with a concentration of 100 µmol/ml were prepared from lyophilised oligonucleotide samples by dilution with deionised water and further diluted to a final concentration of 20 µmol/ml.

Two approaches were used to select the annealing temperature of the primers. The first one is calculated using the formula: T = 2(AT) + 4(GC) (instructions for the ScreenMix reagent), where T is the annealing temperature of the primers; AT is the number of nucleotides adenine and thymine; GC is the number of nucleotides guanine and cytosine included in the primer. The second – by using the online calculator Thermo Fisher (Thermo Fisher Scientific, USA) [19].

Experimental polymerase chain reaction (PCR) assays were performed in a volume of 10 µl of reaction mixture. The PCR components were mixed in the sequence and volumes shown in Table 2, according to the protocol for the ScreenMix reagent (5X ScreenMix, Eurogen CJSC, Russia).

TABLE 2

SEQUENCE AND VOLUMES OF PCR MIX COMPONENTS APPLIED FOR 1 DNA SAMPLE AND TOTAL PCR MIX VOLUME OF 10 ML

Components	Quantity (µL)
Sterile water	5.2
Screen Mix	2.0
PCR primer 1 (L – left)	0.4
PCR primer 2 (R – right)	0.4
DNA matrix	2.0
Total PCR mixture volume	10.0

PCR was performed using the amplifier DT prime (NPO DNA-Technology LLC, Russia) according to the ScreenMix manufacturer's software program presented in Table 3.

The amplicon fragment sizes for each sample were estimated by electrophoresis in a 1.5 % agarose gel in 1xTAE buffer admixed with ethidium bromide, at an electrical voltage of U = 146 V, over a period of 2 hours.

BigDye™ Terminator v. 3.1 Cycle Sequencing Kit (Thermo Fisher Scientific, USA) reagents were used for sequencing according to the manufacturer's instructions. The reaction products were purified by precipitation with 75 % isopropyl alcohol. The nucleotide sequences were determined using the NANOPHOR 05 automatic sequencer (Syntol LLC, Russia). This study has been performed using the equipment of the Center for the Development of progressive personalized Health Technologies of the Scientific Centre for Family Health and Human Reproduction Problems (Irkutsk).

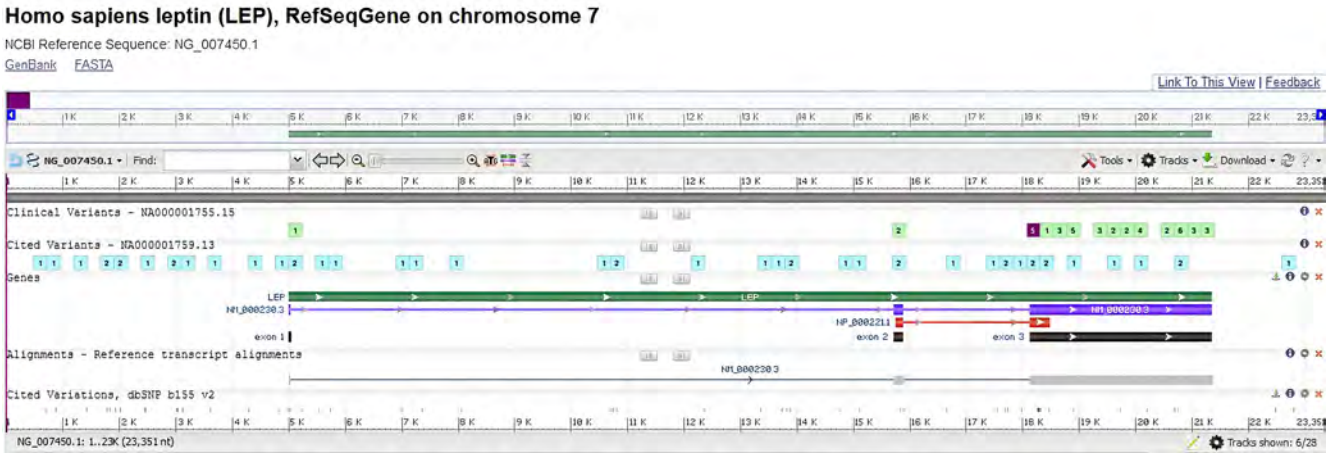

FIG. 1.

Reference Sequence LEP screenshot [18]

TABLE 3
AMPLIFICATION PROGRAM

	Stage	Temperature, °C	Incubation time	Number of cycles
1	Pre-denaturation:	95°	5 min	1
	Denaturation	95°	30 s	
2	Annealing of primers	52–70°	30 s	40
	Elongation	72°	30 s	
3	Storage	4°	–	–

The sequencing results have been bioinformatically processed. The quality of chromatograms was assessed using the UGENE software (Unipro, Russia). The chromatograms were processed using the R v. 4.2.3 software. The obtained nucleotide sequences were aligned to the reference sequence of the *LEP* gene – NG_007450.1 RefSeqGene – and compared with each other in the MEGA 11 software (Pennsylvania State University, USA).

Ethical review

The conduct of this study was approved by the Ethics Committee of the Scientific Centre for Family Health and Human Reproduction Problems (Protocol No. 9 dated October 8, 2014).

RESULTS

At the first stage, the selection of PCR temperature conditions for the analyzed pair of primers (oligonucleotides s16_L891, s16_R891) was carried out. According to the above formula for selecting the annealing temperature of primers, the temperature for both left (s16_L891) and right (s16_R891) primers was 62 °C. A similar calculation using the online calculator Thermo Fisher Scientific (USA) showed an annealing temperature of 63.2 °C. It is known that the annealing temperature gradient should start at a temperature 6–10 °C below the calculated annealing temperature; therefore, three variants of primer annealing temperature have been determined at the first stage of the experiment: 56 °C, 58 °C, and 60 °C.

PCR was performed with 5 DNA samples. The reagents were introduced in the sequence and volumes shown in Table 2. The PCR was performed using the software program shown in Table 3. An electrophoregram with the results of the experiment is shown in Figure 2.

Amplification was satisfactory in all three experimental PCR assays. PCR products with a size of 891 bps are observed in the gel in tracks with DNA amplicons. The annealing of primers at a temperature of 60 °C was the most optimal, because in this mode of amplification, the least amount of non-specific amplification products is observed in the gel.

As a result, the next step in selecting the amplification conditions for the primer pair under study was an experiment in which the number of primers was reduced by 10 % of that indicated in Table 2, the primer annealing temperature was gradiently increased by 2 °C (62 °C annealing) and 4 °C (64 °C annealing) (Fig. 3).

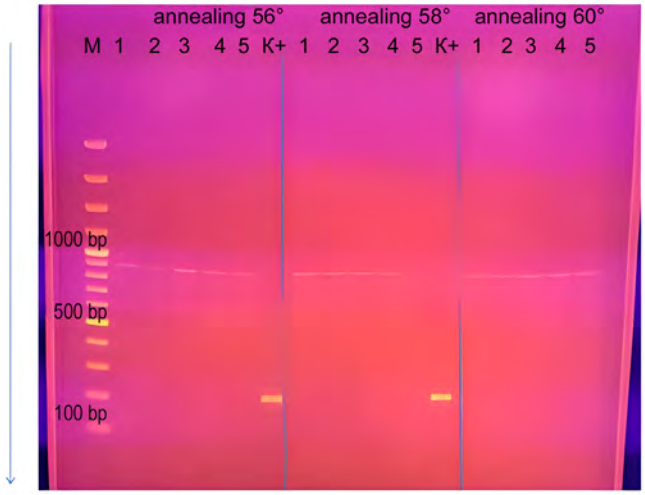

FIG. 2.
Electrophogram: the LEP gene primers – s16_L891, s16_R891; three variants of the temperature shelf for the annealing stage – 56 °C, 58 °C and 60 °C

Analysis of the separation of PCR products shown in Figure 3 showed that a 10 % decrease in the number of primers with primer annealing at all temperature conditions led to the expected result – specific PCR products with a size of 891 bps are present in the in tracks with DNA

amplicons and there are no products of non–specific amplification.

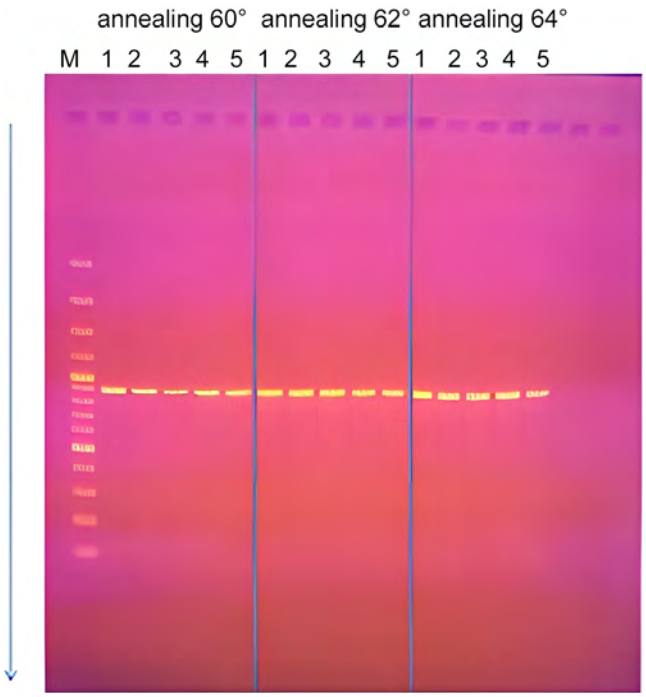

FIG. 3.

Electrophogram: LEP gene primers – s16_L891, s16_R891; the number of primers reduced by 10 %; three variants of the temperature shelf for the annealing stage – 60 °C, 62 °C and 64 °C

Thus, optimal conditions for PCR have been selected for the analyzed fragment of the leptin gene.

The next step of work with the analysed primer pair is amplification of 20 DNA samples using the selected conditions in the volume of reaction mixture equal to 50 µl. Reagents for PCR mixtures were introduced in sequence and volumes, according to Table 4. DNA samples were added at the rate of 10 µl each. The result of PCR is shown in Figure 4.

TABLE 4

SEQUENCE AND VOLUMES OF PCR MIXTURE COMPONENTS APPLIED FOR 20 DNA SAMPLES AND A TOTAL PCR MIXTURE VOLUME EQUAL TO 50 ML

Component	Quantity (µL)
Sterile water	528
Screen Mix	200
PCR primer 1 (L – left)	36
PCR primer 2 (R – right)	36

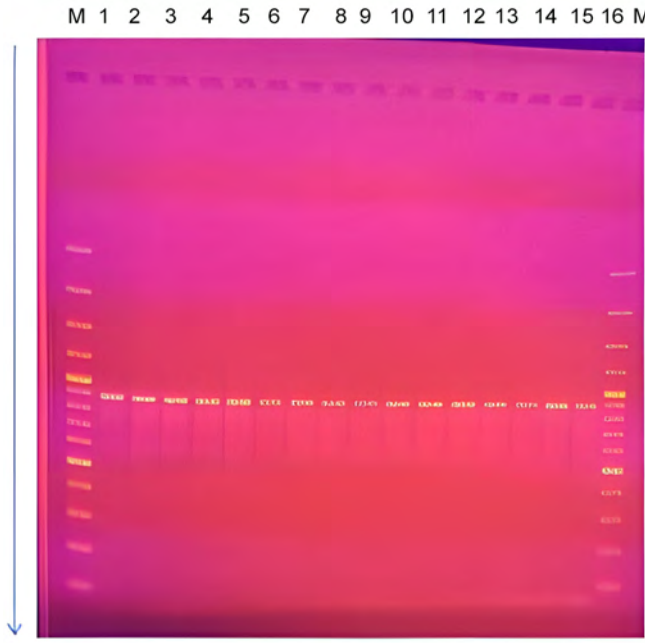

FIG. 4.

Fragment of the electrophogram: LEP gene primers – s16_L891, s16_R891; number of primers reduced by 10 %; primer annealing at 60 °C; DNA samples from 1 to 16

The obtained amplicons were used for sequencing the *LEP* gene using the Sanger method, according to the protocol of the reagent kit manufacturer.

At the next stage, chromatograms were analyzed to search for the SNP leptin *LEP* gene in Caucasian adolescents with different weight status.

We identified 45 SNPs, including 22 SNPs previously reported and 23 SNPs not previously reported in GenBank (01.02.2022) (Table 5), based on the results of aligning the obtained data to the reference sequence of the leptin gene from the NCBI database (NG_007450.1).

Therefore, 14 SNPs of the leptin gene not registered in the GenBank database were found in the group of overweight and obese adolescents.

Moreover, according to the results of sequencing primers s16_L891 and s16_R891 of the *LEP* gene, we identified 12 new nucleotide substitutions in 22 previously registered SNPs (Table 6).

An application has been submitted for registration in GenBank of 23 leptin gene polymorphisms newly diagnosed in the world.

SUMMARY

Sequencing of the *LEP* gene fragment for a pair of primers (s16_L891, s16_R891) revealed both new and previously described single nucleotide substitutions. A total of 45 single nucleotide substitutions were identified in the study groups. SNPs previously registered in GenBank

TABLE 5

LIST OF SNPS OF THE LEPTIN GENE NEWLY DIAGNOSED (NOT REGISTERED IN GENBANK) IN ADOLESCENTS WITH DIFFERENT WEIGHT STATUSES

Seq No.	Position in the gene	Identified nucleotide substitution	The number of adolescents with SDS BMI < 1 (abs.)	Number of adolescents with SDS BMI > 1 (abs.)
1	Ch 7:128254003	G>C,T	2	1
2	Ch 7:128254031	G>T	3	0
3	Ch 7:128254086	A>T,G	3	0
4	Ch 7:128254131	A>T	0	3
5	Ch 7:128254135	G>T	1	3
6	Ch 7:128254139	C>T	0	3
7	Ch 7:128254145	A>T	0	2
8	Ch 7:128254146	G>T,C	2	2
9	Ch 7:128253548	C>T	0	1
10	Ch 7:128253560	A>T	0	1
11	Ch 7:128253561	A>T	0	1
12	Ch 7:128253565	T>C	0	1
13	Ch 7:128253877	G>A	0	1
14	Ch 7:128254051	G>A	1	0
15	Ch 7:128254064	G>C	1	0
16	Ch 7:128254070	C>T	0	1
17	Ch 7:128254083	G>C	1	0
18	Ch 7:128254084	G>A	0	1
19	Ch 7:128254089	G>T	0	1
20	Ch 7:128254094	C>G	1	0
21	Ch 7:128254121	C>T	0	1
22	Ch 7:128254133	C>T	0	1
23	Ch 7:128254138	G>T	0	1

TABLE 6

NUCLEOTIDE SUBSTITUTIONS IN IDENTIFIED LEP GENE POLYMORPHISMS IN ADOLESCENTS WITH DIFFERENT WEIGHT STATUS

Seq No.	Single nucleotide polymorphism	GenBank-registered nucleotide substitution	Identified nucleotide substitution
1	rs1795301406	G>A	G>C
2	rs1795303962	G>A	G>T
3	rs1795304377	A>C	A>T
4	rs1795306221	G>A	G> T
5	rs1795306653	G>T	G> T
6	rs1033530971	C>T	C>A
7	rs1795307023	A>G	A>C
8	rs1010492815	A>G	A>T
9	rs188857788	C>A	C>G
10	rs1795307445	G>A	G>C,T
11	rs1795307524	A>G	A>T
12	rs1430150874	G>A	G>T

were identified in obese adolescents: rs917105894 G>T, rs1795301406 G>A, rs1795303962 G>A, rs1795304377 A>C, rs1795304438 G>T, rs1318987243 G>T, rs3793162 G>A,C,T, rs1795306653 G>T, rs1795307023 A>G, rs1010492815 A>G, rs1795307445 G>A, rs1795307524 A>G, rs1430150874 G>A. SNPs not previously registered with GenBank were identified: Ch 7:128254131, Ch 7:128254139, Ch 7:128254145, Ch 7:128253548, Ch 7:128253560, Ch 7:128253561, Ch 7:128253565, Ch 7:128253877, Ch 7:128254070, Ch 7:128254084, Ch 7:128254089, Ch 7:128254121, Ch 7:128254133, Ch 7:128254138. This study should be continued to evaluate the contribution of these SNPs to metabolic disorders in overweight and obese adolescents by expanding the study cohorts of adolescents and further analysing clinical and metabolic parameters in these study groups.

CONCLUSIONS

Optimal conditions for PCR for a pair of primers (5-AGCCTTGTTCATCATCTGGA, 3-TGGGAGGAATCGCTCTCAGA) of the leptin gene: primer concentration – 0.18 μM; primer annealing at a temperature of 60 °C. Based on the results of *LEP* gene sequencing, 12 new nucleotide substitutions were identified in the SNPs previously registered in GenBank. In the analysed intron fragment of the leptin gene (5-AGCCTTGTTCATCATCATCTGGA, 3-TGGGAGGAATCGCTCTCTCTCAGA), 23 SNPs were identified for the first time.

Conflict of interest

The authors declare no conflict of interest.

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INFECTIOUS DISEASES

FEATURES OF PERIPHERAL BLOOD CELLULAR IMMUNITY PARAMETERS IN PATIENTS WITH LUNG DAMAGE UP TO 30 % IN COVID-19

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ABSTRACT

Background. *The stability of human organism for different kind of infection, including SARS-CoV-2 is significantly defined by the immune system. The mechanisms of the cellular immunity to the SARS-CoV-2 are not exactly defined and are under study.*

The aim. *To study the features of cell immunity parameters in patients with lung damage up to 30 % in COVID-19.*

Material and methods. *73 people were examined during the 2020–2021 pandemic. The study group consisted of 31 patients with lung damage up to 30 % with COVID-19, the comparison group consisted of 42 people not infected with SARS-CoV-2. A complete clinical blood count was carried out using a Medonic M20 hematological analyzer (Boule Medical, Sweden), the level of lymphocyte subpopulations was determined using a FACS Calibur cytometer (BD, USA) and FITC- and phycoerythrin-labeled monoclonal antibodies (Sorbent, Russia). Differences were considered statistically significant at $p < 0.05$.*

Results. *Patients with COVID-19 with lung damage according to computed tomography (CT)≤ 30 % before the treatment had a restructuring in the ratio of lymphocyte subpopulations in 67.7 % of cases. Lymphopenia ($< 1.1 \times 10^9$ cells/L) was detected in 34.4 % of patients: a decrease in the absolute count of $CD3^+$ lymphocytes by 30.8 %, $CD3^+CD4^+$ – by 35 %, $CD3^+CD8^+$ – by 6.7 % ($p < 0.05$), $CD16^+CD56^+$ natural killer (NK) cells – by 29.4 % ($p = 0.009$). The level of $CD95^+$ lymphocytes in COVID-19 is 3.2 times higher than in healthy individuals. Elevated levels of $HLA\text{-}DR^+$ (> 20 %) and $CD3^+$ $HLA\text{-}DR^+$ lymphocytes (> 6 %) are recorded in 60 % and 86.7 % of patients, respectively. Elevated levels of $CD19^+$ B lymphocytes (> 17 %) in COVID-19 are 2.6 times more common than in healthy individuals. Correlation dependences of the count of NK cells with a wide range of T lymphocyte subpopulations were revealed.*

Conclusion. *Cellular immunity indicators in COVID-19 have a number of features that can serve as predictors of the progression of the severity of the disease.*

Key words: *COVID-19, new coronavirus infection, immune state, cellular immunity, lymphocytes, SARS-CoV-2*

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ОСОБЕННОСТИ ПОКАЗАТЕЛЕЙ КЛЕТОЧНОГО ИММУНИТЕТА ПЕРИФЕРИЧЕСКОЙ КРОВИ У ПАЦИЕНТОВ С ПОРАЖЕНИЕМ ЛЁГКИХ ДО 30 % ПРИ COVID-19

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РЕЗЮМЕ

Обоснование. *Успех противодействия организма человека инфекциям в значительной мере зависит от иммунной системы. Механизмы реагирования клеточного иммунитета на вирус SARS-CoV-2 ещё точно не определены и изучаются.*

Цель работы. *Исследование особенностей показателей клеточного звена иммунитета у пациентов с поражением лёгких до 30 % при COVID-19.*

Материал и методы. *Обследовано 73 человека в период пандемии 2020–2021 гг. Группу изучения составил 31 пациент с поражением лёгких до 30 % при COVID-19, группу сравнения – 42 человека, не инфицированных SARS-CoV-2. Общий клинический анализ крови проводили с использованием гематологического анализатора Medonic M20 (Boule Medical, Швеция), уровень субпопуляций лимфоцитов определяли с использованием цитометра FACS Calibur (BD, США) и меченных ФИТЦ и фикоэритрином моноклональных антител (Сорбент, Россия). Различия считались статистически значимыми при $p < 0,05$.*

Результаты. *У пациентов с COVID-19 при поражении лёгких по данным компьютерной томографии (КТ) ≤ 30 % до начала лечения отмечается перестройка в соотношении субпопуляций лимфоцитов в 67,7 % случаев. Лимфопения (< $1,1 \times 10^9$ кл./л) выявлена у 34,4 % пациентов: снижение абсолютного содержания $CD3^+$-лимфоцитов – на 30,8 %, $CD3^+CD4^+$ – на 35 %, $CD3^+CD8^+$ – на 6,7 % ($p < 0,05$), $CD16^+CD56^+$ натуральных киллеров (НК) – на 29,4 % ($p = 0,009$). Уровень $CD95^+$-лимфоцитов при COVID-19 в 3,2 раза выше, чем у здоровых лиц. Повышенные уровни $HLA\text{-}DR^+$- (> 20 %) и $CD3^+$ $HLA\text{-}DR^+$-лимфоцитов (> 6 %) регистрируются у 60 % и 86,7 % пациентов соответственно. Повышенный уровень $CD19^+$ В-лимфоцитов (> 17 %) при COVID-19 бывает в 2,6 раза чаще, чем у здоровых лиц. Выявлены корреляционные зависимости содержания НК-клеток с широким спектром субпопуляций Т-лимфоцитов.*

Заключение. *Показатели клеточного иммунитета при COVID-19 имеют ряд характерных особенностей, которые могут служить предиктором прогрессирования тяжести заболевания.*

Ключевые слова: *COVID-19, новая коронавирусная инфекция, иммунный статус, клеточный иммунитет, лимфоциты, SARS-CoV-2*

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OBJECTIVES

Since the beginning of the COVID-19 pandemic, it has been observed that the infection manifests in humans in a variety of ways, ranging from simple infection without clinical manifestations to severe conditions with multi-organ damage. Progressive lung damage was most often the cause of death in patients. Persons over 65 years of age and those with chronic diseases are most susceptible to the severe course [1]. Lymphocytes and their subpopulation structure play an important role in antiviral immune protection [2]. Viral infections lead to changes in the number and activity of the main subpopulations of lymphocytes (T and B lymphocytes) and natural killer (NK) cells involved in humoral and cytotoxic antiviral immune response [3, 4]. Studies conducted during 2020 have shown that SARS-CoV-2 has a unique pathological effect on the immune system compared to other coronaviruses [5, 6]. Typical characteristics of SARS-CoV-2 infection are a dramatic decrease in lymphocyte counts, changes in the ratios of T lymphocyte subpopulations, including $CD4^+$ and $CD8^+$ T lymphocytes [7]. The severity of changes in the T cell component of immune system determines the severity of the disease [2]. An important point to note is that the success of human resistance to SARS-CoV-2 infection, like the success of vaccination, depends to a large extent on the initial state of the immune system [8]. Studying the development of the macro-organism immune system response to SARS-CoV-2 infection is important for understanding the pathogenesis of the disease, as well as for developing therapeutic strategies and preventing the development of severe conditions caused by COVID-19.

THE AIM OF THE STUDY

To study the features of cell immunity parameters in patients with lung damage up to 30 % in COVID-19.

MATERIALS AND METHODS

Study design. We analysed the results of 73 patients during the 2020–2021 pandemic, including 31 patients on the 5th–7th day from the first signs of infection with SARS-CoV-2 virus with up to 30 % lung damage (study group) and 42 patients not infected with SARS-CoV-2 (comparison group). Study participants underwent an examination including assessment of patients' history, complaints, blood tests, computed tomography (CT) of the chest organs, and polymerase chain reaction (PCR) testing for SARS-CoV-2 infection. The survey data were recorded in a standardised questionnaire.

Compliance criteria. Patients in the study group had positive PCR test results for SARS-CoV-2, lung changes on CT ≤ 30 %, blood oxygen saturation > 95 %, and were not taking antibacterial or hormonal drugs at the time of admission to the hospital. The comparison group had an appropriate gender and age structure and included practically healthy individuals with negative levels of antibodies to SARS-CoV-2 virus at the time of medical surveillance.

Procedure situation. All patients underwent general clinical blood examination using Medonic M20 haematological analyser (Boule Medical, Sweden), including determination of absolute number of leukocytes, platelets, lymphocytes, microscopic determination of leukocyte formula. The percentage and absolute content of T lymphocyte subpopulations ($CD4^+$ T helper cells, cytotoxic $CD8^+$ T lymphocytes, $CD16^+$ T lymphocyte killers, HLA-DR^+ activated T lymphocytes), NK lymphocyte subpopulations ($CD16^+CD56^+$, $CD3^-CD8^+$) were determined, B lymphocytes ($CD19^+$), expression level of HLA-DR^+ and $CD95^+$ markers in the total blood lymphocyte pool (BP) by flow cytofluorimetry using FACS Calibur cytometer (BD, USA) and FITC- (Fluorescein IsoThioCyanate) and phycoerythrin-labelled monoclonal antibodies (Sorbent, Russia).

The leukocyte shift index (LSI) according to N.I. Yabuchinsky was determined by the ratio of the number of granulocytes (neutrophils, eosinophils and basophils) to agranulocytes (lymphocytes and monocytes). The leukocyte T cell index (LTI) according to A.M. Zemskov was determined by the ratio of the absolute number of leukocytes to that of $CD3^+$ T lymphocytes. The immunoregulatory index (IRI) was determined by the ratio of the percentage of $CD3^+CD4^+$ lymphocytes to the percentage of $CD3^+CD8^+$ lymphocytes.

The diagnosis of COVID-19 was verified using a PCR test for SARS-CoV-2, the levels of IgG antibodies (At) to the recombinant structural protein S1 spike of the SARS-CoV-2 virus in BP serum were determined using a semi-quantitative immunoenzymometric assay (IEMA) (Euroimmun AG, Germany), levels IgM-At and IgG-At to the recombinant protein SARS-CoV-2 – using high-quality IEMA (Vector-Best, Russia).

All the studied age, anamnestic and laboratory data were documented in the form of a standardised database (state registration certificate No. 2022620741 dated April 5, 2022) [9].

Ethical review. The study was carried out in accordance with the World Medical Association Declaration of Helsinki "Ethical principles of conducting scientific medical research with human participation as a subject". Written informed consent was obtained from each study participant before performing the procedures. The research protocol was approved by the Bioethics Committee of the Samara State Medical University of the Ministry of Health of the Russian Federation (Protocol No. 211 dated October 7, 2020).

Statistical processing was carried out using the SPSS 22.0 software (SPSS Inc., USA). Median (Me), 25th (Q1, first quartile) and 75th (Q3, third quartile) percentiles, Mann – Whitney rank test, Pearson's χ^2 test, Spearman's rank correlation (*R*). The level of statistical significance (*p*) was assumed to be < 0.05.

RESULTS

Among those examined, 66% were female. The average age 50.8 ± 15.4 years. A comparative analysis of the number of white cells and BP platelets showed that in the majority of patients the median values were within the reference values (Table 1).

The percentage and absolute lymphocyte levels were 23 % ($p = 0.003$) and 26.3 % ($p = 0.003$) lower, respectively, in the COVID-19 patient group than in the comparison group (Table 1). Severe lymphopenia (< 1.1×10^9 cells/L) was observed in 34.4 % of patients and was not observed in healthy individuals. In addition, with a higher neutrophil level (by 17.3 %; $p = 0.004$), there is also a 1.5-fold increased LSI relative to the comparison group ($p = 0.001$). In 21 (67,7 %) patients the pronounced rearrangements in the population structure of BP white cells were noted, among which in 12 (57 %) cases the increase of LSI values due to lymphopenia on the background of neutrophilia was noted (against 4 (10,7 %) healthy persons).

The percentage of atypical neutrophils ranged from 1 to 16 % (mean 8 %) and atypical lymphocytes from 2 to 15 % (mean 4.1 %) during microscopic examination of blood smears in patients with COVID-19. No atypical forms were observed in the comparison group.

When assessing the cellular link of immunity, it was obtained that in most cases the content of the examined subpopulations of T and B lymphocytes did not exceed the reference values (Table 2).

Against the background of the decrease in the absolute content of $CD3^+$ lymphocytes by 30.8 % ($p = 0.001$) in patients with COVID-19 relative to the comparison group there is an increase in LTI by 20 % ($p = 0.002$), which may indicate the presence of a deficiency of T lymphocytes (Table 2).

Relative to the indices of the comparison group, the patients with COVID-19 showed statistically significantly lower values of the absolute content of the examined subpopulations of T and NK lymphocytes (Table 2). While the percentage of $CD3^+CD4^+$ and $CD3^+CD8^+$ lymphocytes in patients with COVID-19 and in healthy individuals did not differ statistically significantly, analysis of individual immunograms indicates significant variability in these lymphocyte parameters. Thus, in the group of patients with COVID-19 there were 1.5 times more frequent cases of reduced content (< 35 %) of $CD4^+$ T lymphocytes: 35.5 % ($n = 11$) versus 26.2 % ($n = 11$) in the comparison group (Table 2).

When studying the distribution of $CD19^+$ B lymphocyte percentage content, it was found that elevated levels (> 17 %) were 2.6 times more frequent in patients with COVID-19 than in the comparison group.

When studying the NK cell system, it was found that the indicator of the absolute number of $CD16^+CD56^+$ lymphocytes was 29.4 % ($p = 0.009$) lower than that of the comparison group (Table 2). Absolute $CD16^+CD56^+$ lymphocyte content in patients with COVID-19 is below the reference value by 0.13×10^9 cells/L in 50 % of cases (versus 21.4 % of the comparison group).

No statistically significant changes in the percentage of both $CD3^+CD16^+$ and $CD3^-CD8^+$ lymphocytes were found during the average period in the group of patients with COVID-19 relative to the comparison group (Table 2). Comparative analysis of the distribution of these lymphocyte subpopulations showed that elevated levels of $CD3^+CD16^+$ and $CD3^-CD8^+$ lymphocytes (> 8 %) were 2-fold more frequent in patients with COVID-19: 21.4 % ($n = 3$) versus 9.4 % ($n = 3$), respectively, and 22.2 % ($n = 6$) versus 9.1 % ($n = 3$) among healthy individuals.

TABLE 1

GENERAL CLINICAL BLOOD EXAMINATION VALUES OF PATIENTS WITH NEW CORONAVIRUS INFECTION (STUDY GROUP) AND HEALTHY DONORS (COMPARISON GROUP)

Indicators [reference values]	Comparison group ($n = 42$)		Study group ($n = 31$)		p
	Me	Q1–Q3	Me	Q1–Q3	
Leukocytes, $\times 10^9$ cells/L [4–9]	6	[5–7]	6	[5–7.8]	0.5
Lymphocytes, % [20–50]	35.1	[28.1–43.6]	27	[12.5–34]	0.003*
Lymphocytes, $\times 10^9$ cells/L [1.13–2]	1.9	[1.6–2.4]	1.4	[0.9–1.9]	0.001*
Platelets, $\times 10^9$ cells/L [180–320]	222	[165–248.2]	204	[173–298]	0.6
Neutrophils, % [48.5–84]	56	[49.2–65.2]	65.7	[58.3–81.4]	0.004
Monocytes, % [3–11]	7.7	[6.6–8.75]	7.2	[4.4–9]	0.3
LSI, units [1.46–2.36]	1.26	[0.95–1.9]	1.9	[1.4–4]	0.001*

Note. p is the level of statistical significance according to the Mann – Whitney test ($p < 0.05$).

No statistically significant changes in the content of HLA-DR$^+$ lymphocytes at the average in the group of patients with COVID-19 relative to the comparison group were observed ($p = 0.1$). In the analysis of individual immunograms, it was noted that in patients with COVID-19, elevated (> 20 %) HLA-DR$^+$ lymphocyte content was observed in 18 (60 %) patients (versus 14 (33.3 %) patients in the comparison group; $\chi^2 = 5$; $p = 0.03$). The level of CD3$^+$ HLA-DR$^+$ lymphocytes in patients with COVID-19 is statistically significantly higher than that in the comparison group (Table 2). Elevated (> 6 %) CD3$^+$ HLA-DR$^+$ lymphocyte content was observed in 36 (86.7 %) COVID-19 patients (versus 18 (58.3 %) comparison group individuals).

A three-fold increase in the CD95$^+$ lymphocyte ratio was observed in the average group of patients with COVID-19 relative to the comparison group (Table 2).

The results of the correlation analysis showed that the percentage level of CD3$^+$CD16$^+$ lymphocytes showed a correlation with a wide range of other parameters of cellular immunity: the reverse – with IRI ($R = –0.62$; $p = 0.02$), the absolute content of CD3$^+$ lymphocytes ($R = –0.6$; $p = 0.02$), the absolute content of CD3$^+$CD4$^+$ lymphocytes ($R = –0.7$; $p = 0.005$); direct – with LTI ($R = 0.6$; $p = 0.02$) and CD3$^+$ HLA-DR$^+$ lymphocytes ($R = 0.63$; $p = 0.016$). The existence of multiple negative correlations between the content of NK cells and T-lymphocyte subpopulations may indi-

TABLE 2

CELLULAR IMMUNITY INDICATORS IN PATIENTS WITH COVID-19 (STUDY GROUP) AND HEALTHY DONORS (COMPARISON GROUP)

Indicators [reference values]	Comparison group ($n = 42$)		Study group ($n = 31$)		p
	Me	Q1–Q3	Me	Q1–Q3	
CD3, % [6–85]	71	[64.5–75]	70	[60.5–76]	0.5
CD3, × 10^9 cells/L [0.94–2.1]	1.3	[11.6–7]	0.9	[0.5–1.4]	0.001*
CD3$^+$CD4$^+$, % [35–55]	40.9	[32.5–46.3]	39	[30.3–45.3]	0.4
CD3$^+$CD4$^+$, × 10^9 cells/L [0.58–1.3]	0.77	[0.56–0.9]	0.5	[0.3–0.9]	0.009*
CD3$^+$CD8$^+$, % [19–35]	23.15	[17–34.1]	23.4	[17.3–31.2]	0.7
CD3$^+$CD8$^+$, × 10^9 cells/L [0.37–1]	0.375	[0.3–0.7]	0.35	[0.17–0.48]	0.02*
CD3$^-$CD8$^+$, %	4.2	[3–6.8]	5.75	[2.8–11.1]	0.14
CD3$^-$CD8$^+$, × 10^9 cells/L	0.09	[0.06–0.14]	0.07	[0.03–0.1]	0.13
CD16$^+$CD56$^+$, % [10–23]	10.25	[7.4–14.25]	9.4	[5.1–14.4]	0.4
CD16$^+$CD56$^+$, × 10^9 cells/L [0.13–0.5]	0.17	[0.14–0.24]	0.12	[0.06–0.19]	0.009*
CD3$^+$CD16$^+$, % [5–8]	3.2	[2–5]	3	[1.6–9.6]	0.8
CD19$^+$, % [7–17]	8	[6–11.3]	9	[6.2–13.6]	0.26
CD19$^+$, × 10^9 cells/L [0.1–0.38]	0.15	[0.1–0.23]	0.13	[0.07–0.2]	0.12
CD3$^+$ HLA-DR$^+$, % [1–6]	7	[5–11.3]	10	[7.4–14.8]	0.025*
HLA-DR$^+$, % [7–20]	18.8	[15.35–22.3]	21.55	[17–27.2]	0.1
CD95$^+$, % [5–43]	9.3	[4.3–33.45]	29.6	[13–39.75]	0.05*
LTI [4-7]	4.5	[3.3–5]	5.4	[4.2–14.1]	0.002*
IRI [1.5–2.6]	1.7	[1–2.6]	1.9	[0.9–2.5]	0.81

Note. p is the level of statistical significance according to the Mann – Whitney test ($p < 0.05$).

cate increased cytotoxicity against the background of $CD3^+$ and $CD3^+CD4^+$ lymphocyte deficiency.

In the study of correlations, it was found that the percentage level of $CD16^+CD56^+$ lymphocytes shows an inverse relationship with the percentage of $CD3^+$ and $CD3^+CD4^+$ lymphocytes ($R = –0.7$ and $R = –0.34$, respectively; $p < 0.05$), a direct relationship with the percentage and absolute levels of $CD3^-CD8^+$ lymphocytes ($R = 0.8$ and $R = 0.5$, respectively; $p < 0.05$). A wide range of correlations between the levels of $CD16^+CD56^+$ NK cells and $CD16^+$ T lymphocytes with other T-lymphocyte subpopulations may indicate a conjugated response of different lymphocyte subpopulations in response to SARS-CoV-2 virus infection.

The level of $HLA\text{-}DR^+$ lymphocytes in patients with COVID-19 showed an inverse correlation with the absolute content of $CD3^-CD8^+$ lymphocytes ($R = –0.49$; $p = 0.007$) and a direct correlation with the percentage level of $CD3^+CD16^+$ lymphocytes ($R = 0.63$; $p = 0.016$). An inverse correlation between the index of the percentage level of $HLA\text{-}DR^+$ lymphocytes and the percentage level of $CD3^+$ lymphocytes was observed ($R = –0.37$; $p = 0.05$).

The $CD95^+$-lymphocyte indices showed inverse correlations with the percentage level of $CD3^-CD8^+$ lymphocytes ($R = –0.48$; $p = 0.02$) and direct correlations with $CD3^+CD4^+$ lymphocytes ($R = 0.43$; $p = 0.037$).

DISCUSSION

In patients with COVID-19, according to the results of the general blood analysis, the average leukocyte level in the group does not exceed the reference values [10], while 67.7 % of patients have marked rearrangements of the percentage of granulocytes and agranulocytes in BP, among which 57 % of cases show lymphopenia on the background of neutrophilia. The evidence of the rearrangements that occurred in patients with COVID-19 is confirmed by both a 1.5-fold increase in the group mean LSI value and the presence of opposite correlations of lymphocyte levels ($R = –0.47$; $p = 0.07$) and neutrophils ($R = 0.45$; $p = 0.012$) with 30 % lung lesion according to CT.

The numbers of atypical neutrophils and lymphocytes were as high as 8 % and 4.1 %, respectively, during the microscopic examination of blood smears in COVID-19. Atypical morphological changes of neutrophils and lymphocytes at the early stage of the disease development were also observed by some foreign researchers [11].

Severe lymphopenia ($< 1.1 \times 10^9$ cells/l) was observed in 34.4 % of COVID-19 cases. Statistically significant decrease in the absolute content of the studied subpopulations of T and NK cells in patients with COVID-19, including the absolute content of $CD3^+CD4^+$ lymphocytes (by 35 %; $p = 0.009$) and $CD3^+CD8^+$ lymphocytes (by 6.7 %; $p = 0.02$) may be used to define this general lymphopenia. Many authors consider a decrease in the absolute content of $CD4^+$ T cells and a change in their internal subpopulation structure to be a characteristic feature of COVID-19 [5, 7, 12]. However, no statistically significant changes in the percentage contents of $CD4^+$ and $CD8^+$ T lymphocytes and IRI were obtained. The involvement of T lymphocytes in the pathogenesis of the disease is considered by many authors to be determinant [5].

In our study, no statistically significant changes in $CD19^+$ lymphocyte indices were found in patients with COVID-19 relative to the comparison group. A possible reason for this may be the considerable variability in both percentage and absolute B-lymphocyte content in COVID-19 patients. In particular, 35.5 % of patients have a decreased (< 7 %) level of B lymphocytes and 13 % have an increased (> 17 %) level. An increase in the percentage of $CD19^+$ B lymphocytes in patients with COVID-19 was revealed in the study of F. Wang et al. (2020) [13].

Alterations in cellular immunity have been identified by many authors as a characteristic feature [1, 14]. A typical characteristic of COVID-19 infection is a dramatic decrease in lymphocyte levels and, in particular, absolute levels of $CD3^+$, $CD3^+CD4^+$ and $CD3^+CD8^+$ lymphocytes [12, 15]. Migration of lymphocytes from the BP to the lungs is considered one of the causes of progressive lymphopenia [5, 16, 17]. Direct cytotoxic action of SARS-CoV-2 virus against the immune cells is considered as another cause of lymphopenia by a number of authors. Viral particles and the SARS-CoV-2 genome were not only found in monocytes and lymphocytes, but also proved their ability to replicate intracellularly in the *in vitro* system [1, 18, 19]. Infection of T lymphocytes and macrophages/monocytes located in lymph nodes, lungs and spleen in autopsy specimens has been reported [1, 20, 21].

Some authors note an increase in the content of cytotoxic cells and their functional activity in patients with COVID-19 [13, 14, 22]. A decrease in NK cell content correlating with the severity of the disease in COVID-19 has been observed in the works of other authors [4, 7]. Our study revealed a 29.4 % ($p = 0.09$) decrease in absolute $CD16^+CD56^+$ lymphocytes content in patients with COVID-19 relative to the comparison group. As concerns peculiarities of $CD3^+CD16^+$ and $CD3^-CD8^+$ lymphocytes level indices distribution, no statistically significant changes in their percentage content were revealed at the average in the group of patients with COVID-19 relative to the comparison group. However, a high variability in the level indices of these subpopulations can be observed, which may level out the average group values. In addition, the presence of multiple negative correlation interdependencies of the content of these populations of NK-cells with those of T lymphocyte subpopulations may indicate an increase in the cytotoxic activity of these cells against the background of $CD3^+$ and $CD3^+CD4^+$ lymphocyte deficiency. High correlation coefficient of the percentage level of $CD3^+CD16^+$ lymphocytes with LTI ($R = 0.6$; $p = 0.02$) may serve as evidence of these findings.

The increasing expression level of activation markers in COVID-19, including HLA-DR, has been documented in a number of studies [12, 13, 23, 24]. Studies show increased expression of a variety of activation markers on T lymphocytes triggering a "cytokine storm" in patients with COVID-19 [6, 18]. A dramatic increase in the level of cells expressing HLA-DR against a statistically significant de-

crease in the levels of $CD3^+CD4^+$ lymphocytes and NK cells has been indicated as a sign of excessive activation of cellular immunity [17]. In the absence of statistically significant differences in the percentage of $HLA\text{-}DR^+$ lymphocytes in patients with COVID-19 relative to the comparison group, the analysis of individual immunograms is 1.8 times more likely to have an increased content of $HLA\text{-}DR^+$ lymphocytes (> 20 %) ($\chi^2 = 5$; $p = 0.03$). In addition, patients with COVID-19 showed a statistically significant increase in the content of $CD3^+$ $HLA\text{-}DR^+$ lymphocytes by 42.8 % ($p = 0.025$). Changes in the indicators of cellular and humoral immunity in COVID-19 reconvalescents within 1.5–2.0 months after the infection were also revealed in the studies of other authors [25, 26].

According to our study, in response to infection of the organism with SARS-CoV-2 there is a change in quantitative and functional indices of cellular immunity, the degree of expression of which and the direction of dynamics largely depend on the initial state of the immune system.

CONCLUSION

Consequently, in 67.7 % of the examined patients with COVID-19 with lung lesions according to CT ≤ 30 % before the initiation of treatment there is a rearrangement in the ratio of lymphocyte subpopulations, among which in 57 % of cases there is lymphopenia on the background of neutrophilia, which is expressed in the increase of LSI values.

Pronounced lymphopenia ($< 1.1 \times 10^9$ cells/L) was observed in 34.4 % of patients with COVID-19; it was caused by a decrease in the absolute content of the main subpopulations of both T lymphocytes ($CD3^+$ – by 30.8 %, $CD3^+CD4^+$ – by 35 %, $CD3^+CD8^+$ – by 6.7 %; $p < 0.05$), and NK cells ($CD16^+CD56^+$ – by 29.4 %; $p = 0.009$), which was accompanied by an increase in LTI by 20 % ($p = 0.002$).

The features revealed in COVID-19 patients, such as a 3.2-fold increased fraction of $CD95^+$-lymphocytes (30 %), high (> 20 %) content of $HLA\text{-}DR^+$-lymphocytes registered in 60 % of patients, and increased (> 6 %) content of $CD3^+$ $HLA\text{-}DR^+$ lymphocytes observed in 86.7 % of patients, may be characteristic of the cellular level of the immune system in the course of SARS-CoV-2 infection. The degree of severity and dynamics of changes in quantitative and functional indices of cellular immunity in the course of SARS-CoV-2 infection are defined by the initial type of immune system response.

The use of immunological screening to determine the levels of $CD3^+CD4^+$, $CD3^+CD8^+$, $CD16^+CD56^+$, $CD3^+CD16^+$, $CD3^+$ $HLA\text{-}DR^+$, $CD19^+$, $CD95^+$ lymphocytes allows to assess the specific features of immune response to SARS-CoV-2 infection and can be recommended as an additional to the general clinical blood test.

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Conflict of interest

The authors of this article declare the absence of a conflict of interest.

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Vdoushkina E.S. – obtaining data for study, discussing the results, writing the text.

Borodulin B.E. – review of publications on the topic of the article, development of study design, discussion of the results.

Povalyaeva L.V. – obtaining data for study, analyzing the data obtained, discussing the results.

CHARACTERISTICS OF NEW CASES OF INFILTRATIVE PULMONARY TUBERCULOSIS IN PATIENTS HAVING HIV INFECTION WITH MULTIDRUG RESISTANCE OF THE PATHOGEN ACCORDING TO MULTI-LAYER SPIRAL COMPUTED TOMOGRAPHY

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ABSTRACT

Background. *Identification of the characteristics of the pulmonary tuberculosis process using multi-layer spiral computed tomography (MSCT) in patients with tuberculosis and HIV infection is important in the diagnosis of tuberculosis, determining the dissemination of the process and its dynamics during treatment.*

The aim. *To determine the initial characteristics and dynamics of infiltrative tuberculosis according to MSCT in patients with and without HIV infection, with and without multidrug resistance (MDR) of Mycobacterium tuberculosis who were treated in a hospital.*

Materials and methods. *126 patients aged 19–59 years with tuberculosis, combined with HIV infection and without HIV infection were examined. For statistical processing, we used MS Excel (Microsoft Corp., USA) software package.*

Results. *Patients with tuberculosis and HIV infection in comparison with patients with tuberculosis and without HIV had more expressed intoxication syndrome and respiratory impairement in the clinical picture ($p < 0.00001$). Patients with coinfection were more likely to suffer from alcohol ($p < 0.05$) and drug addiction ($p < 0.001$).*

According to MSCT, the pathological process in HIV-positive patients with pulmonary tuberculosis was more disseminated ($p < 0.05$), included severe intrathoracic lymphadenopathy ($p < 0.0001$), more common pleural lesions ($p < 0.005$), less common destructive changes (cavities) ($p < 0.001$) and outcomes in form of fibro-cavernous tuberculosis ($p < 0.01$). Process regression was slower in patients with tuberculosis and HIV ($p < 0.005$).

According to MSCT, extensive lung damage, intrathoracic lymphadenopathy were more often found in patients with MDR in coinfection ($p < 0.05$). Cavities and fibro-cavernous tuberculosis outcomes were more common in patients with tuberculosis without HIV infection and with MDR ($p < 0.05$).

Conclusion. *MSCT provides detailed information about the pathological process in the lungs and its dynamics under the treatment of tuberculosis and HIV infection.*

Key words: *infiltrative tuberculosis, tuberculosis combined with HIV infection, computed tomography, multidrug resistance of Mycobacterium tuberculosis*

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ХАРАКТЕРИСТИКА ВПЕРВЫЕ ВЫЯВЛЕННОГО ИНФИЛЬТРАТИВНОГО ТУБЕРКУЛЁЗА ЛЁГКИХ У БОЛЬНЫХ С ВИЧ-ИНФЕКЦИЕЙ С МНОЖЕСТВЕННОЙ ЛЕКАРСТВЕННОЙ УСТОЙЧИВОСТЬЮ ВОЗБУДИТЕЛЯ ПО ДАННЫМ МУЛЬТИСПИРАЛЬНОЙ КОМПЬЮТЕРНОЙ ТОМОГРАФИИ

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РЕЗЮМЕ

Обоснование. *Выявление характеристик туберкулёзного лёгочного процесса методом мультиспиральной компьютерной томографии (МСКТ) у пациентов с туберкулёзом и ВИЧ-инфекцией является важным в диагностике туберкулёза, определении распространённости процесса и его динамики на фоне лечения.*

Цель исследования. *Определить исходную характеристику и динамику инфильтративного туберкулёза по данным МСКТ у пациентов с ВИЧ-инфекцией и без ВИЧ-инфекции с множественной лекарственной устойчивостью (МЛУ) и без МЛУ микобактерий туберкулёза, получавших лечение в стационаре.*

Методы. *Обследованы 126 пациентов 19–59 лет с туберкулёзом, сочетанным с ВИЧ-инфекцией, и без ВИЧ-инфекции. Для статистической обработки использовался пакет прикладных программ MS Excel (Microsoft Corp., США).*

Результаты. *У пациентов с туберкулёзом и ВИЧ-инфекцией в сравнении с пациентами с туберкулёзом без ВИЧ в клинической картине чаще были выражены синдром интоксикации и нарушение функции внешнего дыхания ($p < 0,00001$). Лица с коинфекцией чаще страдали алкогольной ($p < 0,05$) и наркотической зависимостью ($p < 0,001$). Патологический процесс по данным МСКТ у ВИЧ-позитивных пациентов с туберкулёзом лёгких имел большую распространённость ($p < 0,05$), выраженную внутригрудную лимфоаденопатию ($p < 0,0001$), чаще встречалось поражение плевры ($p < 0,005$), реже выявлялись деструктивные изменения ($p < 0,001$) и исходы в фиброзно-кавернозный туберкулёз ($p < 0,01$). Регрессия процесса была замедлена у пациентов с туберкулёзом и ВИЧ ($p < 0,005$). По данным МСКТ распространённое поражение лёгких, внутригрудная лимфоаденопатия чаще регистрировалось у пациентов с МЛУ при коинфекции ($p < 0,05$). Деструктивные изменения и исход в фиброзно-кавернозный туберкулёз чаще наблюдались у пациентов с туберкулёзом без ВИЧ-инфекции с МЛУ ($p < 0,05$).*

Заключение. *МСКТ даёт детальную информацию о патологическом процессе в лёгких и о его динамике под действием лечения при туберкулёзе и ВИЧ-инфекции.*

Ключевые слова: *инфильтративный туберкулёз, туберкулёз в сочетании с ВИЧ-инфекцией, компьютерная томография, множественная лекарственная устойчивость микобактерий туберкулёза*

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In the Russian Federation, there is a tendency to increase the proportion of HIV-infected patients among patients with newly diagnosed tuberculosis, it has a negative impact on the epidemiological process of these infections [1].

Despite the considerable variety of anti-tuberculosis drugs for treating for tuberculosis patients [2], the efficacy of treatment of coinfected patients is significantly lower than of HIV-negative tuberculosis patients [3]. The problem of multidrug resistance (MDR) to anti-TB drugs is also an ongoing issue. In patients with a combination of tuberculosis and HIV infection, MDR is detected much more frequently, cure is more difficult to achieve, and at the same time, a full range of new highly effective antituberculosis drugs is available [4–6].

Patients with co-infection of pulmonary tuberculosis and HIV infection have higher rates of drug resistance and therefore increased mortality according to J.W Wilson et al [7].

The main contribution to the determination of the localization, prevalence, and activity of the tuberculosis process is made by methods of radiation diagnostics: radiography, multi-layer spiral computed tomography (MSCT). MSCT method significantly complements traditional X-ray examination when assessing morphological changes in patients with pulmonary tuberculosis, and in the group of persons with tuberculosis and HIV co-infection in the presence of immunosuppression significantly increases the efficiency of X-ray examination [8].

Consequently, detection of characteristics of tuberculous pulmonary process by MSCT among tuberculosis patients, and especially in patients with tuberculosis combined with HIV infection, is important in primary diagnosis of tuberculosis, determination of process prevalence and its dynamics against the background of specific treatment. A detailed study of the radial semiotics of tuberculosis in co-infected patients, including in their dynamics, will help to improve treatment approaches and contribute to the prevention of relapses.

THE AIM OF THE STUDY

To determine the initial characteristics and dynamics of infiltrative tuberculosis according to multi-layer spiral computed tomography in patients with and without HIV infection, with and without multidrug-resistant *Mycobacterium tuberculosis* treated in hospital.

MATERIALS AND METHODS

Inclusion criteria: microbiologically confirmed infiltrative tuberculosis as the most frequent clinical form of tuberculosis; three MSCT studies of the chest organs – on admission, after 2 and 6 months of treatment.

A total of 126 patients aged 19 to 59 years with newly diagnosed infiltrative tuberculosis were examined: 68 (44 males and 24 females) with isolated pulmonary tuberculosis (TB) and 58 (45 males and 13 females) with TB and HIV co-infection (TB/HIV). The mean age of patients in the TB/HIV group was 32.9 ± 0.9 years and in the TB group 35.9 ± 1.1 years. In the TB/HIV group, all patients had stage 4B of HIV infection, with $CD4^+$-lymphocyte counts ranging from 90 to 420 cells/μL.

Among 68 patients in the group with newly diagnosed infiltrative TB without HIV infection, MDR was detected in 23 (33.8 %), and among 58 patients with TB/HIV coinfection, MDR was detected in 44 (75.8 %; $p < 0.01$).

All patients received inpatient treatment in pulmonary tuberculosis departments and the department for tuberculosis patients with HIV infection of the Irkutsk Regional Clinical Tuberculosis Hospital.

All patients were examined with general clinical and biochemical methods of examination. Microbiological examination methods included molecular-genetic (GeneXpert) with determination of rifampicin resistance, luminescence microscopy, cultures performed on dense and liquid nutrient media. All patients underwent chest MSCT before the initiation of treatment, and in 2 and 6 months of treatment. Human immunodeficiency virus RNA and $CD4^+$ lymphocyte count were obtained in HIV co-infected patients.

In order to identify statistically significant differences, MS Excel application package (Microsoft Corp., USA) was used, the mean (M), standard error of the mean (m) were calculated, and the χ^2 method with Yates correction was applied. The specified critical significance level was $p < 0.05$.

The work was carried out in accordance with the principles of the World Medical Association Declaration of Helsinki. Study protocol was approved by the Committee on Ethics of Scientific Research of the Irkutsk State Medical Academy of Postgraduate Education, a branch of the Russian Medical Academy of Continuing Professional Education of the Ministry of Health of the Russian Federation (Protocol No. 10 dated December 24, 2021).

RESULTS AND DISCUSSION

Infiltrative tuberculosis was diagnosed at preventive fluorographic examination in 41 (60.3 %) tuberculosis patients without HIV infection and 25 (43.0 %) with HIV infection ($p > 0.05$).

During clinical examination, 19 (28.0 %) tuberculosis patients and 6 (10.0 %) coinfected patients had no complaints ($\chi^2 = 2.95$; $p < 0.05$). Moderately severe symptoms of intoxication and a small, low-productive cough were statistically significantly more common in the TB group of patients: 34 (50.0 %) versus 6 (10.0 %) patients in the TB/HIV group ($\chi^2 = 13.4$; $p < 0.0005$). A severe intoxication syndrome, fever, cough, and dyspnoea were only observed in 15 (22.0 %) patients with infiltrative tuberculosis and in the vast majority, 46 (79.0 %), of patients with infiltrative tuberculosis combined with HIV infection ($\chi^2 = 24.2$; $p < 0.00001$).

The social profile of the studied patients is shown in detail in Table 1.

Table 1 indicates that drug and alcohol addiction were statistically significantly more common in the TB/HIV co-infected group of patients. No statistically significant difference was observed between the compared groups in the presence of nicotine addiction, whether or not there is official employment, and time spent in detention facilities.

X-ray examinations play an important role in detecting pathological changes in patients with pulmonary tuberculosis. The radiologist's opinion is considered to be the basis for the formation of a clinical diagnosis according to the current clinical classification. Classical radiological characteristics of tuberculosis in co-infected patients are more common in the early stages of HIV infection. Against the background of severe immunodeficiency, radiological symptoms often begin to acquire an atypical character. In this case, MSCT becomes the main method for detection and evaluation of lung changes against the background of tuberculosis infection. The semiotics of infiltrative tuberculosis according to MSCT is presented in Tables 2–4.

The localisation of the tuberculosis process was «classical» in almost 90 % of the patients in both groups, with lesions in the apical (S1), posterior (S2) and upper basal (S6) segments of the lungs. However, a more prevalent tuberculosis process (involvement of three or more lung segments) was statistically significantly more frequently diagnosed in the TB/HIV group – 70.6 % (41/58) versus 35.3 % (24/68) in the TB group ($\chi^2 = 8.47$; $p < 0.05$).

TABLE 1

CHARACTERISTICS OF THE SOCIAL PROFILE OF PATIENTS IN THE TB AND TB/HIV STUDY GROUPS

Social characteristics	TB group ($n = 68$)		TB/HIV group ($n = 58$)		p
	abs.	%	abs.	%	
Alcohol addiction	19	27.8	30	51.7	$p < 0.05$*; $\chi^2 = 4.21$
Drug addiction	3	4.4	22	37.9	$p < 0.001$*; $\chi^2 = 10.7$
Smoking (more than 10 cigarette per day)	15	22.0	23	40.0	$p > 0.05$
Lack of legal job	43	63.2	42	72.4	$p > 0.05$
Stay in corrective-labour institutions	9	13.2	9	15.5	$p > 0.05$

Note. * – hereinafter differences are statistically significant.

TABLE 2

LOCALISATION AND PREVALENCE OF INFILTRATIVE PULMONARY TUBERCULOSIS ACCORDING TO MSCT IN TB AND TB/HIV GROUPS

Indicators		TB group ($n = 68$)		TB/HIV group ($n = 58$)		p
		abs.	%	abs.	%	
Localization of the pathological process	Typical (S1, S2, S6)	59	86.8	50	86.2	$p > 0.05$
	Atypical (segments of the lower lobe)	9	13.2	8	13.8	$p > 0.05$
The prevalence of the pathological process	1 segment	9	13.2	5	8.6	$p > 0.05$
	2 segments	35	51.4	12	20.7	$p < 0.001$*
	3 segments	20	29.4	20	34.5	$p > 0.05$
	4 segments	4	5.9	14	24.1	$p < 0.005$*
	5 segments	0	0	7	12.0	$p < 0.005$*

TABLE 3

INTRATHORACIC LYMPH NODE ENLARGEMENT BY MSCT IN TB AND TB/HIV GROUPS

The number of groups of enlarged lymph nodes of the mediastinum	TB group (n = 68)		TB/HIV group (n = 58)		p
	abs.	%	abs.	%	
1 group	6	31.5	7	17.9	$p > 0.05$
2 groups	11	57.8	23	58.9	$p > 0.05$
3 or more groups	2	10.5	9	23.0	$p > 0.05$
Total	19	27.9	39	67.2	$p < 0.0001$*

TABLE 4

RADIOLOGICAL SEMIOTICS OF INFILTRATIVE TUBERCULOSIS ACCORDING TO MSCT IN TB AND TB/HIV GROUPS

Indicators	TB group (n = 68)		TB/HIV group (n = 58)		p
	abs.	%	abs.	%	
Thickening of pulmonary pleurae	8	11.7	18	31.0	$p < 0.01$*; $\chi^2 = 7.097$
Pleural fluid	6	8.8	17	29.3	$p < 0.01$*; $\chi^2 = 8.804$
Decay cavity	31	45.6	10	17.2	$p < 0.001$*; $\chi^2 = 5.8$

Patients with a TB/HIV combination have a high incidence of intrathoracic lymphadenopathies with escalating immunodeficiency. The lymph nodes of the following groups are most commonly enlarged: paratracheal, tracheobronchial, bifurcation and bronchopulmonary. Multi-layer spiral computed tomography is a reliable method for detecting the condition of mediastinal lymph nodes. MSCT method enables not only to detect lymph node enlargement (short diameter – more than 10 mm) and their number, but also to assess the structure of lymph nodes, changes in their contours, the perinodular and mediastinal fibre condition. Generally, lymph nodes of mediastinum and lung roots have spindle or oval shape, therefore, it is appropriate to measure the short and long diameters during MSCT, which will coincide only in case of rounded shape of the lymph node.

In our study, enlargement of intrathoracic lymph nodes (ITLNs) was statistically significantly more common in the group of HIV positive patients with infiltrative tuberculosis. No statistically significant difference was observed in the number of affected groups of the ITLNs in patients with infiltrative pulmonary tuberculosis depending on the HIV infection status.

Pathological changes of the pleura in tuberculous lung disease occur in 30 to 50 % of cases as indicated in the literature. Pleural lesions were detected statistically significantly more often in the presence of immunodeficiency in our study. The existence of pleural fluid was also more common in the group of patients with tuberculosis and HIV co-infection.

Destructive processes of lung tissue in HIV-positive patients from the TB/HIV group were diagnosed statistically significantly less frequently than in the group of patients with isolated tuberculosis. The outcome to fibrotic cavernous tuberculosis was also diagnosed statistically significantly more often in the TB group, in 29.4 % (20/68) versus 10.3 % (6/58) in the TB/HIV group ($\chi^2 = 6.949$; $p < 0.008$).

The X-ray dynamics of pathological tuberculosis process in the lungs was assessed according to MSCT after 2 and 6 months of treatment. The data is presented in tables 5 and 6.

According to the data of multi-layer spiral computed tomography, as evident from Tables 5 and 6, against the background of the ongoing treatment, positive dynamics after 2 and 6 months of treatment was registered statistically significantly more often in patients with isolated tuberculosis. After 2 months, almost 60.0 % of patients in both groups had no dynamics on the background of the conducted treatment. After six months, no significant change was observed in every second patient in the TB/HIV group and in 36.7 % (25/68) of the TB group.

Negative dynamics after 2 months was registered in every third patient in the group with TB and HIV co-infection. The negative results obtained in the TB/HIV group require further, more detailed study of this issue.

TABLE 5

DYNAMICS OF PULMONARY TUBERCULOSIS PROCESS AFTER 2 MONTHS OF TREATMENT ACCORDING TO MSCT DATA IN TB AND TB/HIV GROUPS

Dynamics according to MSCT	TB group ($n = 68$)		TB/HIV group ($n = 58$)		p
	abs.	%	abs.	%	
None	39	57.3	35	60.3	$p > 0.05$
Positive	23	33.8	6	10.3	$p < 0.005$*; $\chi^2 = 4.8$
Negative	6	8.8	17	29.3	$p < 0.005$*; $\chi^2 = 6.3$

TABLE 6

DYNAMICS OF PULMONARY TUBERCULOSIS PROCESS AFTER 6 MONTHS OF TREATMENT ACCORDING TO MSCT DATA IN TB AND TB/HIV GROUPS

Dynamics according to MSCT	TB group ($n = 68$)		TB/HIV group ($n = 58$)		p
	abs.	%	abs.	%	
None	25	36.7	29	50.0	$p > 0.05$
Positive	39	57.3	19	32.7	$p < 0.01$*; $\chi^2 = 4.1$
Negative	4	5.8	10	17.2	$p < 0.05$*; $\chi^2 = 3.8$

TABLE 7

RADIOLOGICAL SEMIOTICS OF INFILTRATIVE TUBERCULOSIS ACCORDING TO MSCT IN TB AND TB/HIV GROUPS IN MULTIDRUG-RESISTANT PATIENTS

Indicator	TB + MDR group ($n = 23$)		TB/HIV + MDR group ($n = 44$)		p
	abs.	%	abs.	%	
Atypical localization of the pathological process (lower lobe)	4	17.4	8	18.1	$p > 0.05$
A common process (lesion of 3 or more lung segments)	14	60.8	38	86.3	$p < 0.05$*; $\chi^2 = 4.278$
Intracoracic lymphadenopathy	9	39.1	32	72.7	$p < 0.01$*; $\chi^2 = 7.180$
Thickening of pulmonary pleurae	4	17.3	14	31.8	$p > 0.05$
Pleural fluid	3	13.0	13	29.5	$p > 0.05$
Decay cavity	10	43.4	8	18.1	$p < 0.05$*; $\chi^2 = 3.716$
Outcome in fibrocavitary tuberculosis	12	52.1	5	11.3	$p < 0.005$*; $\chi^2 = 11.218$

Drug-sensitive tuberculosis patients received inpatient treatment with chemotherapy regimen I or III if their TB was confirmed to have DNA with preserved sensitivity to rifampicin before the results of the drug sensitivity test. MDR-TB patients were treated with IV empirical (according to rifampicin-resistant molecular genetic methods) or test (according to culture results on liquid or dense nutrient media) chemotherapy regimens.

Radiation semiotics of drug-resistant patients with isolated infiltrative tuberculosis and those with co-infection of tuberculosis and HIV infection are presented in Table 7.

According to MSCT data, widespread lung damage (3 segments or more) and intrathoracic lymphoadenopathy were statistically significantly more often diagnosed in patients with MDR in the TB/HIV group. As opposed to this, destructive changes and outcome in fibrotic cavernous tuberculosis were statistically significantly more often diagnosed in multidrug-resistant patients in the group with isolated pulmonary tuberculosis. No statistically significant difference was observed in the localisation of the tuberculosis process and the presence of pathological changes in the pleura in drug-resistant patients with isolated infiltrative tuberculosis and in those co-infected with tuberculosis and HIV infection.

CONCLUSION

The data obtained indicate the need for further improvement of work on the early detection of tuberculosis in HIV-infected patients, the use of chest X-ray examinations and immunodiagnostics [7]. Radiological diagnostics in immunodeficient patients should be optimised, with a priority being assigned to thoracic MSCT. Particular attention should be provided to patients from risk groups (drug and alcohol addiction) for tuberculosis towards early detection and timely diagnosis. MSCT methods should be performed to assess the dynamics of the tuberculosis process against the background of treatment, as it is not possible to track the dynamics of specific changes in the lungs of immunodeficient individuals based on radiological examination, which may lead to unwarranted termination of treatment and early relapses of the disease.

Conflict of interest

The authors declare no conflict of interest in relation to this article.

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CARDIOLOGY

LEFT VENTRICULAR DIASTOLIC DYSFUNCTION AND TRANSMITRAL BLOOD FLOW PARAMETERS IN PATIENTS AFTER COVID-19

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ABSTRACT

Background. *People who previously had COVID-19 infection have an increased risk of developing cardiovascular diseases. Left ventricular diastolic dysfunction is an early marker of the development of cardiac pathology. Its early detection is important for the adequate therapy order and dynamic monitoring of patients. In this regard, it seems relevant to study the effect of a recent COVID-19 infection on the left ventricular diastolic function and transmitral blood flow parameters in apparently healthy individuals without clinical and instrumental signs of cardiovascular pathology.*

The aim of the study. *To assess the changes in the diastolic and systolic function of the left ventricle, its anatomical parameters and transmitral blood flow parameters in two groups of apparently healthy individuals: those who had and those who had not COVID-19 infection.*

Materials and methods. *Transthoracic echocardiography was performed according to the standard technique and its results were analyzed in 66 examined patients who were recognized as apparently healthy according to the results of regular comprehensive clinical and instrumental studies. The first group included 30 individuals who underwent an echocardiographic study before or during the COVID-19 pandemic, but did not have a coronavirus infection; the second group consisted of 36 people who recovered from COVID-19. The indicators of the presence of left ventricular diastolic dysfunction and the transmitral blood flow parameters were assessed. The study was approved at a meeting of the Medical Ethics Committee under the Ministry of Health of the Republic of Karelia and of Petrozavodsk State University (Minutes No. 47 of 01.11.2023).*

Results. *The frequency of the left ventricular diastolic dysfunction did not differ in the first and second groups of patients. Statistically significant differences were recorded in the average flow deceleration time for both early and late filling of the left ventricle in people who had and did not have COVID-19 infection. A change in the phase structure of the transmitral blood flow may be an early manifestation of intracardiac hemodynamic disorders in people who have recovered from COVID-19.*

Key words: *COVID-19, diastolic function, left ventricle, transmitral blood flow, echocardiography*

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ДИАСТОЛИЧЕСКАЯ ДИСФУНКЦИЯ ЛЕВОГО ЖЕЛУДОЧКА И ПАРАМЕТРЫ ТРАНСМИТРАЛЬНОГО КРОВОТОКА У ЛИЦ, ПЕРЕНЁСШИХ COVID-19

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РЕЗЮМЕ

Обоснование. *У лиц, перенёсших инфекцию COVID-19, имеется повышенный риск развития заболеваний сердечно-сосудистой системы. Диастолическая дисфункция левого желудочка является ранним маркером развития кардиологической патологии. Её своевременное выявление имеет важное значение для назначения адекватной терапии и динамического наблюдения за пациентами. В связи с этим представляется актуальным исследование влияния перенесённой инфекции COVID-19 на диастолическую функцию левого желудочка и показатели трансмитрального кровотока у практически здоровых лиц при отсутствии у них клинико-инструментальных признаков сердечно-сосудистой патологии.*

Цель исследования. *Оценка изменений диастолической и систолической функции левого желудочка, его анатомических параметров и показателей трансмитрального кровотока в двух группах практически здоровых лиц: перенёсших и не перенёсших инфекцию COVID-19.*

Материалы и методы. *Проведена трансторакальная эхокардиография по стандартной методике и проанализированы её результаты у 66 обследованных, признанных практически здоровыми по результатам регулярных комплексных клинико-инструментальных исследований. В первую группу были включены 30 лиц, прошедших эхокардиографическое исследование до пандемии COVID-19 или в период пандемии, но не болевшие коронавирусной инфекцией; вторую группу составили 36 лиц, переболевшие COVID-19. Оценивались показатели, свидетельствующие о наличии диастолической дисфункции левого желудочка, и параметры трансмитрального потока крови. Исследование одобрено на заседании Комитета по медицинской этике при Министерстве здравоохранения Республики Карелия и ФГБОУ ВО «Петрозаводский государственный университет» (протокол № 47 от 11.01.2023).*

Результаты. *Частота выявления диастолической дисфункции левого желудочка не отличалась в первой и второй группах пациентов. Зарегистрированы статистически значимые различия в средних показателях времени замедления потока как раннего, так и позднего наполнения левого желудочка у лиц, перенёсших и не перенёсших инфекцию COVID-19. Изменение фазовой структуры трансмитрального кровотока может быть ранним проявлением нарушений внутрисердечной гемодинамики у лиц, переболевших COVID-19.*

Ключевые слова: *COVID-19, диастолическая функция, левый желудочек, трансмитральный кровоток, эхокардиография*

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RELEVANCE

The coronavirus (COVID-19) pandemic has provided some new conditions and serious new challenges in the diagnosis and treatment of various diseases. Studies and clinical observations have established that the respiratory tract and lungs are not the only targets for coronavirus; the cardiovascular system is also frequently involved in the pathological process [1-3]. Available publications and medical reports, as well as experts of the World Health Organisation show statistics of higher morbidity and mortality of elderly people with various cardiological diseases against the background of a new infection [4]. Therefore, the cardiovascular implications of COVID-19 continue to be actively under discussion [5].

Patients with coronavirus infection have an increased risk of thrombotic events, including myocardial infarction and pulmonary embolism, myocarditis and other cardiac pathologies [6, 7]. The pathophysiological mechanisms of cardiomyocyte injuries in COVID-19 may be hypoxia, thrombotic complications, side effects of pharmacotherapy, and hemodynamic disturbances in the lesser circulation/pulmonary circulation [8–11].

The majority of studies that have been undertaken in this area have involved predominantly elderly patients, many of whom have previous cardiovascular disease, as well as chronic diseases of other organs [2, 6, 12]. However, there is an evidence of cardiac lesions during COVID-19 in patients without previous cardiovascular disease, which has been obtained from clinical studies, magnetic resonance imaging and echocardiography (EchoCG) [1, 10, 13].

The determination of left ventricular diastolic dysfunction (LV DD) is one of the most used and informative methods to diagnose cardiac lesions of various etiologies at early stages [11, 14]. It is observed in almost all cardiac disorders and often precedes the development of systolic dysfunction, changes in LV morphometric parameters, as well as clinical signs of chronic heart failure syndrome.

Three different types of diastolic dysfunction can be categorised, differing primarily by the degree of its progression – relaxation disorder (type 1), pseudonormal variant (type 2) and restrictive variant (type 3). Assessing of early diastolic disorders in the left ventricle during EchoCG, both morphometric parameters of the left ventricle and left atrium in M- and B-modes and parameters of transmittal blood flow and mitral valve fibrous ring motion in spectral Doppler analysis and tissue Doppler modes should be performed [15–17].

At the same time, as shown in several recent studies, LV DD can be diagnosed in patients without clinical or instrumental signs of cardiac pathology (so-called subclinical dysfunction) [11, 18, 19]. In particular, such changes can be observed in a proportion of patients who have had a coronavirus infection [15]. In COVID-19, diastolic dysfunction can be considered as an early marker of changes in intracardiac haemodynamics as a result of infection in the absence of anamnestic, clinical and instrumental data suggestive of cardiac pathology. Consequently, the LV DD detection may be an important part of a comprehensive assessment of cardiac changes during EchoCG in reconvalescents for risk stratification and development of a more targeted treatment approach [5, 16, 19–21]. One of the most informative indicators for detecting early hemodynamic disorders of non-specific genesis are the parameters of the transmittal blood flow, in particular, the ratio of E/A peaks [16, 19].

However, previous echocardiographic follow-up data are important when assessing the impact of coronavirus infection on the cardiovascular system in previously healthy patients, as pointed out by some authors [1]. Besides, it should be confirmed that they have no cardiological pathology based on a sufficiently complete clinical and instrumental examination. The majority of studies devoted to the determination of LV DD in patients with or after COVID-19 infection have performed echocardiographic studies only when the disease has been diagnosed. This prevents the identified changes from being reliably correlated with the coronavirus infection factor. As a result, it is of interest to study the effect of COVID-19 infection on LV diastolic function in practically healthy individuals with previous EchoCG data in order to detect early abnormalities of intracardiac haemodynamics.

In 2018–2019, before the pandemic of coronavirus infection, we evaluated the presence and degree of LV DD in patients with arterial hypertension, while a group of essentially healthy individuals was examined with transthoracic EchoCG [22]. In all patients, parameters of LV DD were determined, including determination of transmitral blood flow parameters. They were also followed up regularly at our medical facility: this group of individuals formed the basis for the present study.

THE AIM OF THE STUDY

To assess the changes in the diastolic and systolic function of the left ventricle, its anatomical parameters and transmitral blood flow parameters in two groups of apparently healthy individuals: those who had and those who had not COVID-19 infection.

METHODS

The study was conducted on the basis of the Department of Ultrasound Diagnostics of the Clinical Hospital «RZhD-Meditsina» of Petrozavodsk and the Department of Radiation Diagnostics and Radiation Therapy with a course of critical and respiratory medicine of Petrozavodsk State University.

In the period from 2019 to 2022, we performed transthoracic EchoCG according to the standard technique and analysed its results in 66 individuals considered practically healthy according to the annually conducted complex clinical and instrumental examination in the conditions of the therapeutic hospital and polyclinic. The study

participants signed a voluntary informed consent for an ultrasound scan.

All those examined are employed as locomotive drivers or driver's assistants in railway transport and therefore undergo an annual medical examining board to determine their job suitability. Examination of these individuals includes clinical and biochemical analyses, electrocardiography (ECG), daily ECG monitoring, transthoracic EchoCG, triplex scanning of brachiocephalic vessels, integral body rheography, examination by narrow specialists, and, if necessary, coronarography or stress EchoCG (in a central departmental clinic). According to the results, no cardiovascular pathology was recorded in all examined individuals.

All those who entered the study were male and their ages ranged from 21 years to 59 years, with a median of 44 years and a mean age of 43.5 years. The control group (group 1) included 30 individuals who underwent transthoracic EchoCG examination before the COVID-19 pandemic, between 2019 and 2020, and during the pandemic, between 2021 and 2022, but who did not have COVID-19. Group 2 consisted of 36 individuals who had experienced coronavirus infection and were examined between 2021 and 2022. The groups were comparable in age: the minimum age in the group 1 was 21 years, the maximum was 56 years, the mean was 43.3 years, and the median was 45.5 years. In the group 2, the minimum age was 31 years, the maximum was 59 years, the mean was 43.9 years, and the median was 43 years.

Confirmation of the COVID-19 disease in all patients of the group 2 was performed by examination of the patient's history (interview after EchoCG), data of outpatient and inpatient examination records (presence of the diagnosis of coronavirus infection), results of polymerase chain reaction (PCR) study and enzyme-linked immunosorbent assay (ELISA) [23]. Positive PCR test results were available in 100 per cent of the group 2 and ELISA test results in 45 per cent. Individuals with a positive ELISA test and no history of positive PCR, anamnestic and documentary evidence of coronavirus infection were not included in the study. The severity of the disease, as well as the assessment of the presence of lung lesions, was not analysed, as the majority of patients (61 %) were treated as outpatients, and some of them lacked the necessary results, such as SpO_2 level, spiral computed tomography findings, etc.

The absence of a coronavirus infection history was determined according to the results of anamnesis collection, data from outpatient and inpatient records, conclusions of regular medical examinations (2 to 4 times a week) for admission to work in the period preceding the examination, negative results of PCR and ELISA tests.

Transthoracic echocardiographic study with Doppler analysis was performed according to the standard technique of the American Society of Echocardiography (ASE, American Society of Echocardiography) using ACUSON S1000 HELX Evolution series devices (Siemens, Germany) in 2021–2022 and VIVID-3 Expert (General E lectric Healthcare, USA) in 2018-2019 with the determination of a number of generally accepted morphofunctional parameters [16, 19]. To assess LV diastolic function, transmitral flow was studied by pulsed-wave Doppler analysis from apical access in a 4-chamber section with the control volume located at the level of the mitral valve leaflet ends (according to ASE 2009 and 2016 recommendations).

In our study, such morphometric parameters of the heart were evaluated as the left ventricular end-diastolic diameter (LV EDD) and left atrium end-systolic diameter (LA ESD), left ventricular end-diastolic volume (LV EDV) and its index (to body surface area (BSA)), left atrium end-systolic volume (LA ESV) and its index (to BSA), interventricular septum thickness and left ventricle posterior wall in the diastole (T_{ivs} and T_{lvpw}, respectively), left ventricular mass (LVM). The values of ejection fraction (EF) and shortening fraction (SF) in per cent calculated by Simpson's formula in B-mode [19] were assessed as indices of left ventricular systolic function. The following indicators were taken as normal values: 55 % or more for EF, 27 % or more for SF.

In addition, if morphometric indices deviated from the standard, their ratio was determined to detect the presence and type of left ventricular remodelling. If signs of left ventricular myocardial hypertrophy were observed, the LVM and the index of left ventricular relative myocardial thickness (RMT) were used to assess its nature. The mass of the myocardium was determined according to the formula recommended by the ASE: $LVM\ (g) = 0.8 \times (1.04 \times (T)_{ivs} + LV\ EDD + T_{lvpw})^3 - (LV\ EDD)^3 + 0.6$, the relative thickness was calculated using the formula: $RMT = (T_{ivs} + T_{lvpw}) / LV\ EDD$ in M-mode under the control of B-mode. According to the results of the examination, the following types of LV geometry disorders were distinguished: concentric hypertrophy, concentric remodeling and eccentric type of hypertrophy.

From the parameters of the transmittal blood flow, the following were estimated: the maximum flow rate of rapid (early) filling (E, m/s); the maximum flow rate of slow (late) filling (A, m/s); the rate ratio E/A; the acceleration time of the rapid filling phase (AT_E, ms); the deceleration time of the rapid filling phase (DT_E, ms). We also determined the following indices, which are not usually used in studies: the acceleration time of the slow filling phase (AT_A, ms); the deceleration time of the fast filling phase (DT_A, ms). The parameters of the mitral valve ring movement indicated in the 2016 recommendations were not taken into account by us, since they were not determined in patients examined on the VIVID-3-Expert ultrasound machine (General Electric Healthcare, USA) due to the lack of tissue Doppler analysis mode in it. In our opinion, this is not a critical shortcoming of the study, as it is illogical to expect the presence of type 2 or 3 LV DD in the patients with no clinical or instrumentally detectable signs of cardiovascular pathology. In initial (subclinical) disorders of intracardiac haemodynamics, type 1 LV DD may be observed; the estimated indices are sufficient for its detection.

The study was conducted by double-blind sampling. If patients met the requirements for selection for the study (the assessment was performed by a specialist not involved in performing the EchoCG), they were interviewed

and their medical records were reviewed by a physician after the EchoCG. Accordingly, the patient's history was known to the clinician only after all indicators had been assessed and a conclusion had been formed. Patients were also unaware of their participation in the study and which group they belonged to prior to the survey. The analysis of individual dynamics of echocardiographic indices for each patient was not performed since the examined patients underwent examinations in different time periods at various diagnosticians, so the obtained data will have low validity. Here it is necessary to consider the operator's error during measurements, which can be critical in case the estimated parameter exceeds the normal values during individual assessment. During the statistical processing of group indicators it was considered by us using the determination of the standard confidence interval.

We have performed calculations of average values of the assessed indicators by groups and their statistical assessment. Standard tools and formulas of the MS Excel spreadsheet processor from the Microsoft Office 2019 software package (Microsoft Corp., USA) were used for statistical processing. The statistical assessment of all data had a normal distribution (the test for normal distribution was performed using the Shapiro – Wilk criterion). The parametric Student's *t* test was chosen for comparison of mean values; differences in the values of indicators were considered statistically significant at $p < 0.05$. The hypothesis of equality of variance was pre-tested in checking the hypothesis of position (hypothesis of equality of mean values in two samples) using Student's criterion. For this purpose, Fisher's criterion (φ*) was used to compare the indices of the same sample measured in different conditions; differences in the values of indices were considered statistically significant at $p < 0.05$. These results present the data in the form of mean values of the estimated indices with a standard error of mean square.

RESULTS

We obtained the following results during the assessment of morphometric indices at EchoCG, as presented in Table 1. In the groups 1 and 2 of the examined patients, the indicators of LA ESD, as well as LA ESV and its indexed values were almost the same; the differences were not statistically significant ($t > 0.05$). However, the maximum values of these indices were slightly outside the normal range in only one COVID-19 non-diseased and 3 overdiseased patients, respectively.

When left ventricular parameters were assessed, the mean left ventricular EDD values in the group 1 were 50.8 ± 3.3 mm and in the group 2 were 49.8 ± 2.4 mm; the differences were also statistically non-significant ($t > 0.05$). The same results were obtained when compar-

TABLE 1

MORPHOMETRIC AND FUNCTIONAL INDICES OF THE LEFT VENTRICLE AND LEFT ATRIUM IN THE TWO GROUPS OF PATIENTS STUDIED

Indicators	Group 1 (*n* = 30)	Group 2 (*n* = 36)	t-test
LA ESD, mm	35.6 ± 3.3	36.6 ± 2.8	0.25
LA ESV, ml	53.1 ± 6.2	56.6 ± 5.7	0.31
LA IESV, ml/m^2	27.9 ± 2.5	29.8 ± 2.7	0.23
LV EDD, mm	50.8 ± 3.3	49.8 ± 2.4	0.18
LV EDV, ml	122.7 ± 27.8	117.15 ± 24.5	0.45
LV IEDV, ml/m^2	64.5 ± 3.2	61.4 ± 3.9	0.41
$T_{ivs,}$ мм	10.6 ± 1.3	11.1 ± 1.2	0.74
$T_{lvpw,}$ mm	10.3 ± 1	10.3 ± 0.9	0.65
ILVM g/m^2	84.3 ± 5.3	90.2 ± 6.9	0.53
RMT	0.42 ± 0.04	0.42 ± 0.04	0.17
LVEF average value, %	64.0 ± 4	65.3 ± 4.8	0.25
LVSF average value, %	35.1 ± 4.1	36.3 ± 3.9	0.79

Note. LA IESV – indexed left atrium end-systolic volume; LV IEDV – indexed left ventricular end-diastolic volume; ILVM – indexed left ventricular myocardial mass.

ing mean LV end-diastolic volumes and their indexed values – 64.5 ± 3.2 ml/m^2 for the group 1 and 61.4 ± 3.9 ml/m^2 for the group 2.

The analysis of the average values of the T_{ivs} in the groups 1 and 2 also obtained almost identical results. At the same time, signs of minor myocardial hypertrophy of the IVS were detected in three examined patients of the group 1 (T_{ivs} = 12 mm) and in 5 individuals from the group 2 (T_{ivs} = 12–13 mm). When estimating the average value of LVPW diastolic thickness in the group 1, the indicator was 10.3 ± 1 mm, in the group 2 – 10.3 ± 0.9 mm ($t > 0.05$).

LV myocardial mass indices did not differ statistically significantly in the groups 1 and 2 and were within normal values; the average values of RMT in the groups 1 and 2 were almost identical. At the same time, signs of LV remodeling were detected in 3 (10 %) examined from the group 1, of them by type of concentric – in 2 cases, by type of concentric hypertrophy – in 1 case. In the group 2, changes in LV geometry were detected in 7 (19 %) patients, according to the type of concentric remodeling – in 4 examined patients, according to the type of concentric hypertrophy – in 2 cases. Criterion φ* = 1,084, the difference in the frequency of detection of the sign is not statistically significant. In general, deviations of anatomical parameters from the norm out of 66 examined patients were noted in 6 (9 %) individuals who did not have COVID-19 and 8 (12 %) individuals who had coronavirus infection; the difference is not statistically significant (φ = 0.218).

As for the assessment of LV systolic function, the average EF index (Table 1) in the examined groups 1 and 2 were within normal values; the differences were not statistically significant ($t > 0.05$). Similar results were obtained when calculating the SF index, while the minimum values also did not exceed the normal ones.

When evaluating the parameters of the transmittal flow, we obtained the results presented in Table 2; the indicators are presented as an average value with a standard deviation. Differences in the average values of peak E in the groups 1 and 2 were not statistically significant ($t > 0.5$), while indicators above the age norm were noted in 6 examined patients who did not have COVID-19 and in 7 patients who had COVID-19; criterion φ* = 0.556. The calculated average values of AT_E also did not differ significantly in the groups 1 and 2 ($t > 0.05$); this indicator was not taken into account to determine the presence and type of LV DD. The average values of DT_E were 115.1 ± 45.2 ms (group 1) and 84.2 ± 26.3 (group 2); deviation from normal values was detected only in 1 individual who did not have COVID-19. The difference in indicators for AT_E is not statistically significant ($t > 0.05$), for DT_E it is statistically significant ($t < 0.05$).

For A peak, the calculated average values in the groups 1 and 2 were almost the same ($t > 0.05$). The AT_A average values in those who had and did not have coronavirus infection were quite close ($t > 0.5$), while for the DT_A they differed significantly: 115.1 ± 45 ms (group 1) and 84.22 ± 2.3 ms (group 2), respectively. There are no generally accepted normal values for these indicators, so it is not possible to say whether they deviate from the normal values. The difference in the groups in the first case is not statistically significant ($t > 0.05$), in the second – statistically significant ($t < 0.05$).

When assessing the E/A ratio in the groups 1 and 2, the average values were similar ($t > 0.05$), while changes characteristic of LV DD according to type 1 were detected in 18 of the 66 (27 %) examined: 9 (30 %) patients of the group 1 and also in 9 (25 %) individuals of the group 2; the differences obtained in the two groups are not statistically significant (φ = 0.453).

TABLE 2

LEFT VENTRICULAR DIASTOLIC DYSFUNCTION AND TRANSMITTAL BLOOD FLOW PARAMETERS IN TWO GROUPS OF PATIENTS

Parameters	Group 1 (n = 30)	Group 2(n = 36)	t-test
E average value, m/s	0.82 ± 0.15	0.83 ± 0.16	0.89
AT_E average value, ms	60 ± 20.5	78.6 ± 13.5	0.31
DT_E average value, ms	115.1 ± 45.2	84.2 ± 26.3	0.02
A average value, m/s	0.63 ± 0.11	0.64 ± 0.1	0.76
AT_A average value, ms	82.1 ± 14.7	78.6 ± 13.5	0.74
DT_A average value, ms	115.1 ± 45	138.8 ± 29.9	0.01
E/A	1.35 ± 0.28	1.32 ± 0.28	1.32

DISCUSSION

Thus, in our study, there were no statistically significant differences in the frequency of detection of signs of LV DD in the two groups of examined patients. This does not suggest the existence of a correlation between the development of dysfunction and COVID-19 infection. However, when evaluating the results of our study, it is necessary to take into account certain limitations of the sample and the fact that measurements at different points in time for the diagnosis of LV diastolic dysfunction can lead to potential heterogeneity of results [11].

We identified a fairly significant percentage (27 %) of LV diastole disorders in the control group of the examined patients. Consideration and analysis of the causes of LV diastole disorders in echocardiographic examination of practically healthy individuals, demonstrated in our previous study and a number of other works, are beyond the scope of this article [18, 20, 24]. In this case, it is advisable to talk about the so-called subclinical LV DD, which can be an early sign of disorders of intracardiac hemodynamics and subclinical systolic dysfunction, which is not detected by traditional echocardiographic parameters [20, 25].

We have identified statistically significant differences in the average time of slowing down the flow of both early and late filling of the left ventricle in individuals who have and have not had COVID-19 infection. No such differences were found in other parameters of the transmittal flow. This may indicate the initial manifestations of a violation of the phase structure of the transmittal flow in the group 2 of examined patients. At the same time, patients who have had coronavirus infection have signs indicating some statistically significant differences from the control group in the phases of both active and passive relaxation of the left ventricle. Thus, during the period of active filling, there was a shortening of the time of deceleration of the flow of DT_E compared with the group 1 (although its indicators were within the normal range for most of the examined patients), which indicates an increase in LV filling pressure. In the phase of passive filling, an elongation of the DT_A index was noted, which characterizes a deceleration of this process.

The clinical significance of the revealed differences in the parameters of transmittal blood flow in patients with COVID-19 compared with the control group is currently unclear, however, they may indicate initial violations of intracardiac hemodynamics. It cannot be excluded that they are the result of the damaging effect of the COVID-19 virus, which, according to a number of studies, has an affinity to the myocardium and endocardium, and myocardial damage can be detected even after recovery [25–27]. For a more accurate assessment and correlation of the results obtained with the COVID-19 factor, a promising study is needed to determine the parameters of the transmittal blood flow and the movement of the fibrous ring of the mitral valve in the mode of tissue Doppler sonography in the examined group 2.

CONCLUSIONS

Signs of left ventricular diastolic dysfunction were detected in two groups of examined patients, while the frequency of its detection did not differ statistically significantly in those who had and had not suffered COVID-19 infection. In patients with COVID-19, certain statistically significant differences in the phase structure of the transmittal flow were registered compared with the control group, which may be a marker of early disorders of intracardiac hemodynamics. There were no statistically significant differences in the estimated morphometric parameters and indicators of systolic function of the left ventricle in the examined patients with a history of COVID-19 infection and without it.

Conflict of interest

The authors of this article declare the absence of a conflict of interest.

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CLINICAL AND PSYCHOLOGICAL CORRELATIONS WITH TYPE D PERSONALITY IN PATIENTS WITH CHRONIC CORONARY SYNDROME

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ABSTRACT

Recently, it has become common to identify type D personality, which is predisposed to the development of psychological distress. Negative behavioral characteristics of individuals with type D personality contribute not only to the development of cardiovascular diseases, but also to other comorbid pathologies that can influence the progression and prognosis of coronary heart disease.

The aim of the study. *To identify clinical and psychological correlations with type D personality in patients with chronic coronary syndrome.*

Methods. *The study included 113 patients (68 men and 45 women; median age – 64 years) admitted for planned percutaneous coronary intervention at the Research Institute for Complex Issues of Cardiovascular Diseases (Kemerovo, Russian Federation). Based on the results of the DS-14 test, patients were divided into two groups: patients with type D personality (n = 40) and patients without this type (n = 73).*

Results. *In patients with chronic coronary syndrome with type D personality, compared with patients without this type, concomitant diabetes mellitus (35 % and 15 %, respectively; p = 0.018), signs of diastolic dysfunction of left (E/e' ratio 7.1 [6.48; 8.0] and 5.0 [4.55; 5.74], respectively; p = 0.0038) and right (Et/At ratio – 0.8 [0.66; 1.35] and 1.38 [1.28; 1.63], respectively; p = 0.014) ventricles were more often diagnosed. Correlation analysis revealed associations of diabetes mellitus with type D personality (r = 0.243; p = 0.011), severity of negative excitability (r = 0.253; p = 0.008) and social suppression (r = 0.224; p = 0.020), as well as association of ankle-brachial index (ABI) with the severity of negative excitability (r = 0.393; p = 0.004) and social suppression (r = 0.414; p = 0.002).*

Conclusion. *In patients having chronic coronary syndrome with type D personality, concomitant diabetes mellitus, as well as left and right ventricular filling disorders are more often detected. Correlation analysis revealed associations of diabetes mellitus with type D personality and its subscales; the ABI level was associated with subscales of type D personality, but not with the level of anxiety and depression.*

Key words: *type D personality, psychological risk factors, chronic coronary syndrome*

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КЛИНИКО-ПСИХОЛОГИЧЕСКИЕ КОРРЕЛЯЦИИ ПРИ ТИПЕ ЛИЧНОСТИ Д У БОЛЬНЫХ ХРОНИЧЕСКИМ КОРОНАРНЫМ СИНДРОМОМ

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РЕЗЮМЕ

В последнее время принято выделять тип личности Д, предрасположенный к развитию психологического дистресса. Негативные поведенческие особенности лиц с типом личности Д способствуют не только развитию сердечно-сердечных заболеваний, но и другой коморбидной патологии, способной влиять на прогрессирование и прогноз ишемической болезни сердца (ИБС).

Цель исследования. *Выявить клинико-психологические корреляции при типе личности Д у больных хроническим коронарным синдромом.*

Методы. *В исследование включены 113 пациентов (68 мужчин и 45 женщин; медиана возраста – 64 года), поступившие на плановое чрескожное коронарное вмешательство (ЧКВ) в ФГБНУ «Научно-исследовательский институт комплексных проблем сердечно-сосудистых заболеваний». По результатам теста DS-14 пациенты были разделены на две группы: пациенты с типом личности Д (n = 40) и пациенты с его отсутствием (n = 73).*

Результаты. *У больных хроническим коронарным синдромом с типом личности Д по сравнению с пациентами с отсутствием типа Д чаще диагностировали сопутствующий сахарный диабет (35 % и 15 % соответственно; p = 0,018), признаки диастолической дисфункции левого (отношение E/e' – 7,1 [6,48; 8,0] и 5,0 [4,55; 5,74] соответственно; p = 0,0038) и правого (отношение Et/At – 0,8 [0,66; 1,35] и 1,38 [1,28; 1,63] соответственно; p = 0,014) желудочка. При корреляционном анализе выявлены ассоциации сахарного диабета с типом личности Д (r = 0,243; p = 0,011), выраженностью негативной возбудимости (r = 0,253; p = 0,008) и социального подавления (r = 0,224; p = 0,020), а также ассоциации лодыжечно-плечевого индекса (ЛПИ) с выраженностью негативной возбудимости (r = 0,393; p = 0,004) и социального подавления (r = 0,414; p = 0,002).*

Заключение. *У больных хроническим коронарным синдромом с типом личности Д чаще выявляется сопутствующий сахарный диабет, а также нарушения наполнения левого и правого желудочка. При корреляционном анализе выявлены ассоциации сахарного диабета с типом личности Д и его подшкалами, уровень ЛПИ был ассоциирован с подшкалами типа личности Д, но не с уровнем тревожности и депрессии.*

Ключевые слова: *тип личности Д, психологические факторы риска, хронический коронарный синдром*

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INTRODUCTION

Currently, the main trend in medical care is a patient-oriented approach. This is a fairly broad term that includes both personalized medicine (assessment of genetic and epigenetic factors, consideration of gender, ethnic, and social characteristics) and indicators focused on the personal traits of the patient (the effect of treatment on quality of life, the patient's choice of the nature of treatment depending on the profession, psychological characteristics, etc.). In the latter case, it is inevitably necessary to take into account the psychological characteristics of the patient, for example, attitude to the disease [1], ways to overcome stressful situations [1–3], manifestations of psychological distress [2, 4, 5]. Recently, it has been customary to identify a special type of personality predisposed to the development of psychological distress (or type D personality). It is characterized by a combination of pessimistic emotions in response to stressful situations and introversion [6]; people with this type of personality more often develop depressive reactions [2, 7]. The presence of type D personality is associated with poor quality of life [8–10] and with an unfavorable prognosis for various diseases [11, 12]. Cardiovascular diseases have been studied most of all in this regard; exactly in these diseases that this type of personality was first identified and proposed for clinical use [13]. Further studies have made some clarifications to the prognostic effect of type D personality: a negative effect on the prognosis was confirmed in patients with coronary heart disease (CHD) and in younger patients [14, 15] and none was found in chronic heart failure (CHF) and in older age groups [16]. The adverse effect on the prognosis in patients with CHD with type D personality makes us think about both the possible mechanisms through which this prognosis is realized, and ways to correct this effect. In general, two main pathogenetic mechanisms of realization of the negative impact of type D personality in patients are considered: biological (exposure through activation of neurohormonal systems in response to stressful effects, development of endothelial dysfunction, etc. [16–18]) and behavioral (tendency to an unhealthy lifestyle, negative reaction to doctor's recommendations, less frequent seeking medical help [19]). In addition, negative behavioral features of individuals with type D personality contribute not only to the development of cardiovascular diseases, but also to other comorbid pathologies that can influence the progression and prognosis of coronary heart disease [16, 20]. Therefore, there is a need for a broader assessment of the clinical condition of patients in order to identify the possible additional effect of comorbid conditions on the prognosis in patients with coronary heart disease with type D personality. This served as the basis for this study, the aim of which was to identify clinical and psychological correlations in type D personality in patients with chronic coronary syndrome.

MATERIAL AND METHODS

113 patients were included in the study, 68 men and 45 women. The median age was 64 (58.0; 69.0) years. All patients were admitted for elective percutaneous coronary intervention (PCI) in the department of surgical treatment of complex cardiac arrhythmias and pacing of the Research Institute for Complex Issues of Cardiovascular Diseases in the period from October 2020 to October 2021. The inclusion criteria were as follows: stable CHD; planned PCI; ability to adequately complete the questionnaire. Exclusion criteria: acute coronary syndrome; severe concomitant diseases; inability of the patient to complete the questionnaire; refusal of the patient to participate in the study. The study was approved by the Ethics Committee of Research Institute for Complex Issues of Cardiovascular Diseases (extract from meeting No. 8 dated October 10, 2022) and was conducted in accordance with the World Medical Association Declaration of Helsinki. After signing the voluntary informed consent, the patient was included in the study.

At the hospital stage, a standard preoperative examination was performed, including an echocardiographic examination using an extended protocol on Vivid S5 (General Electric Healthcare, USA). The images were obtained using the long and short axes of the parasternal and apical projections. When analyzing left atrium and ventricle of the heart, its structural characteristics were evaluated: left ventricular (LV) end-systolic and end-diastolic volumes, LV mass, and left atrium (LA) maximum transverse diameter in the diastole. The left ventricular ejection fraction (LVEF) was calculated using the Simpson method. When analyzing the structural characteristics of right atrium and ventricle of the heart, the following indicators were evaluated: right ventricle wall thickness in diastole (RVWd), the size of the right ventricle (RV) and right atrium, tricuspid annular plane systolic excursion (TAPSE).

LV functional parameters were studied in the Doppler mode: isovolumic relaxation time (IRT), maximum velocity of early (E) and late transmitral filling (A) of LV and their ratio (E/A). In the analysis of RV diastolic function, velocity of early (Et) and late transtricuspid filling (At) of RV and their ratio (Et/At) were estimated. The velocity of early/late diastolic/systolic excursion of rings of mitral/tricuspid of valves and their ratios (e', a', e'/a', E/e'; e't, a't, e't/a't, Et/e't, s', s't), as well as Tei index of the left and right ventricles was measured in the mode of spectral tissue Dopplerometry. RV diastolic dysfunction was considered to be cases when the value of the Et/At ratio was < 0.8 or > 2.1 and/or the Et/e't ratio was > 6.

All patients in the hospital were additionally examined using volumetric sphygmography (VaSera VS-1000, Fukuda Denshi, Japan). The device automatically calculates vascular stiffness indicators – cardio-ankle vascular index (CAVI), blood pressure, ancle-brachial index (ABI) on the right and left.

During the examination, additional patient questionnaires were conducted. In order to identify patients with type D personality, the DS-14 questionnaire was used, including the subscales NA («negative affectivity») and SI («social inhibition») in 14 questions [21]. Type D personality was diagnosed with 10 points or more for each of the studied subscales. The patients were divided into two groups

TABLE 1
CLINICAL AND DEMOGRAPHIC CHARACTERISTICS OF COMPARED GROUPS

Indicators	Type D (*n* = 40)	Not Type D (*n* = 73)	*p*
Male, *n* (%)	18 (45.0)	27 (37.0)	0.4
Age (years), Me [LQ; UQ]	64.0 [56.0; 70.0]	64.0 [58.5; 69.0]	0.64
BMI (kg/m^2), Me [LQ; UQ]	24.9 [21.8; 27.3]	21.3 [21.3; 27.7]	0.66
Disability, *n* (%)	12 (30.0)	22 (30.1)	0.95
Employed, *n* (%)	14 (35.0)	32 (43.8)	0.365
Retired, *n* (%)	26 (65.0)	41 (56.2)	0.265
Smokers, *n* (%)	16 (40.0)	34 (46.5)	0.38
Arterial hypertension, *n* (%)	33 (82.5)	60 (82.2)	0.735
Diabetes mellitus, *n* (%)	14 (35.0)	11 (15.0)	0.018
Prior stroke, *n* (%)	4 (10.0)	6 (8.2)	0.806
Prior myocardial infarction, *n* (%)	19 (47.5)	43 (58.9)	0.16
Prior coronary bypass surgery, *n* (%)	4 (10.0)	6 (8.2)	0.81
Prior carotid endarterectomy, *n* (%)	2 (5.0)	1 (1.4)	0.26
Angina pectoris, *n* (%)	33 (82.5)	10 (86.3)	0.635
CHF, *n* (%)	37 (92.5)	4 (94.5)	0.7
Laboratory parameters			
Total cholesterol (mmol/L), Me [LQ; UQ]	4.15 [3.4; 4.85]	4.0 [3.5; 5.2]	0.58
HDL (mmol/L), Me [LQ; UQ]	1.26 [0.93; 1.77]	1.03 [0.85; 1.22]	0.18
LDL (mmol/L), Me [LQ; UQ]	2.67 [1.75; 3.84]	2.62 [1.81; 3.03]	0.7
Triglycerides (mmol/L), Me [LQ; UQ]	1.4 [1.1; 1.9]	1.3 [1.1; 1.6]	0.51
Urea (mmol/L), Me [LQ; UQ]	6.85 [5.75; 8.2]	6.4 [5.35; 8.2]	0.38
Creatinine (mmol/L), Me [LQ; UQ]	84.5 [75.0; 99.5]	92.0 [78.0; 114.0]	0.11
Glucose (mmol/L), Me [LQ; UQ]	6.35 [5.15; 7.7]	5.9 [5.3; 6.8]	0.12
Lesion of vascular beds			
Stenosis of one coronary artery, *n* (%)	28 (70.0)	50 (68.5)	0.76
Stenosis of two coronary arteries, *n* (%)	8 (20.0)	16 (21.9)	0.811
Stenosis of three coronary arteries, *n* (%)	3 (7.5)	6 (8.2)	0.892
Stenosis of left main coronary artery, *n* (%)	1 (2.5)	1 (1.37)	0.66
Stenosis of the internal carotid artery more than 50%, *n* (%)	4 (10.0)	9 (12.3)	0.71
Stenosis of the internal carotid artery more than 30%, *n* (%)	3 (7.5)	4 (5.48)	0.67

Note. CHF – chronic heart failure; HDL – high density lipoproteins; LDL – low density lipoproteins.

based on the test results: patients with type D personality (n = 40) and patients without type D personality (n = 73). Additionally, the level of anxiety and depression in patients was assessed using the Hospital Anxiety and Depression Scale (HADS) [22]. Four possible answers correspond to each HADS statement. The higher the overall score, the more pronounced the symptoms of anxiety or depression.

Statistical processing was carried out using the Statistica 8.0 software package (StatSoft Inc., USA). The distribution of quantitative variables for normality was checked using the Shapiro – Wilk test; due to the fact that the distribution was different from normal, these variables are represented as median and quartiles (25th and 75th percentiles). Mann – Whitney and χ^2 (chi-squared) test were used to compare groups with and without type D personality. The Spearman correlation was used to evaluate the associations of clinical indicators with type D personality, its subscales (NA and SI), levels of anxiety and depression. The value of 0.05 was taken as the level of critical significance (p).

STUDY RESULTS

The median age of patients in both groups was 64 years. It should be noted that male patients prevailed in the group with type D personality, in contrast to the group without type D personality (45.0 % vs. 37.0 %, respectively; p = 0.4). Most patients had arterial hypertension. Type 2 diabetes mellitus was more common in patients with type D personality (p = 0.018). A history of myocardial infarction was detected equally often in patients of both groups, as well as a history of stroke. As the results show, there were no statistically significant differences in glucose, creatinine, urea concentrations and lipid profile parameters between the study groups. According to color duplex scanning of extracranial arteries, no differences were found in the groups. According to the coronarography data, no statistically significant intergroup differences were obtained (Table 1).

The analysis of volumetric sphygmography indicators among the entire cohort of patients with coronary heart disease is presented in Table 2.

The median systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate (HR) were within the normal range. The median stiffness index (CAVI) was within the boundary values in patients with type D personality, and in patients without type D personality, the median stiffness of CAVI exceeded 9.0 and was pathological, but no statistically significant difference was achieved in the groups ($p > 0.05$). ABI < 0.9 was more often detected in patients with type D personality ($p > 0.05$).

Table 3 shows the assessment of structural and functional parameters according to echocardiography data.

The median left ventricular ejection fraction in both groups was in the range of normal values. Evaluation of transtricuspid flows in the group of patients

TABLE 2

INDICATORS OF VOLUMETRIC SPHYGMOGRAPHY (VASERA VS-1000, FUKUDA DENSHI, JAPAN)

Indicators	Type D (n = 40)	Not Type D (n = 73)	p
SBP on the right (mmHg), Me [LQ; UQ]	131.5 [117.0; 144.0]	131.0 [119.0; 141.0]	0.63
SBP on the left (mmHg), Me [LQ; UQ]	130.0 [121.0; 144.0]	127.5 [118.0; 139.0]	0.43
DBP on the right (mmHg), Me [LQ; UQ]	80.0 [76.0; 90.0]	80.0 [73.0; 84.0]	0.29
DBP on the left (mmHg), Me [LQ; UQ]	78.0 [72.0; 86.0]	78.0 [73.0; 86.0]	0.83
HR (min), Me [LQ; UQ]	59.0 [54.0; 65.0]	61.0 [55.0; 73.0]	0.12
CAVI on the right, Me [LQ; UQ]	8.5 [7.9; 10.4]	9.2 [7.6; 10.5]	0.614
CAVI on the left, Me [LQ; UQ]	8.3 [7.8; 9.3]	9.3 [7.6; 10.1]	0.252
CAVI > 9.0	12 (30.0)	24 (32.2)	0.192
ABI on the right, Me [LQ; UQ]	1.02 [0.92; 1.16]	1.09 [0.96; 1.18]	0.53
ABI on the left, Me [LQ; UQ]	1.01 [0.85; 1.13]	1.01 [0.92; 1.12]	0.61
ABI < 0.9	13 (32.5)	19 (26.0)	0.563

Note. SBP – systolic blood pressure; DBP – diastolic blood pressure; HR – heart rate; CAVI – cardio-ankle vascular index; ABI – ancle-brachial index.

TABLE 3

ECHOCARDIOGRAPHY INDICATORS DEPENDING ON THE PRESENCE OF THE TYPE D PERSONALITY

Indicators	Type D (*n* = 40)	Not Type D (*n* = 73)	*p*
Ao (mm), Me [LQ; UQ]	3.5 [3.3; 3.7]	3.5 [3.5; 3.9]	0.48
LA (mm), Me [LQ; UQ]	4.4 [4.1; 4.7]	4.3 [3.9; 4.6]	0.21
LV EDS (mm), Me [LQ; UQ]	5.2 [5.0; 5.5]	5.5 [5.1; 6.1]	0.197
LV ESS (mm), Me [LQ; UQ]	3.3 [3.2; 3.5]	3.5 [3.2; 4.2]	0.129
LV EDV (ml), Me [LQ; UQ]	124.0 [118.0; 147.0]	147.0 [124.0; 180.0]	0.079
LV ESV (ml), Me [LQ; UQ]	44.0 [38.0; 51.0]	51.0 [41.0; 79.0]	0.067
LVEF (%), Me [LQ; UQ]	62.0 [58.0; 65.0]	63.0 [51.0; 68.0]	0.73
IE (ml), Me [LQ; UQ]	85.0 [77.0; 96.0]	90.0 [77.0; 96.0]	0.14
LV mass (g), Me [LQ; UQ]	213.0 [181.0; 262.0]	247.0 [200.0; 287.0]	0.311
LV MMI, Me [LQ; UQ]	116.0 [100.5; 136.5]	118.0 [100.0; 151.0]	0.48
ISTd (cm), Me [LQ; UQ]	1.1 [1.0; 1.3]	1.0 [1.0; 1.2]	0.68
LVPVTd (cm), Me [LQ; UQ]	1.1 [1.0; 1.3]	1.0 [1.0; 1.2]	0.311
Indicators of LV diastolic function			
LV IRT (ms), Me [LQ; UQ]	88.5 [88.0; 90.0]	90.0 [88.0; 90.0]	0.162
E (cm/s), Me [LQ; UQ]	65.0 [51.0; 78.0]	54.0 [47.0; 70.0]	0.43
A (cm/s), Me [LQ; UQ]	63.0 [56.0; 80.0]	67.0 [50.0; 88.0]	0.84
E/A, Me [LQ; UQ]	0.81 [0.68; 1.27]	0.77 [0.67; 1.31]	0.72
e' (cm/s), Me [LQ; UQ]	9.8 [7.5; 10.4]	10.6 [9.6; 12.5]	0.21
a' (cm/s), Me [LQ; UQ]	9.4 [8.0; 11.0]	10.0 [7.5; 11.0]	0.66
e'/a', Me [LQ; UQ]	0.86 [0.7; 1.06]	1.32 [1.06; 1.59]	0.082
s' (cm/s), Me [LQ; UQ]	9.2 [8.4; 11.2]	10.4 [9.0; 12.0]	0.19
E/e', Me [LQ; UQ]	7.1 [6.48; 8.0]	5.0 [4.55; 5.74]	0.0038
LV Tei-index, Me [LQ; UQ]	0.29 [0.23; 0.32]	0.27 [0.25; 0.35]	0.71
Indicators of the right ventricle before CABG			
RV (mm), Me [LQ; UQ]	2.0 [2.0; 2.2]	2.0 [1.9; 2.2]	0.81
RVEF (%), Me [LQ; UQ]	50.0 [46.0; 54.0]	50.0 [47.0; 55.0]	0.78
RA (mm), Me [LQ; UQ]	118.0 [109.0; 129.0]	122.0 [113.0; 131.0]	0.39
PAP av. (mmHg), Me [LQ; UQ]	13.0 [11.0; 17.0]	13.0 [12.0; 15.0]	0.68
Indicators of diastolic function			
Et (cm/s), Me [LQ; UQ]	41.0 [36.0; 48.0]	49.5 [45.0; 51.0]	0.013
At (cm/s), Me [LQ; UQ]	44.0 [34.0; 56.0]	36.0 [33.0; 39.0]	0.062
Et/At, Me [LQ; UQ]	0.8 [0.66; 1.35]	1.38 [1.28; 1.63]	0.014
e›t (cm/s), Me [LQ; UQ]	11.6 [10.6; 11.9]	9.8 [8.6; 11.3]	0.033
a›t (cm/s), Me [LQ; UQ]	14.5 [12.5; 15.6]	12.5 [11.1; 15.2]	0.26
e't/a't, Me [LQ; UQ]	0.82 [0.69; 0.95]	0.68 [0.58; 0.95]	0.06
s't (cm/s), Me [LQ; UQ]	13.0 [12.5; 14.6]	12.5 [11.6; 14.6]	0.43
Et/e't, Me [LQ; UQ]	3.58 [3.19; 4.47]	5.0 [4.17; 5.81]	0.014
RV Tei index, Me [LQ; UQ]	0.27 [0.24; 0.29]	0.27 [0.23; 0.29]	0.81

Note. Ao – aorta; LA – left atrium (diameter); EDS – end-diastolic size; LV – left ventricle; ESS – end-systolic size; EDV – end-diastolic volume; ESV – end-systolic volume; EF – ejection fraction; IE – impact ejection; MMI – myocardial mass index; ISTd – interventricular septum thickness in the diastole; LVPVTd– left ventricular posterior wall thickness in the diastole; IRT – isovolumic relaxation time; E – velocity of early diastolic filling of the left ventricle; A – velocity of late diastolic filling of the left ventricle; e' – lateral early diastolic mitral annular velocity; a' – lateral late diastolic mitral annular velocity; s' – lateral systolic mitral annular velocity; RV – right ventricle; RA – right atrium (diameter); PAP av. – pulmonary artery average pressure; Et – rate of early diastolic filling of the right ventricle; At – rate of late diastolic filling of the right ventricle; e't – rate of early tricuspid annular plane diastolic excursion a't – rate of late tricuspid annular plane diastolic excursion; s't – rate of tricuspid annular plane systolic excursion.

with type D personality revealed a decrease in the velocity of early tricuspid annular plane diastolic excursion (e't) compared with the group without type D personality (p = 0.033). The median ratio of early and late diastolic transtricuspid flow (Et/At) was within the normal values in all patients, however, the ratio of Et/At in patients with type D personality was lower compared with the group without type D personality (p = 0.014). It was revealed

TABLE 4

PSYCHOLOGICAL STATUS OF PATIENTS WITH CORONARY HEART DISEASE DEPENDING ON THE PRESENCE OF TYPE D PERSONALITY

Indicators	Type D (n = 40)	Not Type D (n = 73)	p
	Scales of the DS-14 questionnaire		
NA (score)	14.0 [12.0; 16.0]	8.0 [6.0; 9.0]	< 0.001
SI (score)	12.5 [11.0; 14.0]	8.0 [6.0; 9.0]	< 0.001
	HADS scale		
Personal anxiety (score)	7.0 [5.0; 10.0]	5.0 [3.0; 7.0]	< 0.001
Depression level (score)	5.0 [3.0; 9.0]	4.0 [2.0; 6.0]	0.0102

Note. NA – negative affectivity; SI – social inhibition.

TABLE 5

CORRELATIONS OF PSYCHOLOGICAL STATUS WITH CLINICAL INDICATORS

Indicators	Type D		NA		SI		Anxiety		Depression	
	r	p	r	p	r	p	r	p	r	p
Age (years)	0.059	0.550	–0.009	0.921	0.107	0.282	0.215	0.029	–0.009	0.930
Weight (kg)	–0.197	0.046	–0.057	0.567	–0.051	0.616	–0.159	0.109	–0.036	0.716
BMI (kg/m^2)	–0.128	0.195	–0.018	0.854	–0.119	0.229	–0.136	0.172	0.059	0.550
Smoking	0.008	0.935	0.071	0.474	0.148	0.134	0.141	0.155	0.115	0.249
Prior MI	–0.119	0.229	–0.016	0.876	0.069	0.489	0.047	0.662	–0.029	0.768
Prior stroke	0.073	0.466	0.211	0.033	0.091	0.363	–0.097	0.328	0.093	0.350
Prior AH	–0.018	0.855	0.072	0.467	0.106	0.283	–0.117	0.239	–0.014	0.890
Prior diabetes	0.243	0.011	0.253	0.008	0.224	0.020	0.075	0.435	0.018	0.851
SBP	0.153	0.274	0.301	0.028	0.051	0.716	0.144	0.303	0.263	0.057
DBP	0.186	0.182	0.122	0.383	0.006	0.962	0.295	0.032	0.103	0.461
ABI < 0.9	0.266	0.054	0.393	0.004	0.414	0.002	0.181	0.897	0.089	0.526
CAVI > 9.0	–0.104	0.456	0.086	0.539	0.171	0.220	0.058	0.678	–0.009	0.947
HDL	0.246	0.185	0.331	0.073	0.001	0.999	0.144	0.448	0.462	0.01
LDL	–0.133	0.481	–0.389	0.034	–0.228	0.226	–0.062	0.745	–0.189	0.315
Creatinine	–0.107	0.573	–0.011	0.995	–0.039	0.834	–0.124	0.506	0.122	0.521
Glucose	–0.051	0.792	0.215	0.252	–0.105	0.579	–0.081	0.667	–0.047	0.804

Note. NA – negative affectivity; SI – social inhibition; BMI – body mass index; MI – myocardial infarction, AH – Arterial Hypertension, DM – diabetes mellitus; SBP – systolic blood pressure; DBP – diastolic blood pressure; CAVI – cardio-ankle vascular index; ABI – ancle-brachial index; HDL – high density lipoproteins; LDL – low density lipoproteins.

that the ratio of Et/et was statistically significantly lower in the group with type D personality ($p = 0.014$).

In patients with type D personality, the median scores on the scales of «negative affectivity» and «social inhibition» were significantly higher than in patients without type D personality ($p < 0.001$). It was also noted that the level of personal anxiety and the level of depression were higher in the group with type D personality ($p < 0.05$) (Table 4).

When studying the correlations of psychological status with clinical indicators, a negative dependence of weight on the presence of type D personality was established ($r = 0.197$; $p = 0.046$). Associations of diabetes mellitus with type D personality ($r = 0.243$; $p = 0.011$), severity of negative affectivity ($r = 0.253$; $p = 0.008$) and social inhibition ($r = 0.224$; $p = 0.020$) were revealed. Negative affectivity component of type D personality had a statistically significant correlation with a history of stroke ($r = 0.211$; $p = 0.033$), SBP level ($r = 0.301$; $p = 0.028$), ABI < 0.9 ($r = 0.393$; $p = 0.004$) and low density lipoprotein (LDL) levels ($r = -0.389$; $p = 0.034$). An association of an increased level of anxiety with age ($r = 0.215$; $p = 0.029$) and the level of DBP ($r = 0.295$; $p = 0.032$) was established. The level of high-density lipoproteins (HDL) had a statistically significant correlation with an increased level of depression ($r = 0.462$; $p = 0.01$) (Table 5).

DISCUSSION

This study shows that patients having chronic coronary syndrome with type D personality, concomitant diabetes mellitus, as well as left and right ventricular filling disorders are more often detected. Correlation analysis revealed associations of diabetes mellitus with type D personality and its subscales; the level of ABI was associated with the subscales NA and SI, but not with the level of anxiety and depression.

In previous studies, it was shown that with type D personality in patients with coronary heart disease, not only manifestations of psychological distress (anxiety and depression) and a decrease in quality of life are more common, but also certain changes associated with the severity of the process in the cardiovascular system. Thus, in patients with coronary artery disease, coronary angiography revealed a greater degree of coronary artery lesion on a scale with type D personality compared with patients without type D personality (26.21 ± 12.03 and 15.49 ± 8.89, respectively; $p < 0.001$) [23]. In patients with type D personality, optical coherence tomography of the coronary arteries revealed more pronounced signs of instability of atherosclerotic plaques [24]. In the cohort of patients with coronary artery disease before coronary bypass surgery, the presence of type D personality was associated with a greater prevalence of the atherosclerotic process, which was manifested by more frequent detection of multifocal atherosclerosis [20]. In the population cohort of the ESSAY study, more pronounced calcification of the coronary arteries was detected in individuals with type D personality [25]. In this study, we were unable to identify associations of personality type with either the number of affected coronary arteries or with manifestations of peripheral atherosclerosis (the values of ABI and the frequency of stenosis of the internal carotid artery did not differ in the groups). Nevertheless, we have identified more pronounced changes in the filling indices of both the left and right ventricles in patients with type D personality. This is consistent with the data of V.R. Enatescu et al. [23], which revealed in patients with type D personality an increase in the E/e ratio (13.49 ± 4.15 and 10.24 ± 3.25; $p = 0.03$) and left atrium volume (85.79 ± 34.4 and 71.03 ± 26.49 ml; $p = 0.012$) compared with patients without type D personality. In contrast to the present study, in this study, the deterioration of left ventricular filling was accompanied by systolic dysfunction (decrease in left ventricular ejection fraction and s' index), apparently due to the more frequent presence of postinfarction atherosclerosis in this group. The deterioration of the filling of the ventricles in our study is explained by the more frequent detection of diabetes mellitus in patients with type D personality, which was accompanied by a decrease in myocardial elasticity, but did not lead to a decrease in heart pump function.

The presence of somatic manifestations in individuals with type D personality may mediate the influence of various behavioral factors and biomarkers on the prognosis of patients with coronary heart disease. So, Y. Wang et al. showed that patients with coronary heart disease with type D personality had higher levels of tumor necrosis factor α, interleukin 6, total inflammation indices (with additional inclusion of highly sensitive C-reactive protein), kynurenine and the kynurenine/tryptophan ratio (activated kynurenine pathway of tryptophan metabolism is associated with MACE (major adverse cardiac events) in patients with coronary heart disease). In addition, during prospective observation, it was noted that increased total inflammation indices and the level of the kynurenine/tryptophan ratio mediated the influence of type D personality on vulnerable coronary plaques and the prognosis of patients with coronary heart disease [17]. The same group of authors revealed that for patients with type D personality not only was there a low level of fruit and vegetable consumption, but this was largely due to the high incidence of stent restenosis after PCI. It has also been shown that it is the nutrients of fruits and vegetables, including vitamin C, vitamin E and fiber mediated the influence of type personality on the development of stent restenosis [19].

What is the clinical significance of the data obtained? The revealed differences in the clinical manifestations of CHD patients with type D personality emphasize the assumption of N. Kupper and J. Denollet [16] that there is not a chain of events in the influence of type D personality on the prognosis, but a network of potential biological and behavioral mechanisms, as well as a variety of pathophysiological «routes» that can be realized in them. Since coronary heart disease is multifactorial in na-

ture, it is not surprising that type D personality also exerts its influence through a group of interacting mechanisms, each of which contributes.

CONCLUSION

In patients with chronic coronary syndrome with type D personality, compared with those without this type, concomitant diabetes mellitus (35 % and 15 %, respectively; $p = 0.018$), signs of left diastolic dysfunction of left (E/e ratio – 7.1 [6.48; 8.0] and 5.0 [4.55; 5.74], respectively; $p = 0.0038$) and right (Et/At ratio 0.8 [0.66; 1.35] and 1.38 [1.28; 1.63], respectively; $p = 0.014$) ventricle were more often diagnosed. Correlation analysis revealed associations of diabetes mellitus with type D personality ($r = 0.243$; $p = 0.011$), severity of negative affectivity ($r = 0.253$; $p = 0.008$) and social inhibition ($r = 0.224$; $p = 0.020$), as well as association of ABI with the severity of negative affectivity ($r = 0.393$; $p = 0.004$) and social inhibition ($r = 0.414$; $p = 0.002$). These results should be taken into account in rehabilitation programs for psychological problems affecting the course of coronary heart disease.

Conflict of interest

The authors of this article declare the absence of a conflict of interest.

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LECTURES

CURRENT CONCEPTS OF PEYRONIE'S DISEASE (CLINICAL LECTURE)

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ABSTRACT

The article is presented in the format of a lecture. Peyronie's disease (induratio penis plastica) is a progressive fibrotic disorder of the penile tunica albuginea that results in fibrotic penile plaques and can cause penile deformity. The issues of etiology, pathogenesis, clinical picture and diagnosis of Peyronie's disease (PD) are reviewed in the lecture from the modern points of view. PD is frequently associated with penile pain, erectile dysfunction, and a secondary anxiety-depressive state. Despite the existence of this problem for several centuries, no unified concept of the Peyronie's disease pathogenesis can be found in literature. A growing amount of research has shown that PD is a chronical disorder of local wound healing process within the tunica albuginea and the Smith's space. Over the past 40 years, multiple lines of evidence have pointed to a genetic factor that predisposes some men to the development of Peyronie's disease. Treating men with PD remains a challenging problem for clinicians working in urology. Given the high prevalence of PD and its significant impact on affected men, its better understanding is essential. Treatment methods for PD are varied and include oral, local, intralesional and traction therapy, and surgical treatment. Current clinical care standards for PD are aimed at the symptom suppression, as there are currently no treatment for PD that can eliminate its causes or progression. Clostridium histolyticum collagenase has shown its effectiveness in treating PD, but its efficacy and safety remain controversial. Surgery remains the most effective method for PD treatment and is considered to be "gold standard". The choice of the surgical technique depends on the length of the penis, degree of deformity, erectile function, patients' expectations and surgeon's preferences. Various surgical techniques and grafting materials (autologous and non-autologous) can be used for the coverage of the tunica albuginea defect after partial plaque excision or incision.

Key words: *fibroplastic penile induration, Peyronie's disease, curvature of the penis, erectile dysfunction, substitutive corporoplasty, buccal graft*

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АКТУАЛЬНЫЕ ПРЕДСТАВЛЕНИЯ О БОЛЕЗНИ ПЕЙРОНИ (КЛИНИЧЕСКАЯ ЛЕКЦИЯ)

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РЕЗЮМЕ

Статья представлена в формате лекционного материала. Болезнь Пейрони (фибропластическая индурация полового члена) представляет собой прогрессирующее фиброзное заболевание белочной оболочки полового члена, которое приводит к образованию фиброзных бляшек и может приводить к пенильной деформации. В лекции с современных позиций рассмотрены вопросы этиологии, патогенеза, клиники и диагностики болезни Пейрони (БП). БП часто сочетается с болью в половом члене, эректильной дисфункцией и вторичным тревожно-депрессивным состоянием. Несмотря на то, что эта проблема сохраняется не одно столетие, на сегодняшний день в литературе нет единой концепции патогенеза. Растущий объём исследований показывает, что БП представляет собой хроническое нарушение локального процесса заживления ран в белочной оболочке и пространстве Смита. За последние 40 лет многочисленные доказательства указывают на генетический фактор, предрасполагающий некоторых мужчин к развитию БП. Лечение мужчин с БП остаётся сложной проблемой, стоящей перед клиницистами, работающими в области урологии. Методы лечения БП разнообразны и включают пероральную, местную, внутриочаговую и тракционную терапию, оперативное лечение. Действующие стандарты клинической помощи при БП направлены на устранение симптомов, поскольку в настоящее время не разработаны методы лечения, направленные на ликвидацию причин, вызывающих её и способствующих прогрессированию заболевания. Коллагеназа Clostridium histolyticum показала свою эффективность в лечении БП, но её эффективность и безопасность остаются спорными. Хирургия остаётся наиболее эффективным методом лечения БП и считается золотым стандартом. Выбор хирургической техники зависит от длины полового члена, степени деформации, эректильной функции, ожиданий пациентов и предпочтений хирурга. Для закрытия дефекта белочной оболочки после частичного иссечения или разреза бляшки можно использовать различные хирургические методики и трансплантационные материалы (аутологичные и неаутологичные).

Ключевые слова: *фибропластическая индурация полового члена, болезнь Пейрони, искривление полового члена, эректильная дисфункция, заместительная корпоропластика, буккальный трансплантат*

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The strategy of preserving men's health in the Russian Federation is an important and integral part of the health of the nation. In the conditions of modern life, more and more attention in the scientific literature is paid to diseases that lead to a significant decrease in the quality of life of patients and their social maladjustment. This clinical lecture presents generally accepted, effective methods of diagnosis and treatment of an urgent problem of modern uroandrology that causes pathological dysfunction of the penis; it also describes new strategies used in the treatment of this disease, which have successfully proven themselves in clinical practice. Peyronie's disease (PD), as well as erectile dysfunction and premature ejaculation, are attributed to violations of male sexual function. This rather mysterious, although not rare disease was named after the French surgeon, physician in ordinary to Louis XV Francois Gigot de La Peyronie (François Gigot de La Peyronie, 1678–1747) [1]. It should be recognized that much attention has been paid to the problem of fibroplastic penile induration for several centuries, but it is far from being resolved, leaving many unresolved issues related not only to the disruption of the appearance of the erect penis, but also to changes affecting various spheres of a man's life (intimate, psychosocial, functional). The prevalence of erectile dysfunction among PD patients ranges from 40 to 60 % [2]. Another aspect that should be paid attention to is sexual distress, which negatively affects the quality of life not only of the patients themselves, but also of their partners.

In his book on the violation of ejaculation (1743), one of the founders of the French Academy of Surgery, F.G. de La Peyronie wrote about the "lumps" of cavernous bodies in the form of "rosaries", leading to curvature of the penis. However, several centuries before the publication of de La Peyronie pathological curvature of the penis has been studied and described by many surgeons and anatomists: Theodoricus Borgognoni (1205–1298), Guilielmus of Saliceto (1210–1276), Gabriele Falloppio (1523–1562), Andreas Vesalius (1514–1564), Giulio Cesare Aranzi (1530–1589), Claas Pieterzoon Tulp (1593–1674), and Anton Frederik Ruysch (1638–1731). The latter are supposed to have left the first "posthumous" illustration of this pathological condition on a copper engraving (1691). Of all the listed predecessors, F.G. de La Peyronie, only Guillelmus of Saliceto and Falloppio were involved in the treatment of the disease.

DEFINITION AND TERMINOLOGY

Fibroplastic penile induration (induratio penis plastica), or Peyronie's disease, is understood to be a relatively common, acquired, progressive, benign connective tissue disease associated with the uncontrolled formation of fibrous/calcified inelastic plaques on the tunica albuginea and adjacent cavernous tissue of the penis, accompanied by deformation (curvature, shortening) penis in an erect or semi-erect state, with a painful erection, followed by the development of erectile dysfunction (ED) and a decrease in the quality of life of a man. In most cases, PD develops as a result of repeated, often unrecognized and minor micro-injuries of the penis during sexual intercourse. The progression of the condition can occur over several years. In some cases, it can occur as a spontaneously self-healing condition (13 %) [3]. Clinically, PD is manifested by the presence of a palpable plaque, curvature or penis shortening / narrowing, the occurrence of pain discomfort during erection or sexual intercourse, ED, various psychological disorders. Currently, the pathological condition is considered to be a local manifestation of systemic collagenosis.

EPIDEMIOLOGY

To date, there are no cohort data clearly describing the incidence and/or prevalence of PD in the general population, and epidemiological data on the disease in different countries are limited and ambiguous. Different levels of epidemiological studies were conducted in the USA, Germany, Italy, Australia, Turkey, and Japan, while in Eastern Europe, including Russia, epidemiological data on PD is very small. The prevalence rate of PD, according to the first study conducted in the USA in 1991, is 0.38 % (388.6 per 100,000 population), and the average age of patients who sought medical help reached 53 years with a range from 19 to 83 years [4, 5]. The results of the first large-scale cross-sectional study, published in 2001 in the British Urological Journal, allowed us to establish the prevalence of fibroplastic induration of penis in a sample (n = 8000) of surveyed German men aged 30–80 years at the level of 3.2 %, which is much higher than indicated in previous studies [6]. Generalized prevalence of PD in the general adult male population, according to M.G. Manka et al. (2021), ranged from 3 % to 9 %, and in certain groups of the adult population – from 0.39 % to 22.5 % [7]. Modern scientific data suggest that the actual number of patients reaches higher values, and PD itself is usually attributed to an undiagnosed pathology. It was found that in the US population it ranges from 0.5 % to 13 % [8], Italy – 7.1 % [9], Germany – 2.7 % [10], Japan – 0.6 % [11], Turkey – 5.3 % [12]. According to P.A. Shcheplev et al., the prevalence of PD in the Russian Federation reaches 3-8 % in terms of urological care and up to 25 % according to the results of pathoanatomical autopsies [13]. PD is registered everywhere, but the number of cases in the population varies depending on the continent, race, and age. The disease is more common in the population of men of the older age group – usually 50–60 years old. At the same time, personal experience shows that PD occurs at an earlier age: thus, the prevalence in adulthood is about 8 %, is characterized by an acute onset and is accompanied by a lower incidence of ED [14, 15]. The prevalence in the four age groups of adult men (30–39, 40–49, 50–59 and 60–69 years old) was 1.5 %, 3 %, 3 % and 4 %, respectively, and its peak was in men over 70 years old (6,5 %) [14, 15].

RISK FACTORS

The leading risk factors for the development of PD are diabetes mellitus, lipid metabolism disorders, arterial hypertension, ED, smoking, alcohol abuse, androgen deficiency and operations on organs of the genitourinary system (radical prostatectomy, transurethral resection), ischemic cardiomyopathy, autoimmune diseases [16]. PD is detected in 8 % of cases in patients with type 2 diabetes mellitus (DM) and in 20 % of patients with hyperglycemia and ED. The risk of developing PD increases by 16 % among Caucasian men after radical prostatectomy [17]. When screening for prostate cancer in a cohort of 534 men, 6 % of patients reported the presence of penis curvature, and 8.9 % had a Peyronie plaque on physical examination [18]. Smoking is closely related to PD, and a connection has been established between the incidence of the disease and the number of cigarettes consumed per day [19], the early onset of tobacco smoking and its duration [20]. A fairly strong argument in favor of a causal relationship indicating a genetic predisposition of the disease is the identification of the features of the prevalence of PD among different ethnic groups. PD is more common in men of the Caucasian race than in representatives of other nationalities, which suggests the presence of a «founder» mutation effect (loss of genetic variability). Dupuytren contracture is common in patients with PD (8.3–39 %), and PD is detected in 4–26 % of patients with Dupuytren contracture. At the same time, genetic changes in men with both PD and Dupuytren contracture are independent factors supporting the risk of developing cancer of the genitourinary system and gastrointestinal tract compared with men of the same age only with ED and the control group [21, 22]. These data suggest that men with PD should be closely monitored not only after diagnosis, but also after treatment of the disease [22]. Men with PD had an increased risk of developing stomach cancer (hazard ratio (HR) – 1.43; 95% confidence interval (95% CI): 1.06–1.14), testicular cancer (HR = 1.39; 95% CI: 1.05–1.84) and melanoma (HR = 1.19; 95% CI: 1.02–1.38) due to genetic abnormalities contributing to the development of PD: duplications of chromosomes 7 and 8, Y-chromosome deletions and structural changes such as reciprocal translocations 46XY,t(11;12)(q11,p11), 46XY,t(1;5)(q25;q11) and others [23]. However, the specific genetic factors that predispose PD patients to develop not only a number of hereditary diseases, but also malignant neoplasms, have not been definitively established.

Non-gonococcal urethritis, inflammatory diseases of the genitourinary system in a partner, as well as fibromatous lesions of the genitals are among the modifiable risk factors that can be influenced. The study of the role of hormonal shifts as a factor contributing to the development of PD did not lead to convincing evidence, even though low testosterone levels were found in 74.4 % of patients with PD [23]. In 2022, Japanese scientists have identified a correlation between AB0 blood groups and the development of PD: Japanese men with blood type 0 had a high risk of developing PD, while men with group B had a low risk [24].

ETIOLOGY AND PATHOGENESIS

The etiology of PD is multifactorial, and the mechanisms of disease development continue to be insufficiently studied. In most cases, sexual intercourse and vaginal intromission are considered to be provoking PD events with semi-erect penis. Currently, many researchers share the opinion that there are several theories of the pathogenesis of PD: microtraumatic (posttraumatic), endocrine, genetic, connective tissue pathology, chemical and vegetative. From the numerous proposed theories, micro/macrotrauma of the tunica albuginea and damage to the microcirculatory bed of the penis in genetically predisposed men are postulated. Penile injury can be caused by acute and chronic conditions such as accidents or surgical interventions, but it can also be associated with repeated microtrauma during sexual intercourse. Despite the fact that all men are more or less exposed to penis microtrauma during sex, only very few develop PD. An interesting fact indicating the multifactorial nature of the disease is the observation of PD in sexually inactive men who have never had sexual intercourse with penetration [25]. The anatomical location of the vessels crossing the tunica albuginea of the penis is unique. Repeated traumatic damage to the microvessels of the tunica albuginea causes local aseptic inflammation with destruction of elastic fibers and deposition of fibrin. Fibrin deposition is one of the initial consequences of damage to microvessels in the penis. A prolonged inflammatory response leads to the remodeling of connective tissue into dense fibrous plaques, and the formation of the latter causes curvature, which, if severe, can interfere with the copulatory function of a man. The arteries are protected by a cuff made of loose areolar tissue, while the veins are in direct contact with the fibrous membrane. If the fibers of the tunica albuginea of the penis are damaged by micro/macrotrauma with extravasation, then edema and cellular infiltration compress neighboring venous vessels and form a «trap» for an inflammatory reaction. The production of intercellular matrix and collagen fibers is further stimulated by the secretion of leukocytes and macrophages, as well as the release of cytokines. Since inflammation is limited, cytokines cannot disperse and break down, stimulating the production of even more cytokines, which consistently provoke the production of intercellular matrix and collagen [26]. All of the above allows us to consider PD as an abnormal wound healing process in response to local aseptic inflammation (a genetically aberrant healing process), which is limited by layers of the tunica albuginea (Smith space, Buck's fascia). The triggered mechanism with the accumulation of inflammatory cells and the formation of reactive oxygen intermediates, activation of fibroblasts and myofibroblasts, excessive collagen production between the layers of the tunica albuginea leads to a violation of function and structural balance – active growth of collagen fibers, fragmentation of elastic fibers, accompanied by histological changes of an inflammatory nature, which ultimately leads to the re-accumulation of collagen and the formation of a Peyronie fi-

brous plaque. Oxidative stress in the form of free radicals such as superoxide, peroxynitrite and the resulting peroxides leads to lipid peroxidation and further tissue damage [26]. Peroxynitrite, a profibrous compound, has a cytotoxic effect through various mechanisms of lipid peroxidation, DNA fragmentation, damage and nitration of proteins directly on the smooth muscle tissue of the penis cavernous part [26]. Fibrin is a powerful chemoattractant, a chemical that directs the migration of cells sensitive to them. The final process of fibrin replacement in the tunica albuginea of the penis lasts from 12 to 18 months, the curvature of the penis is completely formed, passing through seven successive stages: from injury to the tunica albuginea, loss of fibrinogen, increased local inflammation, growth of myofibroblasts with abnormal location, formation of collagen and deposits, pathological deposition of fibrin and to the formation of plaque [26]. The regulation of collagen synthesis by many endogenous and exogenous peptides plays a key role in the pathogenesis of PD. Tumor growth factor β (TGF-β) in recent studies has interested scientists as a cytokine that affects the deposition of intracellular matrix and induces fibrosis in the tunica albuginea of the penis [27]. TGF-β is also a cause of various chronic fibrotic conditions. Overexpression of TGF-β is also observed in plaques in PD. Some studies have shown that vascular injury leads to the formation of osteoids through osteoblast-like cells originating from the vascular lumen [28]. More recent reports have focused on the activation of certain genes, namely factor 1, specific to osteoblasts, which may be responsible for plaque calcification [29]. At the beginning of the pathological process, inflammation and swelling irritate the nerve endings, causing pain. As the inflammatory reaction subsides, nerve fibers may become ischemic and necrotic. In the chronic phase of the formation of the Peyronie plaque, fibrosis accelerates and prevents the development of an erection, which often leads to ED. Arterial insufficiency caused by direct obstruction by fibrous plaques of nearby cavernous arteries is believed to impede blood flow [30]. In the chronic phase of PD, when the plaque is stable, it often penetrates into the architecture of the penis smooth muscles, which leads to venoocclusive dysfunction.

Numerous studies indicate that there is a genetic contribution that predisposes men to the development of PD: changes in gene expression in plaques; karyotypic abnormality; single nucleotide polymorphism; frequent detection of class II antigens of the HLA – HLA-DR3 and HLA-DQW2 systems [26, 31]. At the same time, despite the progress made in understanding the role of genetic factors in PD, the data remain extremely contradictory, and the genes responsible for the development of the disease have not been identified.

Clinical and experimental studies indicate an important role in the formation of pathological penis deviation the pathology of connective tissue: Dupuytren contracture, Ledderhose disease, scapulohumeral periarthritis, scleroderma, dermomyositis and sclerosis of the auricles, as well as hormonal imbalance (the participation of androgens in collagen metabolism and wound healing) [32].

PHASES OF THE PROGRESSION. CLINICAL MANIFESTATIONS. CLASSIFICATION

Considering PD from the perspective of the dynamic progression of the disease, it is extremely important to distinguish between two phases accompanied by different symptoms, which further determine the choice of treatment method (conservative or surgical). According to international recommendations [33], the first (active) or acute inflammatory phase is distinguished, which is characterized by a variety of dynamically changing clinical signs and symptoms. The duration of the active phase ranges from 12 to 18 months. The first phase is accompanied by penile pain syndrome in the non-erect and erect state; at this stage, soft nodules /plaques form and begin to palpate; sometimes it can simultaneously be accompanied by the development of penis curvature, in some cases, signs of ED appear. A distinctive feature of the first phase is the presence of an inflammatory infiltrate in the tunica albuginea of the penis. Most often, the first manifestation of PD is penis deformation (52–94 %), followed by pain, which is noted in the early stages of the disease by 20 to 70 % of patients. The second phase – fibrotic (chronic, stable) – is characterized by the formation of hard / hard palpable plaques, which can later calcify, bringing the pathological process closer to the stabilization of the disease. This phase is characterized by the disappearance of pain during erection and increased penis curvature for three months. In some cases, stabilization of the process may begin earlier – at the 5th or 7th month from the onset of the disease. Over time, penis curvature increases in 21–48 % of patients, and in 36–67 % of patients it remains stable [33]. Spontaneous improvement of the disease is rare (3–13 %), in most cases there is progression (21–48 %) and stabilization of the disease (36–67 %) [34]. Pain syndrome is usually present at an early stage and in 90 % of cases resolves within the first 12 months [34]. In 39 % of patients, palpable plaques appear first, most often located on the penis dorsal surface [34]. At a later date, there is a lack of tumescence of distal to the penis affected area. In addition to pain and painful erections, penis curvature, erectile dysfunction, the presence of palpable lumps in the penis, the most terrible thing for a man is the inability to participate in sexual relations with penetration. In addition to the physical and sexual symptoms of PD, many men experience emotional and psychological disorders.

According to the coding criteria of the International Classification of Diseases of the tenth revision, fibroplastic penile induration and fibrosis of the cavernous bodies were assigned to one nosological group – plastic penile induration. The stage classification of V.E. Maso (1984) subdivides pathology into four stages. At the first stage, the Peyronie plaque is not palpated or visualized, the only clinical manifestation is pain during erection. The second stage of PD is characterized by the appearance of a palpable fibrous-elastic formation on the tuni-

ca albuginea of the penis. At the third stage of PD, dense fibrous-elastic fibers are detected during histological examination. The appearance of calcifications in the Peyronie plaque indicates the fourth stage of the process [35], which is divided into two periods – painful and functional. The pain period is characterized by the presence of only pain both during arousal and without it, and the functional period is characterized by penis curvature, which prevents sexual intercourse, ED. Researchers F. Iacono et al. (1993) proposed a classification based on the duration of the progression (up to 6 months; from 7 to 12 months; more than a year) of the disease [36]. The classification of A. Kelami (1983) has become widespread, which implies the allocation of three degrees (light, medium, severe): thus, the angle of penis curvature up to 30° and the size of the Peyronie plaque 2 cm is attributed to a mild degree; the angle of penis curvature from 30 to 60° and the size of the plaque from 2 to 4 cm is medium; the angle of penis curvature is greater than 60 ° and the size of the plaque more than 4 cm is severe. [37]. The shape of an erect penis depends on the size of Peyronie's plaques. The classification of PD proposed by I.I. Gorpinchenko and Yu.N. Gurzhenko is the most complete, focused on various aspects of the disease (features of the progression, clinical manifestations, plaque localization, direction of curvature, complications, concomitant diseases), balanced for the choice of surgical tactics [38]. According to the progression, the authors differentiate PD into slowly and rapidly progressing forms. Among the clinical manifestations are: pain (0 – absence; 1 – minor pain during erection; 2 – significant pain, hindering sexual activity; 3 – pain during erection and without erection); curvature (0 – absence; 1 – up to 30° without restriction of sexual activity; 2 – up to 60° with restrictions on sexual activity; 3 – more than 60° with the impossibility of sexual activity); lump (0 – absent; 1 – up to 1 cm in diameter; 2 – 1–3 cm in diameter; 3 – more than 3 cm in diameter). According to the localization of Peyronie plaques, there are: 1 – at the root of the penis; 2 – in the area of the stem of the penis; 3 – at the head of the penis; in the direction of curvature – dorsal, lateral, dorsolateral, dorsolateral right; by the presence of complications – with or without preservation of erectile function; by the presence of concomitant diseases – with or without concomitant diseases.

DIAGNOSTICS

The best diagnostic indicators of the disease are still a detailed medical history and physical examination. The purpose of the clinical examination of patients is to establish the correct diagnosis necessary for the timely initiation of both conservative and surgical treatment, which is the main method at a late stage (stabilization) of the disease. In most cases, the patient actively complains about the curvature and penis shortening, the pain during erection, the formation of lumps/nodules in the penis, as well as the impossibility of introjection, in some cases – the absence of erections and the appearance of psychoemotional disorders. The above complaints are not always the reason for immediate medical attention. The survey allows you to determine not only the trigger that provoked the appearance of complaints, but also the timing of their appearance and disappearance, especially pain syndrome, the first signs of ED, penis curvature and deformation. It is advisable to obtain information about the family history of the disease, cases of PD or other fibrous processes (Dupuytren contracture and Ledderhose disease in close relatives, etc.).

Psychometric analysis makes it possible to evaluate sexual function using the International Index of Erectile Function. An important question should be asked: "Would you be able to have full sexual intercourse if you did not have a penile deformity?". A tool has been developed to assess the progression and severity of symptoms of the disease, known as the Peyronie's Disease Questionnaire, containing 15 questions [39]. In the presence of depression, it is recommended to use the Beck Depressive Inventory scale [39]. The development of ED and psychological changes has a significant influence on the choice of treatment.

The purpose of the physical examination is not only to detect penile deforming changes but also to identify contractures, sclerodermic changes. Vacuum erectors are used to determine the severity of the curvature. The following types of penis deformation are distinguished: dorsal (68 %) – upward curvature; ventral (1 %) – downward curvature; lateral (15 %) – sideways curvature; dorsolateral – upward and sideways curvature; ventrolateral – downward and sideways curvature; «hinge» type deformation – local curvature narrowing and instability of the part of the penis located behind the narrowing; narrowing of the organ along the circumference is an "hourglass" type deformation [39]. Determining the size, localization of the Peyronie plaque, its extent, presence/absence of narrowing, type of curvature are among the main tasks of physical examination. Multiple Peyronie plaques are usually located on opposite sides of the penis. The consistency of plaques varies from soft, which is found in the early stages of the disease, to calcified or even ossified, which is found later with the progression of the disease. Formation of the chondroid/osteoid tissue is an unfavorable prognostic sign of the disease, and therefore determining the consistency of plaques is an important diagnostic technique. In combination with other diagnostic methods, photofixation (autofixation) of penis curvature in both the anterior and lateral planes makes it possible to determine the angle, the surface of the penis curvature (ventral, dorsal, lateral) and its localization relative to the penis longitudinal axis. Measurement of the degree of penis curvature can also be performed on standard photographs using two intersecting lines (A and B) passing through the centers of the distal and penis proximal rods, as well as using a special smartphone application – University of Washington Peyronie's Examination Network [40]. In 70 % of patients with PD, there is objectively a shortening of the penis, which can reach 10 cm, most often it does not exceed 1 cm [39].

When conducting differential diagnosis, it is necessary to recognize diseases in the clinical picture of which there is a curvature/deformation of the penis. If we turn to the list of diseases, penis curvature can occur with developmental abnormalities, dorsal vein thrombosis, posttraumatic cavernous fibrosis, secondary syphilitic lesion, epithelial sarcoma, and sometimes with metastases of malignant tumors from other organs and systems. Laboratory tests are not required to diagnose PD, although, given the link between PD and systemic diseases, including hypogenitalism, chronic hyperglycemia and cardiovascular diseases, screening and examination of these conditions in men may be justified. You also need to get information about previously conducted treatment measures for PD.

PD can be diagnosed by palpation of penis plaques, however, changes in the septum, intracavernous and ventral tunica albugineas of the cavernous bodies cannot be detected. Ultrasound examination, including pharmaco-Dopplerography of penis vessels, is a valuable method in the diagnosis of penis diseases, allowing to determine their early forms and identify non-palpable lesions, the degree of fibrosis and assess the condition of blood vessels [41]. In PD, the method is used not only to confirm the diagnosis; in some cases, penile lesions can only be identified by ultrasound. Uncalcified Peyronie plaques on ultrasound are isoechogenic or hyperechogenic compared to the tunica albuginea. With focal thickening of the pericavernous tissue, plaques are hypoechoic, which is rare. Ultrasonography in shades of gray allows to recognize the involvement of plaques in penis septum. Ultrasound shows 100 % sensitivity in detecting and measuring coarse calcifications in the Peyronie plaque. Peyronie plaques are more often located on the dorsal side of the penis, but they can also be found ventrally or, less often, in other places [41]. Penis pharmaco-Dopplerography allows to determine: the angle of curvature, the shape of deformation, the functional parameters of vascular blood flow and predict the necessary surgical intervention. The assessment of the state of the penile vascular bed with the determination of the erectile response to the injection of a pharmacological vasoactive drug (papaverine, prostaglandin or TriMix, including papaverine, phentolamine and prostaglandin E1) is carried out after achieving a full erection [41]. If necessary, compression of the penis at the base is used to increase the rigidity of the penis and revise the dose of the vasoactive drug. In some patients, under direct observation a significant psychogenic inhibition of the erectile response may occur [41]. The minimum dose is the administration of 10 μg of prostaglandin E1 (caverject, alprostadil). On MRI, plaques look like hypointensive areas of thickening in the tunica albuginea in both T1 and T2-weighted images. Calcification of Peyronie's plaques is better manifested on ultrasound than on MRI [41]. Magnetic resonance imaging and computed tomography, as well as sonoelastography, cavernosography, and scintigraphy, are not routine methods used in clinical practice for the diagnosis of PD.

GENERAL PRINCIPLES OF TREATMENT

There is no adequate universally recognized treatment regimen for PD, so the problem of effective treatment of PD remains a very difficult task. As the evidence base expanded, the range of surgical and non-surgical options for PD patients narrowed. Conservative therapy is indicated for patients in the first phase of the pathological process progression, with moderate penis deviation, small size of the Peyronie plaque (up to 1 cm). Complex drug therapy is considered as a first-line therapy as an additional way to relieve pain, prevent disease progression in patients with contraindications or unwillingness to perform surgical correction of deviation. In this situation, patients are offered several treatment options, including oral therapy, injections into the Peyronie plaque, remote low-energy shockwave therapy, etc. Due to the fact that the results of scientific research on the conservative treatment of PD are contradictory, experts from the European Association of Urology (EAU; 2021) and the American Association of Urology (AUA; 2021) do not give recommendations on its use in routine clinical practice [39]. The EAU task force (2021) does not support the appointment of oral therapy for PD, including pentoxifylline, vitamin E, tamoxifen, procarbazine, potassium paraaminobenzoate (potaba), omega-3 fatty acids, a combination of vitamin E and L-carnitine, due to the lack of their proven effectiveness [39]. The clinical feasibility of prescribing of above mentioned drugs to patients with PD may be limited by the manifestation of side effects; in particular, high doses of vitamin E can lead to cardiovascular disorders. Even in the absence of adverse events from the use of these drugs, inadequate treatment may lead to the later appointment of other more effective methods. The options for conservative treatment in the active phase of the disease, which have a strong evidence level, include only the use of nonsteroidal anti-inflammatory drugs (NSAIDs) (diclofenac, indomethacin in candles up to 100 mg) in order to relieve pain and low-energy shockwave therapy (LEST) [39]. LEST can be used only in the acute phase when there is no plaque: it does not lead to lysis of the Peyronie plaque, it only contributes to a faster relief of pain syndrome.

The scientific literature has published positive results of the effective use of NSAIDs, type 5 phosphodiesterase inhibitors, enzymes (collagenase, etc.), verapamil, nicardipine, interferon (IFN) alpha-2b, hyaluronic acid, botulinum toxin in the treatment of Peyronie's disease [40]. Intralesional injections may be offered to patients who refuse surgical treatment. Even F.G. de La Peyronie himself was the first to use mercury and thermal mineral water (holy water of Bareges) locally to treat plaques, eventually reporting efficacy with regular use of mineral water [41]. In 1901 W.J. Walsham and W.G. Spencer were the first to inject mercury and iodide directly into the plaques on the penis, trying to dissolve them [42]. Modern injectable agents can be divided into two groups, namely anti-inflammatory/proliferative agents and lytic agents. In addition to the above, there is evidence of a positive effect of intraocular administration of clostridial collagenas-

es, purified Clostridium histiolytica collagenase (0.58 mg with a difference of 24–72 hours every 6 weeks for 4 cycles) on reducing the degree of penile deviation (in 32–42.9 %) [43]. Clostridial collagenase became the first treatment for PD approved by the U.S. Food and Drug Administration (FDA) in 2013 [41]. Collagenase is injected directly into the primary plaque at the point of maximum bending of the penis, using only one puncture point to deliver the drug. Injections into the plaque (5×10^6 units of IFN-α-2b in 10 ml of saline solution 2 times a week for 12 weeks) can reduce the penile curvature, the size of the plaque and its density, as well as pain compared with placebo, therefore IFN-α-2b is recommended for the treatment of PD in the second stable phase [39].

Traction therapy of penis (> 5 hours a day for 6 months) or devices for vacuum erection (promotes the expansion of the cavernous sinuses) can be used as monotherapy or in combination with other treatment methods to reduce the penile deviation, however, the «promising» effectiveness has yet to be proven [43].

Surgical correction of PD with or without prosthetics remains the main method of PD treatment and the gold standard for correction of penile deformation [44]. No surgical method has been singled out as the standard of medical care. Surgical operations performed to correct penile deviation are divided into interventions performed without preservation (operations with resection on the convex side of the tunica albuginea of the penis – Nessbit, Essed – Schroeder, STAGE, etc.) and with preservation of the length of the penis (operations on the concave side of the tunica albuginea using a graft), as well as operations with simultaneous the installation of penile implants with or without lengthening of the penis in the presence of ED [44]. The patient should understand that the purpose of the operation is to make his penis "functionally straightened"; in clinical practice, after most interventions, there is a penis residual deviation of more or less 20°. All surgical correction procedures involve some loss of length. Based on the opinion of experts from the Russian and European Associations of Urologists, it is recommended that clinicians evaluate and begin surgical treatment of patients with PD only if they have sufficient experience and the medical institution has the appropriate technological capabilities [45, 46]. In the stable stage of the disease, the formation of further surgical intervention tactics depends on the presence/ absence of ED, the effectiveness of previously performed conservative therapy, the initial erectile status of the patient, the initial penile length / thickness, the angle and the presence of complex forms of penile deviation. Important criteria influencing the choice of surgery for PD are the surgeon's experience and the patient's preference [45, 46].

Shortening methods of surgical correction include operations with and without opening the tunica albuginea (plication techniques). The first method (with opening of the tunica albuginea) includes the Nesbit surgery, Nesbit – Saalfeld (longitudinal incisions no more than 1 cm long on the curved side of the tunica albuginea of the penis and suturing in the transverse direction), Nesbit – Lemberger (longitudinal incision on the curved side of the tunica albuginea of the penis with suturing in the transverse direction), Nesbit – Hellstrom (excision of several flaps depending on the degree of curvature), Nesbit – Yachia. The second method (without opening the tunica albuginea or plication techniques) includes Nesbit – Shcheplev surgery, Essed – Schroder (invagination of the tunica albuginea using screwing seams), Nesbit – Lue [45].

The advantages of these surgery include the straightening of the penis (it is possible to achieve in 80 % of patients), a low incidence of recurrences of curvature, ED and decreased sensitivity [45]. A significant disadvantage is that 85 % of patients after Nesbit surgery have a shortening of the penis; at the same time, it does not exceed 1–1.5 cm and does not lead to a significant violation of sexual function. Shortening techniques should be the method of choice and be performed in patients with PD and a curvature angle of less than 45° [45].

Tunica albuginea lengthen surgery are preferred in patients with severe shortening of the penis, pronounced curvature and/or complex deformations (like an hourglass or screw), but without the initial ED. A curvature of more than 60° is considered to be pronounced. An important aspect when performing lengthening operations on the penis is the dissection of the Peyronie plaque along the line of the maximum angle of curvature. Complete removal or excision of the Peyronie plaque is associated with a high rate of ED due to the development of venous leakage, but in the case of severe calcification, partial excision is acceptable. It is necessary to inform patients about the significant risk of ED development up to 50 % after surgery [45]. The risk of shortening can be minimized by penis stretching. No less significant is the characteristic of the material used to replace the tunica albuginea defect. Traditionally, grafts are the main material. There are four types of grafts: autologous transplants from the patient himself (dermis, vein, temporal fascia, broad fascia, tunica albuginea and cheek mucosa); allografts from a cadaveric donor (pericardium, broad fascia and dura mater); xenografts (taken from various animal tissues – bovine pericardium, submucosal layer of pig intestine, bovine and pig dermis, horse collagen matrix); synthetic grafts [47, 48]. Synthetic grafts are not recommended for use in PD [39]. For more than forty years, it has been proposed to use the cheek mucosa as an autologous material during reconstructive urological operations, including for penis deformation correction in PD [47, 48]. Substitutive corporoplasty allows to achieve satisfactory aesthetic and functional results in 90 % of patients and is the preferred method for curvature angles of more than 45–90° and significant size of the Peyronie plaque.

In patients with Peyronie's disease and ED, with the ineffectiveness of pharmacotherapy and severe penile deformation, implantation of penile prostheses with additional techniques (modeling, plication, dissection or excision of plaque with transplant surgery) is indicated [39, 49]. A strict program of postoperative rehabilitation reduces the risk of recurrence of penis curvature and shortening.

THE MAIN POINTS OF THE LECTURE

Peyronie's disease has been haunting humanity for centuries.

The key to successful treatment of the disease is the knowledge underlying its pathophysiology, which is still not fully understood, and the lack of such knowledge leads to the inability to prevent the onset and progression of the disease, which makes fibroplastic induration of penis difficult to treat the disease. Perhaps a vital part of the underlying mechanism is still eluding us, and when it is discovered, it will allow us to differentiate patients whose disease can resolve spontaneously from those whose disease can progress.

The main focus of treatment should be on relieving acute pain and restoring sexual function by reducing penile curvature and associated erectile dysfunction.

Many of the proposed conservative treatment options have either failed or produced contradictory results. NSAIDs and LEST are indicated for the treatment of penile pain syndrome, but should not be used to eliminate penis deformation/curvature or Peyronie plaque.

Intralesional injection therapy IFN-α-2b and clostridial collagenase are recommended for the treatment of PD in the second stable phase in specially selected patients.

Surgery to correct penile deformation in PD with an emphasis on preventing organ length loss remains a difficult task, despite the fact that various techniques have appeared over the past few years. Penis reconstructive surgery is mainly based on the restoration of the functional state of the organ, that is, its straightening while maintaining sufficient rigidity and the possibility of sexual intercourse.

Conflict of interest

The authors of this article declare the absence of a conflict of interest.

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MORPHOLOGY, PHYSIOLOGY AND PATHOPHYSIOLOGY

AUTOMATED MORPHOMETRIC STUDIES OF COLLAGEN FIBERS AS AN AUXILIARY METHOD FOR DIAGNOSING COLD INJURY

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ABSTRACT

Background. *Cold injury is one of the most significant problems for the northern regions of the Russian Federation. Frostbite is defined as a complex of changes caused by the action of low temperatures, which lead to morphological changes in damaged tissue structures. As a result, the skin with underlying tissues and the intercellular matrix, the components of which are collagen fibers, are damaged, which eventually leads to remodeling and a protracted course of the wound process. Morphometric studies in combination with quantitative analysis of microphotographs (histological specimens) using GIS technologies make it possible to distinguish altered collagen fibers under the influence of low temperatures from relatively healthy tissues.*

The aim of the study. *To assess the possibility of using computer analysis of microphotographs in a complex of morphometric studies of collagen fibers in local cold injury.*

Materials and methods. *The study included 84 patients with III and IV degree frostbite of the lower extremities. Morphological study of tissues and microphotography were performed using Leica DM2500 microscope (Leica, Germany). The thickness of collagen fibers was measured based on visual measurements of characteristic areas of the microphotograph. Computer analysis of tissue microphotographs of the zone of cryoinjury was performed using the ArcINFO software (Esri, USA). Statistical processing of the study results was carried out using the SPSS Statistics software package (IBM Corp., USA). Graphs and diagrams were constructed using MS Office Excel (Microsoft Corp., USA).*

Results. *With frostbite, severe tissue damage occurs, accompanied by the destructive processes of the extracellular matrix components. Low temperatures contribute to changes in the width and orientation of collagen fibers in the damaged area. In this regard, a change in the texture of the histological specimen image leads to a change in the numerical characteristics of the standard deviation of the curvature coefficient in the studied area of the microphotograph. Thus, in the late reactive period, the described complex of morphometric studies makes it possible to classify particular microphotograph as having pathological signs or as a sample of healthy tissue.*

Key words: *morphometry, collagen fibers, cryoinjury, frostbite, standard deviation, curvature coefficient, raster image*

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АВТОМАТИЗИРОВАННЫЕ МОРФОМЕТРИЧЕСКИЕ ИССЛЕДОВАНИЯ КОЛЛАГЕНОВЫХ ВОЛОКОН КАК ВСПОМОГАТЕЛЬНЫЙ СПОСОБ ДИАГНОСТИКИ ХОЛОДОВОЙ ТРАВМЫ

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РЕЗЮМЕ

Актуальность. *Холодовая травма является одной из наиболее значимых проблем для северных регионов нашей страны. Отморожения определяются как комплекс изменений, возникающих вследствие действия низких температур, которые приводят к морфологическим сдвигам в повреждённых тканевых структурах. В результате повреждаются кожа с подлежащими тканями и межклеточный матрикс, компонентами которого являются коллагеновые волокна, что в конечном итоге приводит к ремоделированию и затяжному течению раневого процесса. Морфометрические исследования в комплексе с количественным анализом микрофотографий (гистологических препаратов) с помощью ГИС-технологий позволяют отличить изменённые коллагеновые волокна под действием низких температур от относительно здоровых тканей.*

Цель исследования. *Оценить возможность применения компьютерного анализа микрофотографий в комплексе морфометрических исследований коллагеновых волокон при местной холодовой травме.*

Материалы и методы. *В исследование включены 84 больных с отморожениями нижних конечностей III и IV степени тяжести. Морфологическое изучение тканей и микрофотосъёмка выполнялись на микроскопе Leica DM2500 (Leica, Германия). Измерение толщины коллагеновых волокон выполнено на основе визуальных измерений характерных участков микрофотографии. Компьютерный анализ микрофотографий тканей зоны криоповреждения выполнен в программе ArcINFO (Esri, США). Статистическая обработка результатов исследования осуществлялась с помощью пакета программ SPSS Statistics (IBM Corp., США). Построение графиков и диаграмм выполнено с помощью пакета MS Office Excel (Microsoft Corp., США).*

Результаты. *При отморожениях происходит выраженное повреждение тканей, сопровождающееся деструктивными процессами компонентов внеклеточного матрикса. Низкие температуры способствуют изменению ширины и ориентации коллагеновых волокон в зоне повреждения. В этой связи изменение текстуры изображения гистологического препарата приводит к изменению численных характеристик стандартного отклонения коэффициента кривизны в исследуемой области микрофотографии. Таким образом, в позднем реактивном периоде описанный комплекс морфометрических исследований позволяет отнести ту или иную микрофотографию к числу имеющих патологические признаки либо к образцу здоровых тканей.*

Ключевые слова: *морфометрия, коллагеновые волокна, криотравма, отморожения, стандартное отклонение, коэффициент кривизны, растровое изображение*

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INTRODUCTION

Frostbite is one of the most severe types of cold injury and often leads to a high level of disability and death of victims [1]. During cold alteration, severe dystrophic, inflammatory, and necrotic changes develop after tissue warming, which contribute to damage to cells, intercellular matter, and connective tissue [2, 3].

One of the most characteristic symptoms of the development of pathological processes in tissues is a change in the structure of collagen fibers. In general, the changes include a change in the thickness of the fibers and a transition from a highly dispersed fine-fiber structure to a coarse-dispersed one with thickened linear structures. The listed changes in collagen fibers are visually recorded during morphometric studies.

In order to reduce the subjective component in the evaluation of tissue samples, it seems advisable to apply some numerical estimates of microphotographs, in particular, the study of the texture of the sample. This approach would reduce the time for mass evaluation of samples and eliminate the features introduced by the level of training of the specialist performing the diagnosis. The general purpose of this approach is to develop a set of numerical characteristics that would allow us to classify a sample as having pathological features or as a healthy tissue sample. Similar studies are known in the work on the assessment of the structure and condition of collagen by the second harmonic generation signal, which provides the results of numerical characterization of collagen fibers in a bundle and the mutual organization of bundles of collagen fibers [4]. It should also be noted that there is no universal method to quantify microphotographs suitable for any collagen structure [4].

Any raster image, in particular microphotography of collagen fibers, can be represented as a surface, the height of which corresponds to the numerical characteristic of brightness [5]. In this case, the black pixel (brightness 0) will correspond to a height of 0 m, and the white pixel (brightness 255) will correspond to a height of 255 m. Taking the pixel size as a fixed spatial value (for example, also for 1 m), we get a three-dimensional surface. It should be noted that the direct assessment of the spectral characteristics of the samples (color) is not a reliable source of classifying features. The color may vary depending on the reagents used and the characteristics of lighting. Therefore, texture assessment is the most reliable and promising approach.

For surface analysis, there is a fairly set of geoinformation technology tools [6-8], traditionally used for spatial analysis (regardless of spatial coverage). It is justified to use such a surface characteristic as curvature coefficient at a given point.

The change in the organization of collagen fiber bundles, their width and length is accompanied by a large number of brightness changes, i. e. (in the representation described above) local surface bends within a certain studied area. The more bends such a surface has, the higher the total and average values of the curvature coefficient (CC) will be. It is expected that the CC value will be significantly higher for an image of a histological specimen (transformed into a surface) with a large number of thin contrast fibers than for an image with a smaller number of thicker fibers. In this regard, the authors of the article proposed an algorithm that allows using the CC value as a reliable numerical criterion for classifying a sample as having pathologies or no pathologies.

THE AIM OF THE STUDY

To assess the possibility of using computer analysis of microphotographs in a complex of morphometric studies of collagen fibers in cold injury.

MATERIALS AND METHODS

The study included 84 patients with frostbite of the lower extremities of the III and IV degrees of severity in the period of granulation and epithelialization (day 30). The age of the victims ranged from 25 to 45 years. Men made up the majority of the group of patients – 78 %, women – 22 %. All the victims with local cold injury of the distal segments of the feet underwent surgical treatment at the regional center for thermal trauma at the Chita City Clinical Hospital No. 1 in the period from 2016 to 2020. All the patients gave written voluntary informed consent. Tissue fragments measuring $1 \times 1 \times 0.5$ cm were taken with a scalpel under local anesthesia during surgical treatment in the affected area at the border of damaged and healthy tissues (in the demarcation zone). The study was performed in accordance with the Regulations of Declaration of Helsinki of the World Medical Association (rev. 2013).

Autopsy material was used as a control. Tissue sampling from the lower extremities was performed within 24 hours after the biological death of the patients. The control group consisted of 50 patients who died from acute trauma, without severe concomitant diseases, of a similar age category.

Exclusion criteria: age – under 18 y. o. and over 50 y. o.; tuberculosis; cachexia of various etiologies; sepsis; chronic obstructive pulmonary disease; diabetes mellitus; vascular and nerve diseases of extremities; rheumatism; premorbid heart rhythm disorders; acute inflammatory diseases; atherosclerosis; acute cerebral circulation disorder.

Histological specimens were prepared according to a generally accepted method. Sections of 5–10 μm thickness were stained with hematoxylin, eosin and picrofuxin according to Van Gieson. Histological microslides of tissues of patients with local cold injury and tissues of patients of the control group were examined using Olympus CX21 microscope (Olympus, Japan), microphotography was performed using a Leica DM2500 microscope (Leica, Germany), at the Department of Pathological Anatomy of the Chita State Medical Academy.

Morphometric studies to determine the thickness of collagen fibers were performed for the control group

and patients with frostbite on day 30 with local cold injury. The measurement of the thickness of collagen fibers was performed on the basis of visual measurements of characteristic areas of microphotograph, the areas for measurements were selected based on diagnostic experience. For each microphotograph, at least 10 measurements of the thickness of collagen fibers were performed at the same magnification of the microscope (× 5). The complex of morphometric studies includes computer analysis of images of collagen fibers, which suggests the author's method of evaluating microphotographs in the field of view. The following sequence of actions was adopted for processing microphotographs:

Step 1. Creating surfaces. To perform this task, the RGB image in JPG format was converted to grayscale by channel-by-channel averaging (the numerical values of the brightness of each pixel in R, G, B channels are added and divided by 3). Contrast, gamma and histogram correction, other digital brightness correction methods were not applied in order to ensure the most homogeneous, content-independent spectral brightnesses of the samples. The resulting gray image is transformed into an ArcINFO GRID type surface (matrix with height values), where each cell is assigned a height numerically equal to the brightness of the gray image on a scale from 0 to 255.

Step 2. Surface filtration. The resulting image may contain digitization (technical features of the microscope) and compression (defects in the JPG algorithm) defects. Samples from different researchers may have different types of artifacts. For this purpose, the final surface was filtered. The sliding window method with a radius of 2 pixels is used for filtering, window shape – circle. The idea of the filtering algorithm is that a circle with a radius of 2 pixels is built around the current cell of the surface, the brightness of all pixels inside the circle is added and divided by the number of cells. The resulting average value is assigned to the current cell. Next, the analysis window moves one pixel to the side.

Step 3. Calculation of the curvature surface. The surface is calculated using the ArcGIS package using the Curvature function. The calculation of the numerical characteristic of the curvature goes along a 3 × 3 cell section, the final results are assigned to the central cell. The calculated curvature coefficient is multiplied by 100 for ease of use – it allows us to work with whole numbers, not fractional numbers. The result is a surface of CC values, the range of values is from 0 to 1800.

Step 4. Calculation of Curvature statistics. For each image, a block structure was formed in the form of squares measuring 100 × 100 pixels (which corresponds to a 64 × 64 μm matrix cell). The size of the block is determined by the size of the digital image, the field of view and the size of the collagen fibers. It is necessary to follow the principle when the block size is at least an order of magnitude larger than the average fiber thickness and when the field of view is at least an order of magnitude larger than the block. The size of 100 × 100 pixels satisfies these conditions.

Statistics of CC values were calculated for each image and for each block. The «Average» value is not representative, as it is close to 0 for all blocks and images – this is a consequence of peculiarities of the algorithm itself. However, the value of the standard deviation for CC values shows the presence of two well-defined classes correlating with the presence or absence of pathologies in the images. Calculating the values of standard deviations not for the entire images, but for blocks (with their subsequent filtering) allows us to exclude from consideration tissue samples and structures present on tissue samples, but not the subject of analysis (background, extraneous inclusions, accompanying tissues, etc.).

Step 5. Displaying the results. The results of the computer analysis of microphotographs are presented in the form of classified images by class: Control → Transitional class → Patients.

Statistical processing of the study results was carried out using the SPSS Statistics software package v. 25.0 (IBM Corp., USA). The results of the study are presented in the form of median, quartiles 1 and 3 – Me (Q1; Q3). Taking into account the number of the studied groups, the assessment of the normality of the distribution of quantitative characteristics was carried out using the Kolmogorov-Smirnov test. The statistical significance of the differences in the indicators was assessed by comparing the calculated and critical values of the Kruskal-Wallis test, followed by determining the significance level of p.

The results are presented in the form of boxplots of collagen fiber thickness and values of CC standard deviations in the studied area of the microphotograph for the groups (Control, Patients) in the figures. The image of the results of the computer analysis of microphotographs is presented in the form of classified images by class: Control → Transitional class → Patients in the pictures.

THE RESULTS OBTAINED AND DISCUSSION

As a result of the study, a significant increase in the thickness of collagen fibers was revealed in a group of patients with cold injury in the late stages of cryoinjury relative to the control group.

Figure 1 shows an example of the results of determination the thickness of collagen fibers for microphotographs from the control group and the group of patients.

In the field of view of the microphotograph, significant differences are observed in the form of changes in the thickness and orientation of the collagen fibers of the cryoinjury zone. This fact confirms the significant damage to the tissues of the frostbite zone at a late date (on day 30). Destructive processes in collagen fibers manifest themselves in the form of changes in their thickness and orientation. The so-called tissue disorganization of the cryoinjury zone occurs.

In the late reactive period, in patients with cryoinjury, the fiber thickness is 2.8 times higher relative to the control group ($p < 0.001$).

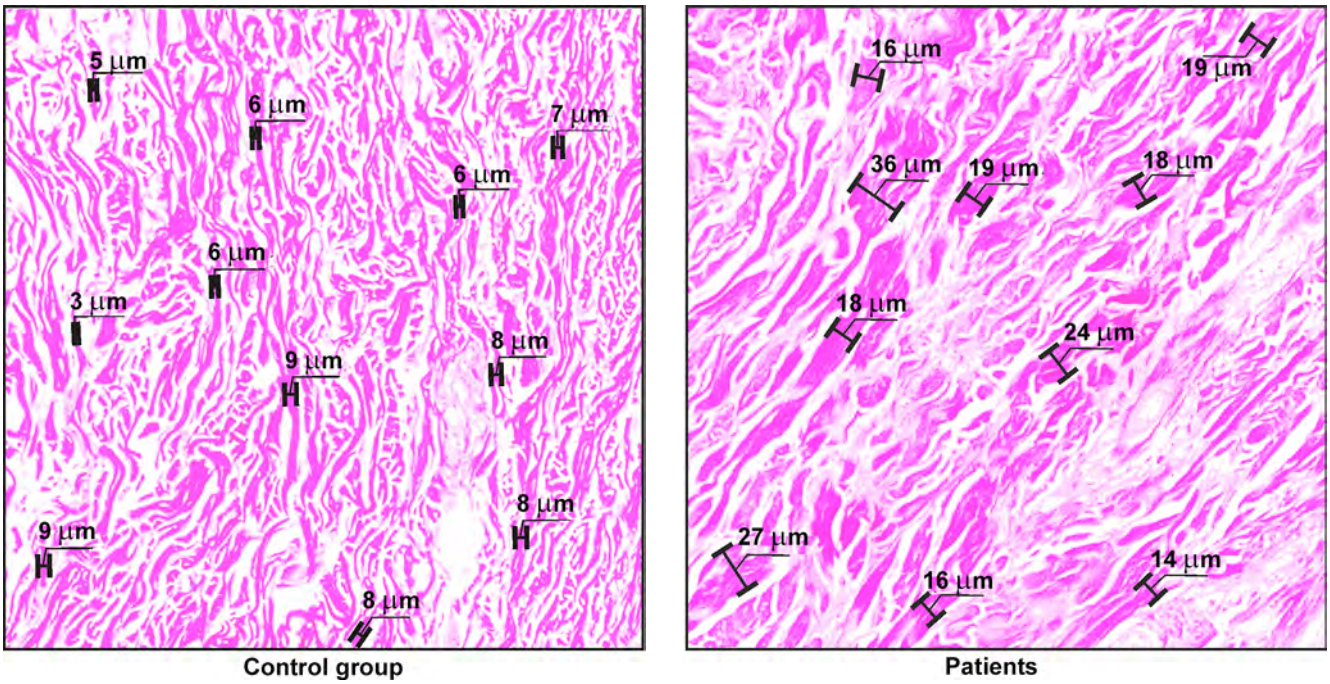

FIG. 1.
Thickness of collagen fibers in the control group and in the group of patients with frostbite. Van Gieson picrofuxin staining. Magnification during microphotography ×5. Photo made by the author

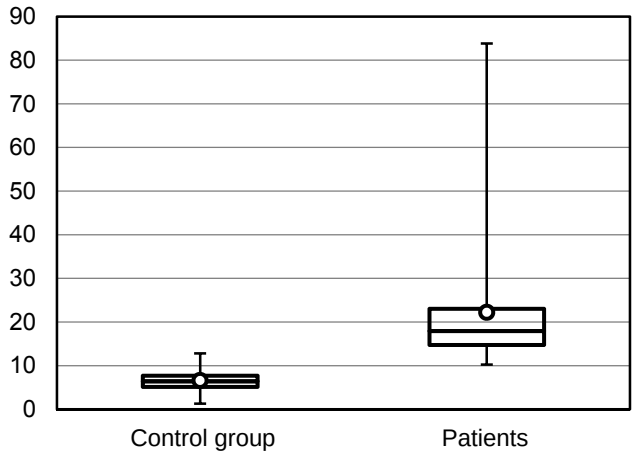

FIG. 2.
Fiber thickness (microns) in the control group and in the group of patients with local cold injury

Collagen disorganization has enzymatic and non-enzymatic mechanisms. Enzymatic mechanisms are carried out by the interaction of collagenases and their inhibitors, which regulate the intensity of catabolic processes. Non-enzymatic mechanisms are associated with the formation of additional intermolecular links under the influence of end-products of glycation of UV irradiation or mechanical pressure (edema), as well as a result of carcinogenesis. Different regulatory pathways and degradation mechanisms intersect with each other at different stages, rather than working independently of each other [9–11].

Thus, secondary dystrophic and inflammatory changes in tissues under the influence of low temperatures lead to damage to collagen and its disorganization. This phenomenon pathogenetically explains the large number of late complications in patients with frostbite and the characteristic prolonged course of reparative processes.

As a result of computer analysis of images of collagen fibers for the control group and the group of patients, the standard deviation of the curvature coefficient in the studied area of the microphotograph was determined.

In the late reactive period, in patients with cryoinjury, the standard deviation of CC in the studied area of the microphotograph is 1.8 times lower relative to the control group ($p < 0.001$).

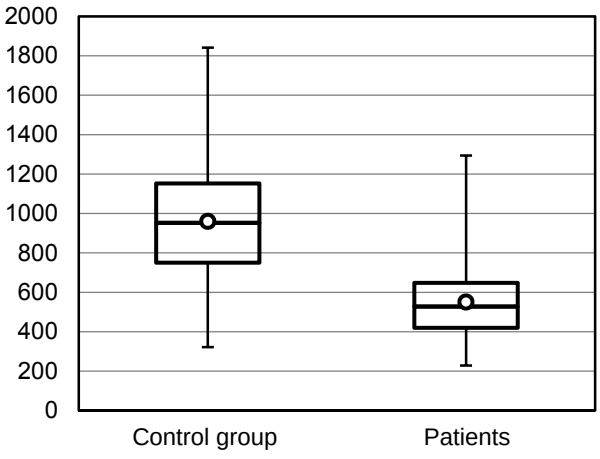

FIG. 3.
Standard deviation of the curvature coefficient (standard units) in the studied area of microphotograph

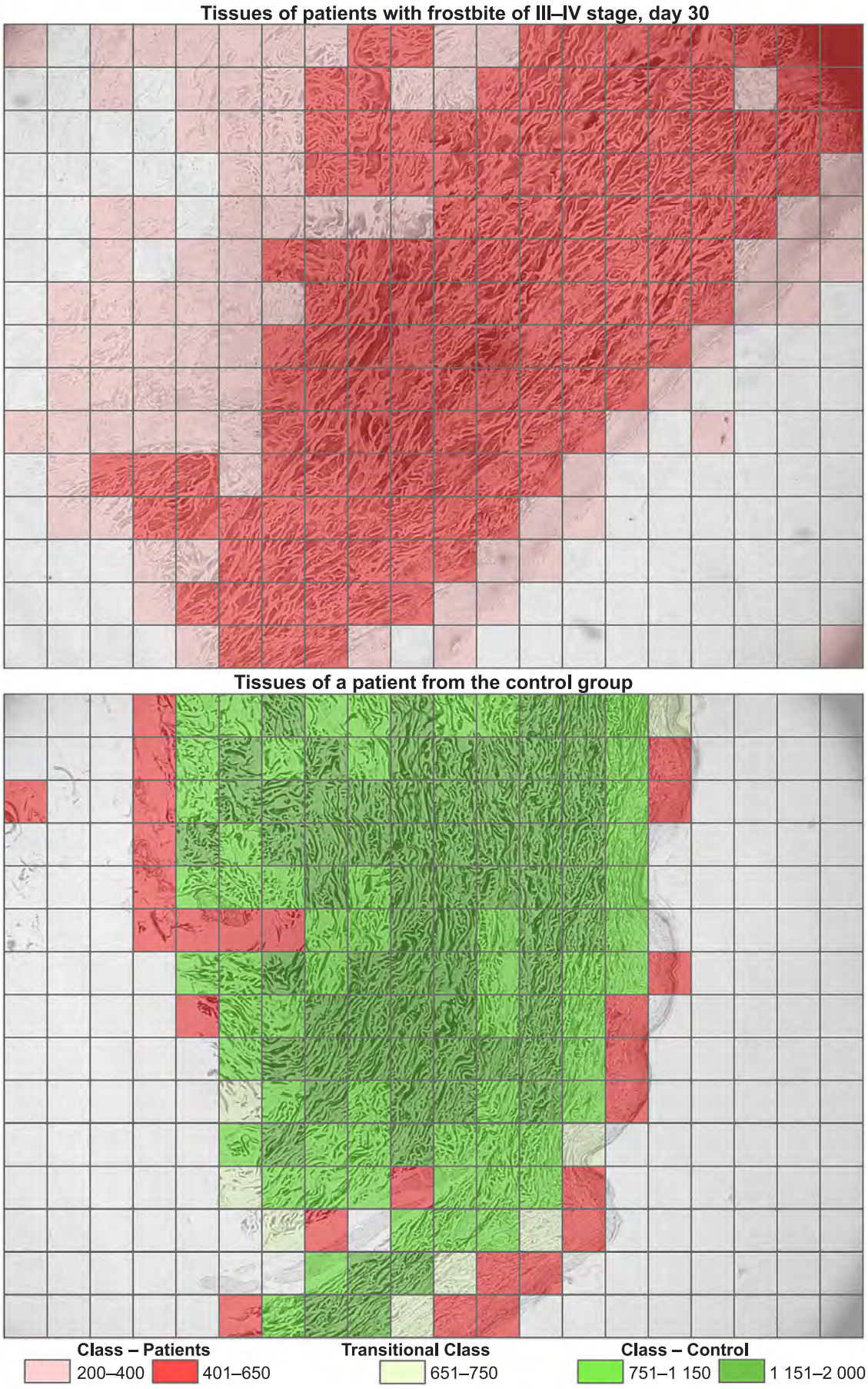

FIG. 4.

Classification of microphotographs according to the values of the standard deviation of the curvature coefficient. Photo made by the author

With significant cryoinjury of tissues, changes in the thickness and orientation of collagen fibers occur, which is well matched in the field of view of micrography. In this regard, a change in the texture of the microphotograph leads to a change in the numerical characteristics of the standard deviation of CC in the studied area. This is confirmed by a strong inverse correlation between the measured thickness of collagen fibers and the standard deviation of CC in the studied area of the microphotograph ($r_S = -0.75$; $p < 0.001$).

Thus, the characteristic of the standard deviation of the CC allows us to attribute one or another microphotograph to the number of pathological signs or to a sample of healthy tissues. This fact makes it possible, based on the obtained statistical indicators of the standard deviation of the CC, to classify the field of study of microphotograph by classes: Control → Transitional class → Patients (Fig. 4).

Based on the classification obtained, the difference between the microphotograph of tissues of patients with frostbite of III–IV stage on day 30 and the control group is clearly demonstrated (Fig. 4).

The number of colored blocks in each class characterizes the area of damage to collagen fibers in the field of view of the microphotograph.

CONCLUSIONS

Low temperatures lead to qualitative changes in collagen fibers and contribute to a change in their width and orientation in the area of damage. With frostbite, severe tissue structure damage occurs, accompanied by the destructive processes of the extracellular matrix components. Changes in the tissue structure and their organization are important for the morphological characteristics of tissues in various physiological conditions and pathologies, including cold injury.

Computer analysis of microphotographs allows classifying the image of a histological preparation by the value of CC and classifying one or another microphotograph as having pathological signs or as a sample of healthy tissues. Thus, the characteristic changes in collagen fibers during local cold injury in the late reactive period are a factor that makes it possible to perform automated computer diagnostics of microphotographs using GIS technologies.

Conflict of interest

The authors of this article declare the absence of a conflict of interest.

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MORPHOLOGICAL DETERMINANTS FOR THE LOCAL HEMOSTATIC EFFECT OF EXOGENOUS FIBRIN MONOMER IN ITS SYSTEMIC ADMINISTRATION AFTER INJURY WITH INHIBITION OF PLATELET AGGREGATION IN THE EXPERIMENT

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ABSTRACT

Background. *In our previously published studies, we demonstrated a high hemostatic activity of a low dose of exogenous fibrin monomer during its systemic administration in a model of dosed liver injury with preliminary inhibition of platelet aggregation. However, the analysis of platelet involvement in the mechanisms of local fibrin formation has not been analyzed.*

The aim of the study. *To conduct a comparative analysis of the cellular composition of venous and wound blood, as well as blood in the wound vessels to assess the contribution of platelets to the hemostatic effect of exogenously administered fibrin monomers in dosed liver injury under conditions of pharmacologically determined thrombocytopathy.*

Methods. *In a model of dosed liver injury in rabbits after inhibition of platelet aggregation by acetylsalicylic acid in combination with clopidogrel, the effect of the administration of fibrin monomer was evaluated in comparison with the use of tranexamic acid. We studied the number of platelets in venous and wound blood smears, as well as in the contents of wound vessels.*

Results. *It has been established that with the systemic administration of exogenous fibrin monomer, the number of platelets in wound blood smears decreases by 17.2 % in comparison with free circulating venous blood. Platelets in wound blood form aggregates and are in an activated state. In the wound vessels, the number of these cells was maximum (150 per lower field) compared with the number of platelets in the placebo and tranexamic acid groups (55 and 84 per lower field, respectively). Also in the wound blood, erythrocytes with altered forms (echinocytes, schistocytes, stomatocytes and ovalocytes) were found.*

Conclusion. *Systemic administration of exogenous fibrin monomer affects the redistribution of platelets between the systemic circulation, wound vessels and wound blood, determining its hemostatic effect and local wound fibrin formation in dosed liver injury. The presence of receptor-mediated platelets recruitment due to fibrin monomer in the wound vessels with the participation of damaged erythrocytes is assumed.*

Key words: *fibrin monomer, acetylsalicylic acid, clopidogrel, tranexamic acid, platelets, hemorrhage control*

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МОРФОЛОГИЧЕСКИЕ ПРЕДПОСЫЛКИ ЛОКАЛЬНОГО ГЕМОСТАТИЧЕСКОГО ЭФФЕКТА ЭКЗОГЕННОГО ФИБРИН-МОНОМЕРА ПРИ ЕГО СИСТЕМНОМ ВВЕДЕНИИ ПОСЛЕ ТРАВМЫ ПРИ ПОДАВЛЕНИИ АГРЕГАЦИОННОЙ ФУНКЦИИ ТРОМБОЦИТОВ В ЭКСПЕРИМЕНТЕ

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РЕЗЮМЕ

Обоснование. *В опубликованных нами ранее исследованиях была продемонстрирована высокая гемостатическая активность низкой дозы экзогенного фибрин-мономера (ФМ) при его системном введении на модели дозированной травмы печени при предварительном угнетении агрегационной функции тромбоцитов. Однако анализ участия тромбоцитов в механизмах локального фибринообразования не анализировался.*

Цель исследования. *Провести сравнительный анализ клеточного состава венозной и раневой крови, а также крови в прираневых сосудах для оценки вклада тромбоцитов в обеспечение гемостатического эффекта экзогенно введённого ФМ при дозированной травме печени в условиях фармакологически обусловленной тромбоцитопатии.*

Методы. *На модели дозированной травмы печени у кроликов после угнетения агрегационной функции тромбоцитов ацетилсалициловой кислотой в сочетании с клопидогрелом оценивали влияние введения ФМ в сравнении с применением транексамовой кислоты (ТК). Изучали количество тромбоцитов в мазках венозной и раневой крови, а также в содержимом прираневых сосудов.*

Результаты. *Установлено, что при системном введении экзогенного ФМ количество тромбоцитов в мазках, полученных из раневой крови, снижается на 17,2 % в сравнении со свободно циркулирующей венозной кровью. Тромбоциты в раневой крови образуют агрегаты и находятся в активированном состоянии. В прираневых сосудах количество этих клеток было максимальным (150 в поле зрения) по сравнению с числом этих клеток в группах плацебо и с применением ТК (55 и 84 в поле зрения соответственно). Также в раневой крови встречались эритроциты с изменёнными формами – эхиноциты, шизоциты, стоматоциты и овалоциты.*

Заключение. *Системное введение экзогенного ФМ влияет на перераспределение тромбоцитов между системным кровотоком, прираневыми сосудами и раневой кровью, определяя его гемостатический эффект и локальное прираневое фибринообразование при дозированной травме печени. Предполагается наличие рецепторно-опосредованного привлечения тромбоцитов за счёт ФМ в прираневых сосудах с участием повреждённых эритроцитов.*

Ключевые слова: *фибрин-мономер, ацетилсалициловая кислота, клопидогрел, транексамовая кислота, тромбоциты, остановка кровотечения*

Для цитирования: Вдовин В.М., Сузопов Е.В., Шахматов И.И., Бобров И.П., Орехов Д.А., Долгатов А.Ю., Момот А.П. Морфологические предпосылки локального гемостатического эффекта экзогенного фибрин-мономера при его системном введении после травмы при подавлении агрегационной функции тромбоцитов в эксперименте. *Acta biomedica scientifica*. 2023; 8(4): 155-164. doi: 10.29413/ABS.2023-8.4.17

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OBJECTIVES

The concept of the hemostasis system as a multicomponent cascade reaction aimed at the formation of fibrin strands with the participation of coagulation factors, endothelial cells and formed elements of blood has undergone certain changes in recent years. First of all, it concerns the regulation of this complex process.

Modern approaches to the study of blood coagulation regulation are based on studies of spatial thrombogenesis *in vitro* [1], the "cellular theory" of M. Hoffman and D.M. Monroe [2–4], within the framework of which new data obtained by analyzing the effect of one of the fibrinogen derivatives, des-AABB-fibrinogen (fibrin monomer (FM)) – on the hemostasis system under physiological conditions, as well as with pharmacologically determined suppression of blood coagulation reactions. Previous studies carried out in our laboratory have shown the presence of unique hemostatic and hemostasiological effects with systemic administration of FM without activation of blood coagulation reactions and pathological thrombosis [5, 6].

From our point of view, the detected regulatory effects of FM are realized outside the set of coagulation reactions leading to the thrombin formation, but preceding fibrin formation. It is indicated by studies conducted under conditions of mediated inhibition of thrombin by unfractionated heparin [7, 8], as well as with direct suppression by dabigatran [9]. In both experimental models, FM demonstrated the effect of minimizing post-traumatic blood loss. At the same time, it contributed to local fibrin formation without correction of hypocoagulation caused by the administration of anticoagulants.

Based on the results presented above, our research group suggested that the mechanisms of the hemostatic effect of FM are realized with the participation of blood cells, primarily platelets, despite the pharmacological suppression of their functional activity. The latter is indicated by the results of studies conducted using dual antiplatelet therapy and correction of post-traumatic bleeding with tranexamic acid (TA) or FM [10]. In this work, a high local hemostatic activity of FM comparable to the effects of TA with a concomitant 16-fold decrease in the intensity of ADP-induced platelet aggregation compared with control values was demonstrated. In the same series of experiments, the authors carried out morphological analysis of tissues in the area of injury, where high activity of wound fibrin formation was revealed [11]. In the above materials, no sufficient attention was paid to assessment of the regularity of platelet redistribution between venous and wound blood, which seems necessary for a possible decoding of the mechanisms of the hemostatic effect of FM *in vivo*.

THE AIM OF THE STUDY

To conduct a comparative analysis of the cellular composition of venous and wound blood, as well as blood in the wound vessels to assess the contribution of platelets to the hemostatic effect of exogenously administered fibrin monomers in dosed liver injury under conditions of pharmacologically determined thrombocytopathy.

METHODS

The study included 48 healthy mature male Chinchilla rabbits weighing 3.0–4.5 kg. Animal experiments were carried out in accordance with the European Convention and Directives for the Protection of Vertebrates Animals used in the experiment 86/609/EEC, as well as the World Medical Association Declaration of Helsinki and the "Regulations for Animal Use in Biomedical Research". The work was approved by the local Ethics Committee of the Altai State Medical University (Protocol No. 12 dated November 12, 2015).

To suppress platelet aggregation function, a mixture of acetylsalicylic acid (Thrombo ACC®; Lannacher Heilmittel GmbH, Austria) at a dose of 2.0 mg/kg and clopidogrel (Plavix®; Sanofi Winthrop Industry, France) at a dose of 8.0 mg/kg dissolved in water was administered to all animals *per os*. As is known, acetylsalicylic acid causes inhibition of platelet cyclooxygenase-1 and a subsequent decrease in the production of A_2 thromboxane. Clopidogrel is a prodrug, which is transformed into its active form through metabolism in the liver and acts as an antagonist of $P2U_{12}$ platelet receptors [12].

Next, all the animals were divided into three groups using the random number method. In 1 hour after taking these antiplatelet drug, animals were injected intravenously (IV) into the marginal vein of the ear with aqueous solutions of the following drugs: group No. 1 (n = 13) – placebo solution (3.75 M urea solution corresponding to its concentration in FM solution) with a volume of 0.5 ml; group No. 2 (n = 22) – tranexamic acid solution (Tranexam®; Moscow Endocrine Plant, Russia) at a dose of 15 mg/kg; group No. 3 (n = 13) – FM at a dose of 0.25 mg/kg (Technologiya-Standart LLC, Russia). All animals underwent standard liver injury under general anesthesia with Telazol (Zoetis, Russia; 10 mg/kg IV) in accordance with available recommendations: groups No. 1 and No. 3 – 1 hour after administration of placebo and FM, animals of group No.2 – 30 minutes after administration [13]. As it was shown earlier, FM showed its maximum effects when administered 1 hour before injury [6]. According to the manufacturer, with a high risk of bleeding, tranexamic acid is injected into the systemic circulation 20-30 minutes before the intervention (Instructions for use, ЛСР-001709/07).

To determine the morphology of erythrocytes, the number and aggregation function of platelets in animals, blood was obtained after incision of the marginal vein of the ear (by gravity) twice – before administration of hemostatic drugs or placebo and before liver injury. To assess the aggregation function of platelets, blood was placed in plastic tubes containing 0.11 M (3.8 %) sodium citrate solution (blood-stabilizer ratio 9:1). The production of platelet-rich blood plasma was carried out ac-

cording to a generally accepted method. Platelet aggregation in it was evaluated using a Chronolog 490-2D aggregometer (CHRONO-LOG Corporation, USA) when using adenosine diphosphate (ADP) aggregation agonist at an initial concentration of 10 μm.

To count the number of platelets and evaluate the morphology of erythrocytes, smears of blood from the marginal vein of the ear and blood from the wound surface 3–5 minutes after injury were obtained. The smears were stained according to Romanovsky–Giemse followed by oil immersion microscopy on a Nikon Eclipse E-200/F binocular microscope with a Nikon Digital Sight 1000 camera (Nikon, Japan) at a total optical magnification of ×1000. The number of platelets by Phonio ($\times 10^9$/L), their morphological characteristics (shape, presence of granules and inclusions), as well as the presence of aggregates of these cells in the smears were assessed. Along with this, the morphology of erythrocytes (size and shape) was evaluated.

After spontaneous cessation of post-traumatic bleeding, liver tissue, including its wound part and a fragment of an intact surface, was taken as part of histological studies, followed by fixation in a 10 % solution of neutral formalin according to Lilly. Material histologic processing was carried out using isopropyl alcohol using a TISSUE-TEK VI PTM6 carousel-type tissue processor (Sakkura, Japan). Paraffinization was carried out using the TISSUE-TEK TEC 5 embedding console (Sakkura, Japan). Histological sections 4–5 μm were obtained using a semi-automatic rotary microtome Accu-Cut SRM (Sakkura, Japan), the preparations were stained with hematoxylin and eosin in a TISSUE-TEK Prisma slide stainer with film encapsulation in TISSUE-TEK Film machine (Sakkura, Japan). The count of platelets (the number in the field of view) was carried out on microslides, including large vessels (venous or arterial), in five fields of view at magnification × 1000, under oil immersion, followed by calculation of the average number of cells in the fields of view. Microphotography was performed using Leica DM 750 E200 microscope with Leica EC3 digital video camera (Leica Microsystems CMS GmbH, Germany). The image analysis was performed using the "Image Tool 3.0" software.

The distribution of features in the samples was evaluated according to the Shapiro – Wilk test. Depending on the distribution of features, the Student's t-test and Mann – Whitney U-test or Wilcoxon test were used. Differences in the mortality rate of animals in the groups were established using the Fisher's exact test. The differences were considered statistically significant at $p < 0.05$. The experimental data were processed using the statistical software MedCalc 17.9.7 (license BU556-P12UT-BBS55-YAH5M-UBE51). The data obtained are presented in the form of median (Me), 25th and 75th percentiles (Q): Me [$Q_{25} \div Q_{75}$].

RESULTS

The use of dual antiplatelet therapy in animals led to an expected decrease in the intensity of ADP-induced aggregation of venous blood platelets in study groups No. 1, No. 2 and No. 3 by 4.5, 3.0 and 16.6 times, respectively (Table 1).

At the same time, in the control group (group No. 1), the number of platelets in the systemic bloodstream after administration of antiplatelet agents and placebo decreased moderately (by 19.8 %) compared with the baseline value (Table 2).

These cells were usually in an inactive state, their aggregates were absent (Fig. 1a). In rare cases, there were single activated platelets (up to 1–3 in the field of view). The appearance of erythrocytes in the systemic circulation was featureless. The number of platelets in wound blood was 32.8 % less than in circulating venous blood before traumatic exposure, and 46.0 % less than the same indicator before the introduction of antiplatelet agents. In the wound blood, platelets were visualized in an activated, partially aggregated state. The granules of the blood plates were tightly "condensed" (tightly fitting to each other), which also indicated the activation of these cells. The number of platelets in the aggregates varied from 6 to 10 cells (Fig. 1b). During the assessment of the morphology of erythrocytes in wound blood

TABLE 1

INDICATORS OF PLATELET AGGREGATION IN THE EXPERIMENTAL ANIMAL GROUPS, ME [Q25÷Q75]

Indicators	Group No. 1 Antiplatelet agents and placebo		Group No. 2 Antiplatelet agents and TA		Group No. 3 Antiplatelet agents and FM	
	before administration (1a)	after administration (1b)	before administration (2a)	after administration (2b)	before administration (3a)	after administration (3b)
ADP-induced platelet aggregation, %	20.1 [18.4÷45.9]	4.5 [0.6÷7.0] **$p_{1a-1b} = 0.001$; $\Delta_{1a-1b} = -4.5$ times**	24.0 [19.0÷46.5]	8.0 [4.9÷10.1] **$p_{2a-2b} < 0.001$; $\Delta_{2a-2b} = -3.0$ times**	19.9 [13.3÷20.1]	1.2 [1.0÷2.0] **$p_{3a-3b} = 0.001$; $\Delta_{3a-3b} = -16.6$ times**

Note. p is the level of statistical significance of the differences in the compared indicators; Δ is the difference in indicators.

the presence of echinocytes up to 5–8 pieces (in the field of view), single schizocytes, stomatocytes and ovalocytes were noted. Histological analysis of the liver parenchyma revealed that the count of platelets was about 55 cells in the field of view in the vessels next to the wound surface.

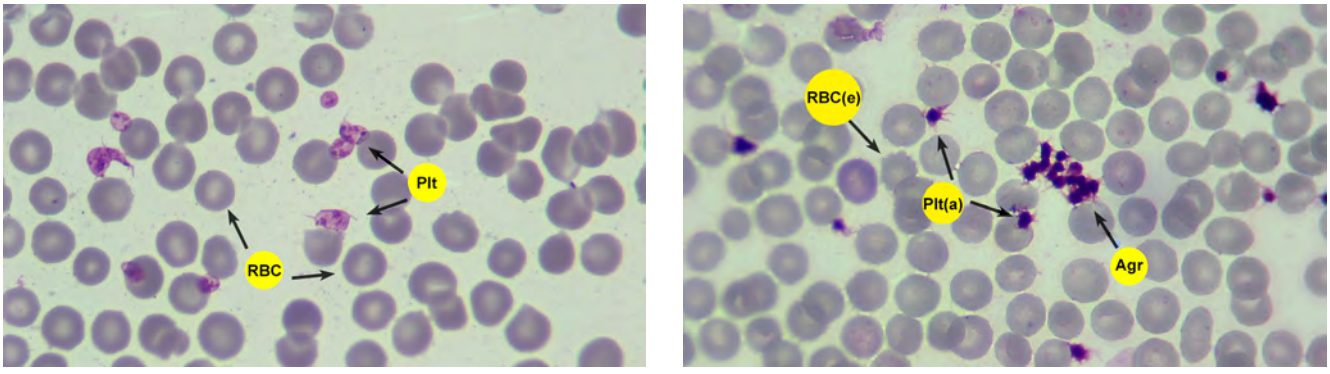
In the group of animals that received a fibrinolysis inhibitor along with antiplatelet agents (group No. 2), the number of platelets in the systemic bloodstream remained stable both before and after TA (Table 2). Platelets under microscopy were predominantly in an inactive state, without visible aggregation (Fig. 2a). The appearance of erythrocytes also corresponded to the physiological norm, although there were single echinocytes in the field of view. In wound blood, the number of platelets did not significantly differ from the count of blood plates in circulating venous blood taken before the injury. During visualization, activated platelets were noted in the wound blood, and their granules were densely "condensed". Along with this, platelet aggregates (2–20 cells) were detected (Fig. 2b). Chang-

TABLE 2

INDICATORS OF PLATELET COUNT IN SYSTEMIC BLOOD, INJURY BLOOD AND IN THE VESSELS NEAR THE WOUND SURFACE, ME [Q25÷Q75]

Groups	Platelet count in venous blood (smear)		Platelet count in wound blood (smear), count/FOV (c)	Platelet count in large vessels near the wound (morphometry on the section), count/FOV (d)
	before the administration of drugs, count/FOV (a)	after the administration of drugs, count/FOV (b)		
Group No. 1 Antiplatelet agents and placebo	605.0 [571.3÷648.5]	485.0 [436.0÷540.0] **p_{1a-1b} = 0.028; Δ = –19.8 %**	327.0 [273.0÷419.5] **p_{1b-1c} = 0.008; Δ = –32.6 %** **p_{1a-1c} = 0.028; Δ = –46.0 %**	55.0 [50.8÷60.0]
Group No. 3 Antiplatelet agents and tranexamic acid	574.5 [531.3÷648.5]	597.0 [451.8÷708.5] p_{2a-2b} = 0.754 p_{b-2b} = 0.109	602.0 [403.0÷841.5] p_{2b-2c} = 0.695 p_{2a-2c} = 0.388	84.0 [82.0÷89.5] **p_{1g-2g} = 0.0003; Δ = +52.7 %**
Group No. 3 Antiplatelet agents and fibrin monomer	514.0 [477.8÷560.5]	530.0 [470.5÷546.0] p_{3a-3b} = 0.756 p_{1b-3b} = 0.403	439.0 [427.0÷443.0] **p_{3b-3c} = 0.021; Δ = –17.2 %** **p_{3a-3c} = 0.028; Δ = –13.8 %**	150.0 [113.5÷201.0] **p_{1d-3d} = 0.001; Δ = +172.7 %** **p_{2d-3d} = 0.029; Δ = +78.5 %**

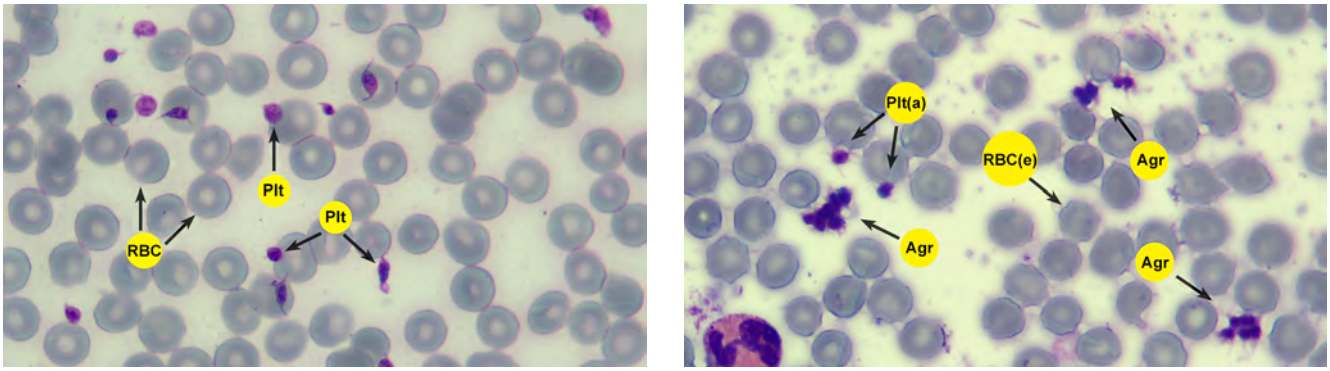
Note. *p* is the level of statistical significance of the differences in the compared indicators; FOV is the field of view; Δ is the difference in indicators.



a ***b***

FIG. 1.

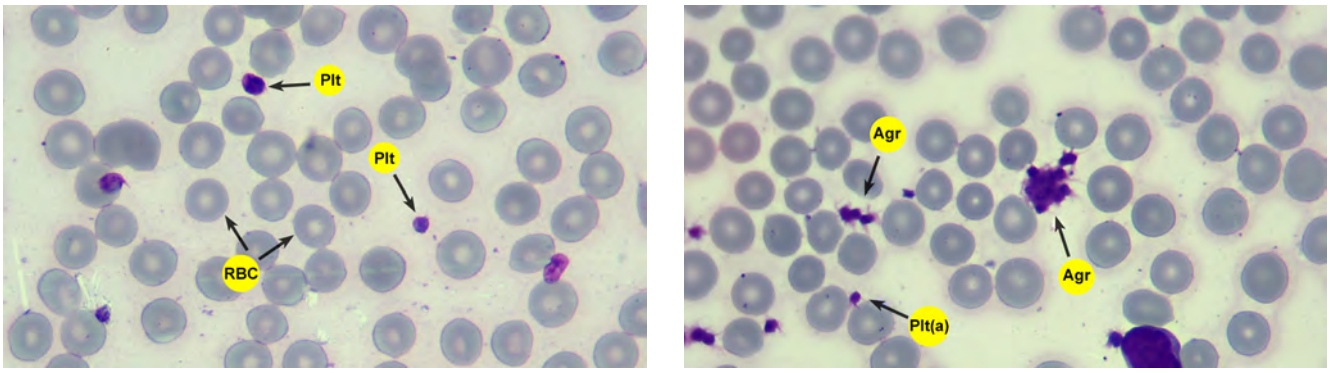
Visualization of rabbit blood from group No. 1 (antiplatelets and placebo): ***a*** *– systemic blood smear after drug administration (non-lysed red blood cells and platelets: Plt – non-activated platelets; RBC – normal red blood cells);* ***b*** *– injury blood smear (modified cell morphology: Agr – platelet aggregate; Plt(a) – activated platelets; RBC(e) – lysed red blood cell (echinocyte)). Hereinafter: Romanovsky – Giemsa staining; light microscopy, magnification × 100*



a *b*

FIG. 2.

*Visualization of rabbit blood from group No. 2 (antiplatelets and tranexamic acid): **a** – systemic blood smear after drug administration (non-lysed red blood cells and platelets: Plt – non-activated platelets; RBC – normal red blood cells); **b** – injury blood smear (modified cell morphology: Agr – platelet aggregate; Plt(a) – activated platelets; RBC(e) – lysed red blood cell (echinocyte))*



a *b*

FIG. 3.

*Visualization of rabbit blood from group No. 3 (antiplatelets and FM): **a** – systemic blood smear after drug administration (non-lysed red blood cells and platelets: Plt – non-activated platelets; RBC – normal red blood cells); **b** – injury blood smear (modified cell morphology: Agr – platelet aggregate; Plt(a) – activated platelets)..*

es in the morphology of erythrocytes concerned the formation of a large number of echinocytes (25–30 cells in the field of view). Morphometry of liver tissue revealed that in the vessels next to the wound surface, the count of platelets was about 84 cells in the field of view.

In the group of animals that received exogenous fibrin monomer along with antiplatelet agents (group No. 3), the number of platelets in the systemic bloodstream remained unchanged regardless of pharmacological effects (Table 2). They were mostly in an inactive state, and aggregates of blood plates were not observed (Fig. 3a). The appearance of erythrocytes in the systemic bloodstream practically did not differ from the physiological norm, although 10–15 echinocytes per the field of vision were found among erythrocytes. The number of platelets in the systemic circulation was 17.2 % higher than in wound blood. Activated and aggregated platelets with densely "condensed" granules were also found there. The number of platelets in the aggregates varied from 5 to 9 cells (Fig. 3b). Single echinocytes, ovalocytes and schistocytes (up to 3 in the field of view) were found among erythrocytes. During microscopy of histological preparations about 150 platelets were noted inside the wound vessels in the field of view.

DISCUSSION

As a result of the study, we reproduced a model of coagulopathy caused by oral administration of two antiplatelet agents with different mechanisms of action. It was confirmed by a decrease in ADP-induced platelet aggregation in animals in all experimental groups.

After the use of antiplatelet agents, the number of platelets in the systemic venous bloodstream in animals treated with TA or FM did not change. On the contrary, in the control group (without the use of systemic hemostatics), the number of blood plates in the systemic circulation decreased statistically significantly from the initial level, which corresponds to the data obtained in the work of O.D. Ostroumova et al. [14]. Moreover, platelets of the systemic blood flow in all groups were in an inactive state, the formation of aggregates in the smears was not noted.

An important aspect necessary for studying the mechanisms of local hemostasis when using FM, in our opinion, was the study of the cellular composition of wound blood and wound vessels. It was determined that in the TA group, the number of platelets in wound blood remained unchanged, compared with the number of these cells in the systemic circulation. On the contrary, in the placebo and FM groups, wound blood contained fewer blood plates compared to systemic blood flow. Wound blood platelets in all the observed groups were in an activated state, with densely "condensed" granules, and formed aggregates, which is natural for traumatic effects. As is known, the process of convergence of granules (condensation) of platelets leads to a change in the shape of platelets, flattening of their cytoplasm and signals readiness for thrombus formation [15]. A relatively higher number of cells in aggregates were observed in the group with TA.

From our point of view, the number of platelets in the wound vessels during histological examination of microslides of the wound surface of the liver may reflect the degree of involvement of cells from the general circulation to the injury area. The smallest number of blood plates in these vessels was observed in the control group, while their maximum value was recorded in the FM group – 2.7 times.

On the other hand, a decrease in the number of platelets in wound blood may be explained by their involvement in the process of local thrombosis. To explain this phenomenon, we can refer to the results of our previous studies [11]. Thus, in the placebo and antiplatelet agents groups, we observed a low-intensity formation of thrombotic masses in the area of injury, consisting of fibrin strands and unchanged erythrocytes. On the contrary, the use of TA and FM against the background of pharmacologically induced thrombocytopathy led to an increase in the thickness of thrombotic wound deposits (with a maximum value in the group with FM) and fibrin strands in their composition.

Thus, the use of modern antiplatelet agents was not accompanied by the attraction of platelets to the wound surface and their inclusion in the process of local thrombus formation. A similar conclusion can be drawn for the TA group. As is known, TA is used for increased bleeding associated with thrombocytopathy/thrombocytopenia [16, 17]. It is also known that the hemostatic effect of TA is mediated by a change in the hemostatic balance towards thrombosis due to a disruption of the plasminogen activation in plasmin [18]. Consequently, against the background of the use of TA, the role of platelets in local thrombosis (taking into account the suppression of their function by antiplatelet agents) is insignificant. This is also evidenced by their stable content in wound blood (in comparison with systemic blood flow) and a moderate increase in their number in the wound vessels.

A new result obtained in the course of this study is the fact of platelet redistribution in the case of FM application. Probably, a decrease in the number of these cells in wound blood with an increase in their number in the wound vessels indicates their targeted attraction to the site of injury when exogenous FM is introduced into the bloodstream.

The assumption of the inclusion of platelets in wound fibrin formation with the introduction of FM is supported by studies that demonstrate the independent ability of this fibrinogen derivative to enhance the aggregation activity of platelets [19–23] and erythrocytes [24]. As is known, FM is a derivative of fibrinogen with a similar molecular structure, differing only in the absence of four fibrinopeptides (2A and 2B), as a result of the action of thrombin. Fibrinogen is able to bind to GP IIb/IIIa platelet receptors to form molecular bonds between cells during their aggregation. The binding centers with these receptors are present in the FM molecule and are localized at the C-end of γ-chain (conservative sequence of Liz-Gln-Ala-Gli-Asp-Val at position 400–411) [25, 26] and α-chain (tripeptide Arg-Gli-Asp at position 572–574) [27, 28]. Consequently, FM, like fibrinogen, has the ability to bind to platelets through GP IIb/IIIa receptors and form fibrin-like bridges between cells to stimulate their aggregation function. In addition, it is known that platelets in the presence of collagen of damaged vessels and thrombin are able to produce microvesicles enriched with a large number of receptors, including IIb/IIIa, necessary for interaction with fibrin, fibrinogen and fibronectin [29, 30].

Thus, we suggest that in animals with pharmacologically determined thrombocytopathy, the use of exogenous FM may be accompanied by receptor-mediated involvement of blood plates in the mechanisms of local thrombosis.

Another important aspect of this study was the assessment of the morphology of erythrocytes to determine the contribution of these cells to the hemocoagulation process. The appearance of erythrocytes in the systemic circulation in the observation groups corresponded to the physiological norm. In cases involving the use of TA and FM, the presence of single echinocytes was noted, which may be represented by artifacts formed during the preparation of blood smears [31].

There were erythrocytes with altered forms in the wound blood of animals – echinocytes, schistocytes, stomatocytes and ovalocytes. After passing the formed elements through the damaged vessels of the wound surface, a disruption of the structure of their cell membrane is noted. Previous studies have shown that thrombotic formations in the placebo group included unchanged erythrocytes, forming mixed-type thrombi (fibrin-erythrocyte) [11]. On the contrary, thrombotic masses in the wound when using TA and FM consisted of a large number of predominantly hemolysed erythrocytes. In general, it can be assumed that damage to the cell membranes of erythrocytes, up to hemolysis, creates an additional condition for the activation of blood coagulation, including against the background of the use of TA and FM.

CONCLUSION

The results obtained on the model of pharmacologically conditioned thrombocytopathy demonstrated the effect of platelet redistribution between the systemic

bloodstream, wound vessels and wound blood when exogenous FM is injected into the bloodstream. It is assumed that the targeted attraction of injected FM to the injury site has a receptor-mediated mechanism for local thrombosis, despite the action of antiplatelet agents. The study also showed a positive contribution of erythrocytes to the formation of wound thrombotic masses. The detailed mechanisms of exogenous FM vector accumulation on the wound surface will have to be clarified in subsequent studies.

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Conflict of interest

The authors declare that there is no conflict of interest in the submitted article.

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OPHTALMOLOGY

CORNEAL EDEMA CAUSED BY UNDIAGNOSED IRIDOCORNEAL ENDOTHELIAL SYNDROME AFTER SMILE

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ABSTRACT

The aim. *To present a clinical case of a patient with corneal edema caused by undiagnosed iridocorneal endothelial syndrome after SMILE surgery.*
Iridocorneal endothelial syndrome is characterized by abnormal proliferation of the corneal endothelium beyond the Schwalbe line, blockade of the iridocorneal angle, specific changes in the shape of the pupil and the formation of synechiae between adjacent structures of the anterior chamber angle of the eye, which is accompanied by the development of pretrabecular retention of intraocular aqueous humour, increased intraocular pressure (IOP) and subsequent development of glaucoma in 46–82 % of patients. One of the forms of the disease is Chandler's syndrome, which clinically in the initial stages may be accompanied by the formation of only moderate ectropion. At the same time, endothelial dysfunction is characterized by a significant decrease in the number of cells, a change in their shape and the appearance of epithelioid cells with a hyper-reflective nucleus (the so-called "ICE (iridocorneal endothelial) cells"), with a violation of their natural pumping function. The article presents a clinical case of a patient who underwent a standard examination and symptomatic treatment after laser keratorefractive surgery for myopia correction. Low visual acuity, diffuse corneal edema, IOP decompensation of unclear genesis were observed in one operated eye.
Conclusion. *Keratorefractive operations can act as a trigger that stimulates the transition of the latent form into a clinically developed pathological process, which causes a decrease in corrected visual acuity due to the formation of corneal edema and glaucoma optic neuropathy.*

Key words: *iridocorneal endothelial syndrome, keratorefractive surgery, corneal edema, postoperative complications*

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ОТЁК РОГОВИЦЫ, ВЫЗВАННЫЙ НЕДИАГНОСТИРОВАННЫМ ИРИДОКОРНЕАЛЬНЫМ ЭНДОТЕЛИАЛЬНЫМ СИНДРОМОМ, ПОСЛЕ SMILE

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РЕЗЮМЕ

Цель работы. *Представить клинический случай отёка роговицы, вызванного недиагностированным иридокорнеальным эндотелиальным синдромом, у пациента после SMILE.*

Иридокорнеальный эндотелиальный синдром характеризуется аномальной пролиферацией эндотелия роговицы за пределы линии Швальбе, блокадой иридокорнеального угла, специфическими изменениями формы зрачка и образованием синехий между соседними структурами угла передней камеры глаза, что сопровождается развитием претрабекулярной задержки внутриглазной водянистой влаги, повышением внутриглазного давления (ВГД) и последующим развитием глаукомы у 46–82 % пациентов. Одной из форм заболевания является синдром Чандлера, который клинически на начальных стадиях может сопровождаться формированием лишь умеренного эктропиона. В то же время эндотелиальная дисфункция характеризуется значительным уменьшением количества клеток, изменением их формы и появлением эпителиоидных клеток с гиперрефлексивным ядром (так называемые «клетки ICE» (iridocorneal endothelial)) с нарушением их естественной насосной функции.

В статье представлен клинический случай пациента, прошедшего стандартное обследование и симптоматическое лечение после лазерной кераторефракционной операции по коррекции близорукости. На одном прооперированном глазу наблюдались низкая острота зрения, диффузный отёк роговицы, декомпенсация ВГД неясного генеза.

Вывод. *Кераторефракционные операции могут выступать в качестве триггера, стимулирующего переход скрытой формы в клинически развившийся патологический процесс, который вызывает снижение скорректированной остроты зрения из-за формирования отёка роговицы и глаукомной оптической нейропатии.*

Ключевые слова: *иридокорнеальный эндотелиальный синдром, кераторефракционная хирургия, отёк роговицы, послеоперационные осложнения*

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Refractive surgery is a popular direction in ophthalmology, and its main task is to provide improved quality of life, safety, and high visual acuity. The results of keratorefraction surgeries are usually evaluated according to the following criteria: efficiency, predictability, stability and safety, determined by the resulting visual acuity and the presence of complications. Complications associated with surgery techniques, or with the peculiarities of the early and late postoperative period, become the main cause of patients' disappointment. The most common problems are the development of a dry eye syndrome, inadequate postoperative wound healing with fibroplasia, epithelial ingrowth, insufficient correction of ametropia, various valve adaptation disorders, etc. [1, 2]. The competent intraoperative tactics or adequate postoperative therapy can help to avoid many of the complications mentioned above.

However, in clinical practice there are situations caused by violations of the patients' preoperative examination algorithm. Thus, undiagnosed during the fundus ophthalmoscopy holes and peripheral degeneration of retinal tissue can lead to the development of retinal detachment. Lack of gonioscopy in the examination may lead to untimely detection of pathological changes in the outflow pathways of aqueous humour, the formation of ophthalmic hypertension and glaucoma in patients with congenital and acquired syndromes and diseases accompanied by disorders of the eye hydrodynamics. At the same time, keratorefractive operations may act as a trigger stimulating the transition of the disease from a latent form to a clinically developed pathological process.

The aim of this work is to present a clinical case of the corneal edema formation after SMILE (Small Incision Lenticule Extraction) myopia correction in a patient with an undiagnosed iridocorneal endothelial syndrome.

CLINICAL CASE

Patient B., 35 years old, applied to an ophthalmology clinic for myopia correction. Preoperative diagnostics revealed myopic refraction, SE (spherical equivalence) –4.5 D on both eyes, BCVA (best corrected visual acuity) – 1.0, IOP (intraocular pressure) – 14/14 mmHg. No pathological changes during biomicroscopy and ophthalmoscopy. The patient underwent SMILE surgery on both eyes.

One day after surgery UCVA (uncorrected visual acuity) of the right eye – 1.2, of the left eye – 0.1, the refraction of both eyes was SE = +0.5 D, IOP = 18 mmHg. Insufficient visual acuity of the left eye was explained by the presence of diffuse corneal epithelial edema.

Optical coherence tomography (OCT) of the cornea of the left eye revealed insufficient adhesion of the walls of the intrastromal space that caused the necessity to wash it with a BSS solution and perform a tupfer massage. Positive dynamics has not been achieved.

The patient was consulted in the Irkutsk Branch of the S. Fyodorov Eye Microsurgery Federal State Institution. During biomicroscopy, a local inversion of the pigment border (vascular ectropion) and segmental compaction of the iris stroma were determined at 6 o'clock position (Fig. 1), which could demonstrate the presence of iridocorneal endothelial (ICE) syndrome.

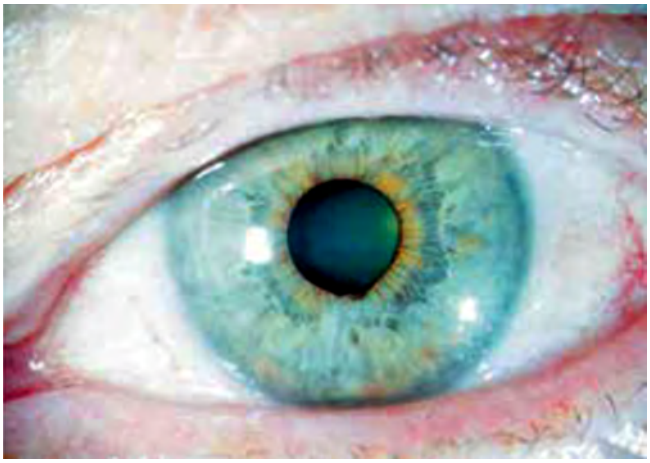


FIG. 1.

Photorecording of the anterior segment of the left eye: vascular ectropion, segmental destruction of the iris stroma at 6 o'clock position

Gonioscopy revealed a high planar goniosynechia in the projection of the inversion of the pigment border, occupying 1 quadrant of the angle of the anterior chamber, confirmed by ultrasound biomicroscopy data.

OCT revealed significant differences in the postoperative corneal intrastromal interface: a linear stroma seal corresponding to the removed lenticule was determined in the right eye in the cornea, additional microcystic spaces, corresponding to the corneal hydration zones in the left eye (Fig. 2).

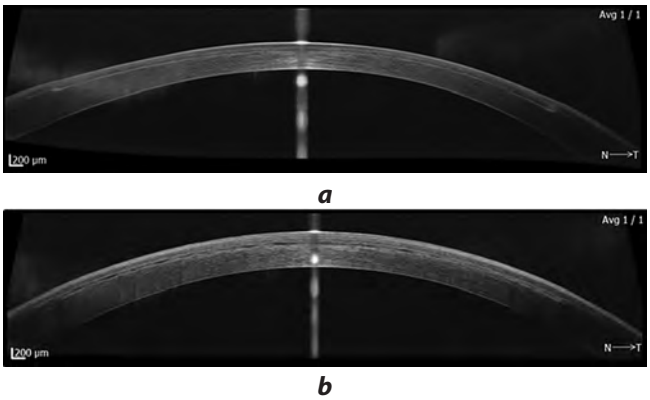
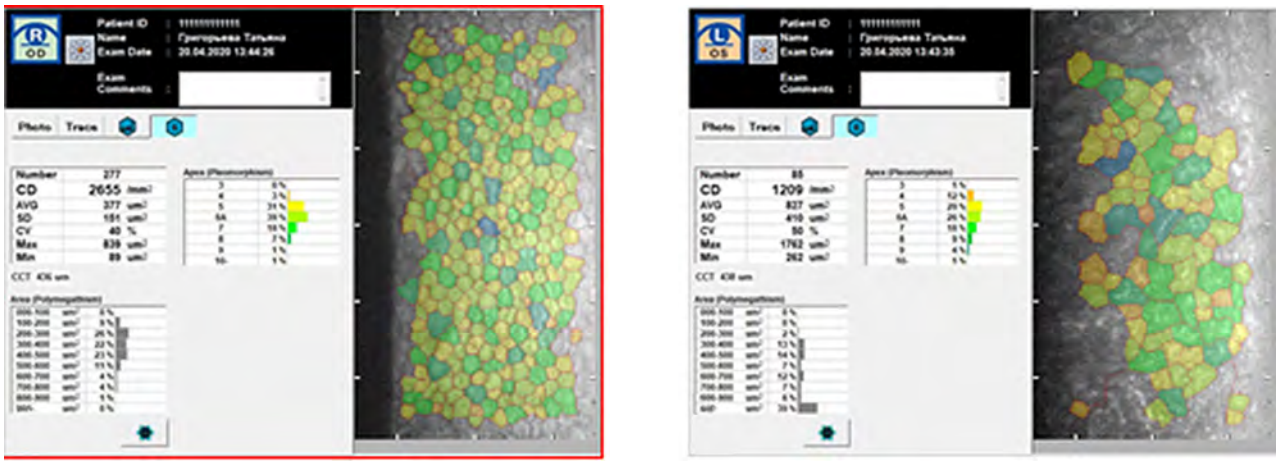


FIG. 2.

*OCT of the cornea: **a** – right eye, linear compression of the stroma corresponding to the removed lenticule; **b** – left eye, insufficient adhesion of the walls of the intrastromal space, small-cystic formations*

Endothelial microscopy of the left eye showed the reduction of endothelial cells number up to 1209/mm^2 (right – 2655/mm^2), a change in their shape and the appearance of epithelioid cells with a hyper-reflective nucle-



a *b*

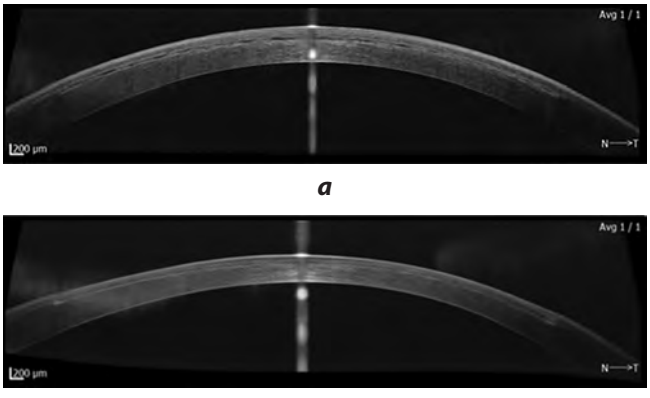
FIG. 3.

*Endothelial microscopy protocol: **a** – right eye, the number of endothelial cells is 2655 per mm^2, the shape and size are not changed; **b** – left eye, the number of endothelial cells is 1209 per mm^2, polymegatism – cells with an area of more than 900 um^2 make up 39 %*

us (Fig. 3), the so-called "ICE cells", which once again confirmed the preliminary diagnosis.

The results of the conducted examination allowed us to state the final diagnosis: iridocorneal endothelial syndrome (Chandler's syndrome), secondary endothelial corneal dystrophy, the condition after SMILE.

To reduce the hydrodynamic load on the corneal endothelium hypotensive therapy was prescribed as symptomatic therapy: drops suppressing the production of intraocular aqueous humour – a fixed combination of timolol 0.5 % and dorzolamide 1 % twice a day. After 1 month: IOP = 18/14 mmHg, UCVA = 1.2/1.0, transparent cornea, OCT showed complete adaptation of the walls of the intrastromal space (Fig. 4).



a *b*

FIG. 4.

*OCT of the cornea of the left eye: **a** – before hypotensive therapy, insufficient adhesion of the walls of the intrastromal space; **b** – after hypotensive therapy, complete adaptation of the walls of the intrastromal space, restoration of corneal transparency*

DISCUSSION

In this clinical case, vascular ectropion, fibrous segmental changes in the iris stroma did not alert doctors at the stage of preoperative diagnosis, and laser refractive surgery served as a trigger for the transition of the syndrome into a clinical form of the disease.

It is known that the pathogenetic mechanisms underlying the clinical changes observed in ICD are based on abnormal proliferation of the corneal endothelium [3, 4]. In postnatal age, endothelial cells are postmitotic and do not divide under normal conditions. The pathogenic trigger in IES causes endothelial cells to lose control of the cell cycle. With reduced regulation of expression of cyclin-dependent kinase inhibitors, endothelial cells can proliferate and behave like epithelial cells without undergoing malignant transformation. In this regard, the endothelial cells of the cornea are primarily affected by ICE, their proliferation begins, and the ability to migrate to surrounding tissues also appears [5, 6].

It is known that the appearance of corneal endothelial cells with morphological characteristics resembling the epithelial phenotype, called by E.S. Sherrard and his colleagues in 1985 "ICE cells", which differ from the norm in pleomorphism, polymegatism and the presence of a hyperreflective nucleus, is characteristic of the ICE.

Regardless of the etiological factor, abnormal endothelial cells migrate posteriorly beyond the Schwalbe line, block the iridocorneal angle, move into the anterior chamber of the eye, cover the anterior surface of the iris, form an abnormal basement membrane, which eventually contracts, causing changes in the shape of the pupil, atrophic damage to the iris and the formation of synechiae between neighboring structures. The adhesive process in the corner of the anterior chamber is accompanied by the development of pretrabecular retention of intraocular moisture, an increase in intraocular pressure and the subsequent development of glaucoma in 46–82 % of patients [7].

To date, there are three clinical subtypes of ICE that have a single pathogenetic mechanism, but differ in the nature of pathological changes in the iris. Chandler's syndrome is accompanied by the formation of vascular ectropion (inversion of the pigment border). With progressive atrophy of the iris (essential mesodermal atrophy), the formation of "stretching" or "melting" through ruptures occurs,

with Cogan – Reese syndrome, in addition to ectropion, nevus-like formations of the iris are visualized.

There is no true treatment for iridocorneal endothelial syndrome, so all measures can be attributed to symptomatic therapy [8]:

1) treatment of corneal decompensation and related complications;

2) solving the problem of iris atrophy and its cosmetic and visually significant defects;

3) control of glaucoma associated with ICE.

In the initial stages of the disease, corneal edema can be treated with hypertensive ophthalmic drugs, such as glucose solution 20–40 % or NaCl solution 3 %. A decrease in IOP, even at initially normal values of intraocular pressure, also improves the condition of the cornea. In advanced cases with stable corneal edema with well-controlled IOP, endothelial and descemet membrane transplantation is possible, which is considered today as a procedure of choice to achieve a better functional effect of vision, reduce immunological rejection of the graft and reduce pain [9].

Despite the positive effect of drug and surgical reduction of IOP, the prognosis for the preservation of visual functions is not favorable enough, taking into account the chronic progressive course of iridocorneal endothelial syndrome and its accompanying complications.

CONCLUSION

Manifest changes in the anterior segment of the eye in many syndromes accompanied by the formation of glaucoma make it possible to identify hydrodynamic disorders at the earliest stages and prevent the destructive effect of increased IOP. The diagnosis of a refractive patient should include standard examination approaches: a thorough assessment of changes in the iris, gonioscopy, ophthalmoscopy at maximum mydriasis with an assessment of the state of the optical disc, macular zone and peripheral parts of the retina. The complex and meaningful application of modern imaging methods provides an objective assessment of pathological changes in the eye, and also allows to determine indications and contraindications to refractive surgery.

Conflict of interest

The authors of this article declare the absence of a conflict of interest.

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DISSOCIATION OF BIOLOGICAL AGE AND BLOOD INTERLEUKINS IN PATIENTS AGED 45–59 YEARS WITH DIABETIC RETINOPATHY IN TYPE 2 DIABETES MELLITUS

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ABSTRACT

Background. *The development of diabetic retinopathy is favoured by immunological factors such as interleukins (IL) and chemokines. However, analysis of blood interleukins in patients aged 45–59 years with diabetic retinopathy in type 2 diabetes mellitus, who have biological age acceleration, has not yet been presented in publications.*

The aim of the research. *To study the content of blood interleukins in patients aged 45–59 years with diabetic retinopathy in type 2 diabetes mellitus, who have an excess of biological age over chronological age.*

Materials and methods. *241 patients aged 45–59 years with diabetic retinopathy in type 2 diabetes mellitus were examined in a clinical setting. Biological age acceleration over chronological age was found in 148 patients, biological and chronological age concorded in 51 patients. The content of interleukins in the blood was studied in all patients using an enzyme-linked immunoassay.*

Results. *The concentration of blood interleukins in patients with biological age exceeding chronological, compared with patients aged 45–59 years with concordance of biological and chronological age, was statistically significantly different for most blood interleukins and especially for IL-6, the concentration of which was 20.8 ± 1,2 pg/ml versus 3.9 ± 0.6 pg/ml, respectively ($p < 0.001$). IL-13, IL-17 were significantly increased among patients with biological age acceleration over chronological; their concentrations were 2.1 ± 0.4 and 16.5 ± 0.6 pg/ml versus 0.5 ± 0.2 and 7.9 ± 0.7 pg/ml in the comparison group ($p < 0.001$). In contrast, IL-4 and IL-10 levels were higher in patients aged 45–59 years with diabetic retinopathy in type 2 diabetes mellitus and with concordance of biological and chronological age.*

Conclusion. *IL-6, IL-8, IL-13, IL-17, IL-4 and IL-10 may serve as markers of biological age dissociation in patients aged 45–59 years with diabetic retinopathy in type 2 diabetes mellitus.*

Key words: *biological age, chronological age, diabetic retinopathy, type 2 diabetes mellitus, blood interleukins, adulthood*

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ДИССОЦИАЦИЯ БИОЛОГИЧЕСКОГО ВОЗРАСТА И ИНТЕРЛЕЙКИНОВ КРОВИ У ПАЦИЕНТОВ 45–59 ЛЕТ С ДИАБЕТИЧЕСКОЙ РЕТИНОПАТИЕЙ ПРИ САХАРНОМ ДИАБЕТЕ 2-ГО ТИПА

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РЕЗЮМЕ

Введение. *Развитию диабетической ретинопатии способствуют иммунологические факторы, такие как интерлейкины (IL) и хемокины. Однако анализ интерлейкинов крови у пациентов 45–59 лет с диабетической ретинопатией при сахарном диабете 2-го типа, имеющих ускорение биологического возраста, до настоящего времени в публикациях не представлен.*
Цель исследования. *Изучение содержания интерлейкинов крови у пациентов 45–59 лет с диабетической ретинопатией при сахарном диабете 2-го типа, имеющих превышение биологического возраста над хронологическим.*
Материалы и методы. *В клинических условиях обследован 241 пациент 45–59 лет с диабетической ретинопатией при сахарном диабете 2-го типа, среди которых выявлено превышение биологического возраста над хронологическим у 148 пациентов, у 51 пациента – соответствие биологического и хронологического возраста. У всех пациентов изучено содержание интерлейкинов в крови иммуноферментным анализом.*
Результаты. *Концентрация интерлейкинов крови у пациентов, имеющих превышение биологического возраста над хронологическим, по сравнению с пациентами 45–59 лет с соответствием биологического и хронологического возраста статистически значимо различалась по большинству интерлейкинов крови и особенно по IL-6, концентрация которого составляла 20,8 ± 1,2 пг/мл против 3,9 ± 0,6 пг/мл соответственно (p < 0,001). Значительно повышенными среди пациентов с превышением биологического возраста над хронологическим были IL-13, IL-17, концентрация которых составила соответственно 2,1 ± 0,4 и 16,5 ± 0,6 пг/мл против 0,5 ± 0,2 и 7,9 ± 0,7 пг/мл в группе сравнения (p < 0,001). Напротив, уровни IL-4 и IL-10 были выше у пациентов 45–59 лет с диабетической ретинопатией при сахарном диабете 2-го типа с соответствием биологического и хронологического возраста.*
Заключение. *IL-6, IL-8, IL-13, IL-17, IL-4 и IL-10 могут служить маркерами диссоциации биологического возраста у пациентов 45–59 лет с диабетической ретинопатией при сахарном диабете 2-го типа.*

Ключевые слова: *биологический возраст, хронологический возраст, диабетическая ретинопатия, сахарный диабет 2-го типа, интерлейкины крови, зрелый возраст*

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RELEVANCE

Diabetic retinopathy (DR) is considered one of the main complications of type 2 diabetes mellitus (DM), with an increase in incidence concurrent with the rising incidence of type 2 DM [1]. According to the International Diabetes Federation, the number of patients with diabetes mellitus has reached 463 million worldwide, and by 2045 the number of patients is expected to increase up to 700 million, with the predominance of people with type 2 diabetes mellitus [2, 3]. In our country, the prevalence of type 2 diabetes mellitus is steadily increasing, reaching 3148.5 cases per 100 thousand population. DR in type 2 diabetes mellitus leads to loss of vision and remains a serious cause of deterioration in the daily household and social activity of patients, as well as the quality of life [4, 5]. The progression of DR and a decrease in the functional activity of patients occur against the background of constant hyperglycemia and insulin resistance, which cause neurodegenerative processes and increase inflammaging [6–8].

Studies concerning changes in systemic interleukins (IL) in patients with DR in type 2 diabetes mellitus with both physiological and accelerated aging are inconsistent and ambiguous. Some publications report the involvement of IL-6, IL-10 and tumor necrosis factor α (TNF-α) in the aging process of patients with DM and DR and other somatic pathology without differentiation of aging variants [9, 10]. Other systemic interleukins, when considering the processes of premature aging in the discussed ophthalmological complication of type 2 diabetes mellitus, are usually not analyzed, although a correlation of type 2 diabetes mellitus with an increase in biological age is shown compared with chronological age, reflecting accelerated aging [11]. In this regard, it is relevant to study a wide range of systemic interleukins in patients aged 45–59 years with DR in type 2 diabetes mellitus with premature aging.

THE AIM OF THE STUDY

To study the content of blood interleukins in patients aged 45–59 years with diabetic retinopathy in type 2 diabetes mellitus, and who have an excess of biological age over chronological age.

MATERIALS AND METHODS

Clinical studies included 241 patients aged 45–59 years with DR with type 2 diabetes mellitus at Tambov Branch of the S. Fyodorov Eye Microsurgery Federal State Institution in 2020–2021. Diagnosis of type 2 diabetes mellitus and DR was carried out based on the results of a comprehensive laboratory and instrumental examination of patients and taking into account the criteria of clinical recommendations «Diabetes mellitus: diabetic retinopathy, diabetic macular edema» [12].

The verification of the diagnosis of DR was confirmed by the data of a comprehensive ophthalmological examination, including optical coherence tomography (OCT HS-100, Canon Medical, Japan), fluorescence angiography (Spectralis HRA + OCT, Heidelberg Engineering Inc., Germany), pulsed Doppler ultrasonography (Voluson 730 Pro, General Electric Healthcare, USA), determination of visual acuity without correction and maximum corrected visual acuity. Among those included in the study, 96 patients had a non-proliferative stage or form of DR, 78 people had a preproliferative stage of DR and 67 people had a proliferative stage of DR. The compared groups had no statistically significant differences in concomitant pathology. The leading concomitant diseases among patients with matching parameters of biological and chronological ages and in the group with dissociation of chronological and biological ages were arterial hypertension (34.3 ± 3.2 % and 38.5 ± 3.0 %, respectively; $p > 0.05$), respiratory diseases (18.6 ± 1.9 % and 20.3 ± 2.1 %, respectively). Kidney stone disease (13.2 ± 1.6 % and 10.9 ± 1.8 %, respectively), diseases of the musculoskeletal system (14.8 ± 2.2 % and 12.4 ± 2.0 %, respectively) were less common. Polymorbidity index in the compared groups was 2.9 ± 0.5 and 3.1 ± 0.6, respectively ($p > 0.05$). Information about concomitant pathology is obtained from official medical documentation. The patients of the compared groups also did not differ statistically significantly in the duration of type 2 diabetes mellitus.

Among 241 examined patients aged 45–59 years with DR in type 2 diabetes mellitus, biological age was determined according to the method of V.P. Voitenko et al. [13], taking into account the sex of the patient. When determining biological age by V.P. Voitenko et al. [13] method, the following indicators were determined and used: body weight (BW), systolic (SBP) and diastolic (DBP) blood pressure, pulse blood pressure (PBP), timed inspiratory capacity (TIC), static balance (SB) and self-assessed health (SAH), – according to which biological age was calculated, using the appropriate formulas for men ($BA = 26.985 + 0.125 \times SBP - 0.149 \times TIC + 0.723 \times SAH - 0.151 \times SB$) and women ($BA = -1.463 + 0.415 \times PBP + 0.248 \times BW + 0.694 \times SAH - 0.14 \times SB$). Proper biological age (PBA) was calculated using the formula: women – $PBA = 0.581 \times CA + 17.24$; for men, $PBA = 0.694 \times CA + 18.56$, where CA is the chronological age. The coefficient of aging rate was determined by the formula: BA/PBA.

Biological age was interpreted as the level of development, change or wear of the structure or function of an element of the body, a functional system, an organism as a whole, expressed in a unit of time [14]. It is believed that biological age correlates with premature (accelerated) aging [15–17], and therefore the excess of biological age over the value of the chronological age was considered by us in this work as an integral indicator of premature (accelerated) aging of patients 45–59 years old with DR in type 2 diabetes mellitus.

Based on the difference between biological and chronological (passport) age, an aging variant was established: physiological – with a difference between biological and chronological age in the range from –2.9 to +2.9 years,

accelerated (premature) – with a difference between biological and chronological age in the range of more than 3 years [14]. Taking this into account, among the examined 241 patients 51 patients with physiological aging and 148 patients with accelerated aging were identified, in whom, in accordance with the purpose of the study, the study of the systemic interleukin profile was performed using a single technique. The patients of the compared groups were comparable in terms of concomitant somatic diseases, which were in the compensation stage and could not distort the results of this study.

The content of interleukins in blood plasma was determined by enzyme immunoassay using Protein Contour kits (St. Petersburg). Blood sampling was performed before the procedures and taking medications in the morning in fasting state. The content of IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-13, IL-17 was determined in blood plasma.

The examination of patients was carried out taking into account the principles of Good Clinical Practice and obtaining written informed consent of patients (Protocol No. 148-Э dated February 9, 2022).

The Statistica 10.0 programme (StatSoft Inc., USA) was used for statistical processing and the non-parametric χ^2 test was used.

RESULTS

The average chronological age in the examined cohort of patients aged 45–59 years with DR in type 2 diabetes mellitus was 51.28 ± 2.06 years (Fig. 1), and the biological age of the same group was 59.72 ± 3.41 years and statistically significantly differed from the chronological age ($p < 0.01$), exceeding the value of the latter by 8.44 ± 0.16 years. Along with other characteristics and criteria of biological age, this indicates premature aging of patients aged 45–59 years with DR in type 2 diabetes mellitus.

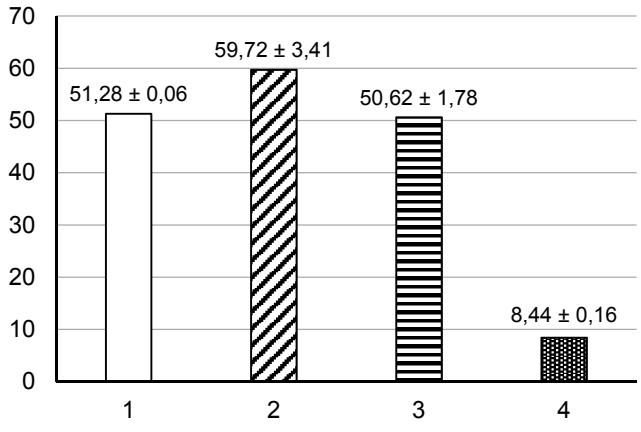

FIG. 1.

The ratio of chronological and biological age in patients aged 45–59 years with diabetic retinopathy in type 2 diabetes mellitus (M ± m, years): 1 – chronological age; 2 – biological age; 3 – proper biological age; 4 – the difference between biological and chronological age

Premature aging of mature patients with DR with type 2 diabetes mellitus is confirmed by other criteria reflecting biological age of the examined category of persons. In particular, biological age was statistically significantly higher than proper biological age, and the difference between biological and proper biological age was +9.11 ± 0.69 years. The fact that the above-mentioned difference turned out to be more than 5 years indicates accelerated aging of patients 45–59 years with DR in type 2 diabetes mellitus in accordance with the accepted grading. The statement about premature aging of patients aged 45–59 years with DR in type 2 diabetes mellitus is also based on the value of the aging rate coefficient, which reached 1.18 ± 0.02.

Biological age exceeding over chronological age reflects premature aging. It was established in 61.41 cases per 100 examined patients aged 45–59 years with DR in type 2 diabetes mellitus.

The correspondence of chronological and biological age in the same group was observed in 21.16 cases per 100 examined, which indicates the rate of normal or physiological aging, and the excess of chronological age over biological age in 17.43 cases per 100 examined, which indicates slow aging.

Consequently, type 2 diabetes mellitus and DR contribute to the acceleration of aging in most patients aged 45–59 years, realized at the cellular level by pathophysiological metabolic processes resulting from hyperglycemia and insulin resistance. Another reason for the accelerated aging of patients aged 45–59 years with DR in type 2 diabetes mellitus may be changes in the systemic interleukin profile, which we established during the study (Table 1).

Among patients 45–59 years old with DR in type 2 diabetes mellitus with biological age exceeding the chronological age, higher levels of most systemic pro-inflammatory interleukins were found relative to patients 45–59 years old with DR in type 2 diabetes mellitus with matching biological and chronological ages. Thus, the acceleration of biological age is accompanied by an increase in IL-1a, IL-3, IL-5, IL-8, IL-13, IL-17. A multiple increase in expression in patients with a biological age exceeding the chronological age is characteristic of pro-inflammatory IL-6.

However, among the anti-inflammatory blood interleukins in patients aged 45–59 years with DR in type 2 diabetes mellitus with biological age exceeding over chronological age, there was a statistically significant decrease in both IL-4 and IL-10 compared with patients 45–59 years old with DR in type 2 diabetes mellitus with matching chronological and biological ages.

The acceleration of biological age in patients aged 45–59 years with DR in type 2 diabetes mellitus did not have a statistically significant effect on the level of IL-1β, IL-2 and IL-17, the content of which was practically equivalent in both clinical groups ($p > 0.05$).

The considered results suggest an increase in the pro-inflammatory activity of systemic interleukins and inhibition of anti-inflammatory activity in patients 45–59 years of age with DR in type 2 diabetes mellitus with accelerated aging caused by biological age exceeding over chronological age, that is, due to premature aging.

TABLE 1

LEVELS OF INTERLEUKINS IN THE BLOOD OF PATIENTS AGED 45–59 YEARS WITH DIABETIC RETINOPATHY IN TYPE 2 DIABETES MELLITUS WITH ACCELERATION AND CONCORDANCE OF PARAMETERS OF BIOLOGICAL AND CHRONOLOGICAL AGE (M ± m, PG/ML)

Blood interleukins	Patients with matching biological and chronological ages	Patients with mismatch of biological and chronological ages	*p*
IL-1α	17.2 ± 0.6	20.8 ± 0.7	< 0.05
IL-1β	13.4 ± 0.9	14.6 ± 0.7	> 0.05
IL-2	2.6 ± 0.4	3.1 ± 0.5	> 0.05
IL-3	3.3 ± 0.4	5.8 ± 0.4	< 0.01
IL-4	4.2 ± 0.4	2.0 ± 0.3	< 0.001
IL-5	3.6 ± 0.2	6.2 ± 0.5	< 0.001
IL-6	3.9 ± 0.6	20.8 ± 1.2	< 0.001
IL-7	4.8 ± 0.4	6.0 ± 0.5	> 0.05
IL-8	5.6 ± 0.7	11.5 ± 0.8	< 0.001
IL-9	8.7 ± 0.6	10.0 ± 0.7	> 0.05
IL-10	17.4 ± 0.7	6.2 ± 0.6	< 0.001
IL-13	0.5 ± 0.2	2.1 ± 0.4	< 0.001
IL-17	7.9 ± 0.7	16.5 ± 0.6	< 0.001

DISCUSSION

Aging-related inflammation in conditions of systematic (chronic) hyperglycemia causes activation of pro-inflammatory cytokines [18]. In the blood plasma of elderly patients with type 2 diabetes mellitus and DR, the content of pro-inflammatory cytokines, including TNF-α, IL-6, IL-8, IL-1β, significantly increases compared with patients with a similar pathology of mature age [1]. It is noted that the levels of pro-inflammatory cytokines TNF-α, IL-6 in the blood are associated with the aging process, apoptosis, especially in patients with type 2 diabetes mellitus and DR [9, 19]. Pro-inflammatory cytokines such as TNF-α, IL-1, IL-6, IL-8 and interferon γ (IFN-γ) are the main factors of immune inflammation in DR in elderly patients with type 2 diabetes mellitus. Elevated concentrations of TNF-α, IL-1, IL-6, IL-8 and IFN-γ were found not only in the blood plasma of elderly patients with DR in type 2 diabetes mellitus, but also in aqueous humor and vitreous humor, and their change may be associated with the severity of DR [1]. At the same time, the concentration of TNF-α in patients with DR in type 2 diabetes mellitus differs significantly from that in healthy people [1]. The contribution of IL-1β to the development of DR, the level of which is increased in blood serum and aqueous humor, has been shown [20]. However, in the present study, in patients with DR in type 2 diabetes mellitus with biological age exceeding over chronological age, no statistically significant differences in the content of IL1-β were found, and there was only a tendency to increase in blood plasma.

According to researchers [21], IL-6 plays a leading role in the development of such an ophthalmological complication of type 2 diabetes mellitus as DR. It has also been shown that human aging is accompanied by a 2–4-fold increase in IL-6 in the blood [10], which can exhibit both pro-inflammatory properties, stimulating the production of antibodies and inducing an acute inflammatory process, and anti-inflammatory properties, blocking the synthesis of inflammatory cytokines [10]. The priority value of IL-6 in the accelerated aging of patients with DR in type 2 diabetes mellitus was also established in this study, which is consistent with the above-mentioned leading role of IL-6 in human aging. IL-6, from the point of view of gerontology and aging, can not only contribute to accelerated aging, but also act as a risk factor for reducing the functional reserve of the immune system and the development of life limitations [9, 10]. In addition, higher levels of IL-6 in the elderly can be considered as markers of age-associated diseases associated with accelerated aging.

In addition to the established multiple increases in IL-6 in blood plasma of patients with DR in type 2 diabetes mellitus with biological age exceeding over chronological age, statistically significant changes in other pro-inflammatory interleukins accompanied by an increase in the concentration of IL-13, IL-17, IL-8, IL-5 were diagnosed. This fact certainly indicates the involvement of the above-mentioned systemic interleukins in accelerating the aging of patients with DR in type 2 diabetes mellitus. However, it remains unclear whether inflammatory interleukins play a causal role in the pathological process of accelerated aging or act

as mediators [9]. Previous studies have shown that higher levels of inflammatory cytokines are associated with various manifestations of human aging, but these results turned out to be inconsistent and the conclusions were ambiguous [19]. Differences in the content of IL-6, TNF-α and IL-10 were found in patients with type 2 diabetes mellitus and DR compared with other somatic diseases.

Premature aging of the human body can be caused by a decrease in IL-10 in blood plasma with existing insulin resistance [7], including those established by us in patients with DR in type 2 diabetes mellitus with biological age exceeding over chronological age. It was the decrease in IL-10 among anti-inflammatory interleukins at the systemic level that turned out to be more significant in patients with DR in type 2 diabetes mellitus with accelerated aging. IL-10, produced mainly by macrophages, is responsible for suppressing the pro-inflammatory response and prevents inflammation, as well as the release and activity of inflammatory cytokines such as IL-6, TNF-α and IL-1β [6]. On the contrary, we found a lower level of IL-10 in the blood plasma of patients 45–59 years old with DR in type 2 diabetes mellitus with dissociation of biological and chronological ages, that is, with accelerated (premature) aging. At the same time, the concentration of another anti-inflammatory cytokine IL-4, whose inhibition of production was lower than IL-10, was also lower in this group. An experimental study has shown that IL-10 prevents aging-related inflammation and insulin resistance. According to this study, anti-inflammatory IL-10 plays a potential therapeutic role in the prevention of premature aging and the treatment of aging-mediated insulin resistance and helps reduce elevated levels of inflammatory interleukins circulating in the blood in the elderly against the background of aging processes.

Information on the effect of other systemic interleukins on human aging, including patients with DR in type 2 diabetes mellitus, is extremely limited, and some results were obtained on a relatively small sample of patients, the volume of which was not representative [6, 13].

CONCLUSION

Among 45–59-year-old patients suffering from DR in type 2 diabetes mellitus, with biological age exceeding over chronological age, i. e., accelerated aging occurs in more than half of cases. Patients with DR in type 2 diabetes mellitus with accelerated aging are characterized by statistically significant differences in plasma concentrations of most of the studied systemic interleukins. In patients with DR in type 2 diabetes mellitus with accelerated aging compared with patients with DR in type 2 diabetes mellitus with physiological aging, a statistically significant increase of IL-6, IL-8, IL-13, IL-17 and a decrease in the level of anti-inflammatory interleukins IL-4 and IL-10 in blood plasma. The established dissociation of interleukins shows the involvement of the immune system in accelerating the aging of patients with DR in type 2 diabetes mellitus, and the above-mentioned systemic interleukins can be used as immunological predictors of accelerated aging in such patients.

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Conflict of interest

The authors of this article declare the absence of a conflict of interest.

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MATHEMATICAL MODELING OF THE REFRACTIVE EFFECT OF SMILE SURGERY IN HIGH DEGREE MYOPIA CORRECTION

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ABSTRACT

The aim of the study. *To develop a mathematical model of changes in corneal refraction during femtosecond laser-assisted lenticule extraction through a small surgical incision and, on this basis, to propose a technology for modified calculation of surgical parameters and to prove its effectiveness.*

Material and methods. *The study included 191 patients with high myopia. They were divided into two groups: group 1 consisted of 55 patients who were had SMILE (SMall Incision Lenticule Extraction) surgery with standard calculations; group 2 included 136 patients who had SMILE surgery with a modified calculation of surgical parameters based on the developed mathematical model of the refractive effect of the surgery.*

Results. *When assessing the refractive effect of patients who were operated using standard technology, it was found that it was possible to achieve a refraction different from emmetropia for ± 0.5 D only in 51 % of cases; in the remaining patients, the planned residual refractive effect was obtained and averaged –1.96 ± 0.29 D. In patients operated using the modified technology, a statistically significantly better refractive result was achieved already on the first day. A refractive error of more than ± 1.0 D was obtained in only 1 % of cases; a deviation from the calculated refraction of ± 0.5 D was achieved in 82 % of cases, with the average values by 1 year –0.24 ± 0.57 D.*

Conclusions. *The developed technology of a modified calculation of the parameters of the SMILE surgery for high myopia correction makes it possible to obtain an optimal refractive effect in compliance with safety rules when the structural and functional parameters of the eye are initially unfavorable for refractive surgery.*

Key words: *high myopia, SMILE, mathematical model, regression equation*

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МАТЕМАТИЧЕСКОЕ МОДЕЛИРОВАНИЕ РЕФРАКЦИОННОГО ЭФФЕКТА SMILE В КОРРЕКЦИИ МИОПИИ ВЫСОКОЙ СТЕПЕНИ

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РЕЗЮМЕ

Цель исследования. *Разработать математическую модель изменения рефракции роговицы при фемтолазер-ассистированной экстракции лентикулы через малый операционный доступ и на этой основе предложить технологию модифицированного расчёта параметров операции и доказать её эффективность.*

Материал и методы. *В исследование вошёл 191 пациент с миопией высокой степени. Все пациенты были разделены на две группы: первую группу составили 55 человек, которые были прооперированы методом SMILE (SMall Incision Lenticule Extraction) по стандартным расчётам; во вторую группу – 136 пациентов, прооперированных методом SMILE, с модифицированным расчётом параметров хирургического вмешательства на основе разработанной математической модели рефракционного эффекта операции.*

Результаты. *При оценке рефракционного эффекта пациентов, прооперированных по стандартной технологии, установлено, что достичь рефракции, отличающейся от эмметропии на ± 0,5 дптр, удалось лишь в 51 % случаев, у остальных пациентов был получен планируемый остаточный рефракционный эффект, который в среднем составил –1,96 ± 0,29 дптр. У пациентов, прооперированных по модифицированной технологии, уже в первые сутки удалось достичь статистически значимо лучшего рефракционного результата. Отклонение рефракции более ± 1,0 дптр было получено лишь в 1 % случаев, отклонение от расчётной рефракции ± 0,5 дптр было достигнуто в 82 % случаев, при этом средние значения к году составили –0,24 ± 0,57.*

Выводы. *Разработанная технология модифицированного расчёта параметров операции SMILE для коррекции миопии высокой степени позволяет получить оптимальный рефракционный эффект с соблюдением правил безопасности при исходно неблагоприятных для рефракционной хирургии структурно-функциональных показателях глаза.*

Ключевые слова: *миопия высокой степени, SMILE, математическая модель, регрессионное уравнение*

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Currently, myopia is the most common refractive error, the incidence of which tends to increase due to a significant increase in visual load, especially at close range [1, 2]. According to the World Health Organization, about 290 million people in the world have myopia. In the Russian Federation alone, 3.1 million people suffer from myopia, which is 2,158.2 per 100,000 population, and 70 % of these patients are people aged 20–40 years [3].

In this regard, correction of refractive errors, in particular myopia, is an urgent problem of modern ophthalmology. A special place in the correction of myopia of varying degrees is occupied by keratorefractive surgery using both excimer and femtosecond lasers.

Starting from 2010–2011, microinvasive technologies based on femtosecond laser-assisted formation and subsequent removal of the corneal lenticule, and first of all, SMILE surgery (SMall Incision Lenticule Extraction), began to occupy a leading position among other keratorefractive interventions [4–6]. The key difference between this method and other laser vision correction technologies is the possibility of changing the refractive power of the cornea without forming an extensive corneal flap and/or superficial keratectomy, which are necessary stages of LASIC, FemtoLASIC and photorefractive keratectomy methods.

Modern studies of the effectiveness of SMILE surgery demonstrate the possibility of correcting moderate myopia with a deviation of the calculated refraction of ± 1.0 D in 100 % of cases, ± 0.5 D in 93–97 % of cases [7–9].

However, with high myopia, it is not always possible to achieve a qualified refractive effect, which is due to the initial disproportion between a high degree of ametropia and insufficient corneal thickness.

This determined **the aim of the study** – to develop a mathematical model of changes in corneal refraction during femtosecond laser-assisted lenticule extraction through a small surgical incision and, on this basis, to propose a technology for modified calculation of surgical parameters and to prove its effectiveness.

MATERIALS AND METHODS

The study included 191 patients with high myopia, who were divided into two groups. Group 1 consisted of 55 patients who underwent SMILE surgery according

TABLE 1

DEMOGRAPHIC AND CLINICAL CHARACTERISTICS OF PATIENTS (M ± S)

Parameters	Group 1 (standard technology)	Group 2 (modified technology)	p_U
Number of eyes	55	136	
Age, years	29.71 ± 4.71	27.83 ± 6.06	0.28
Sex M, %	46.4 %	53.7 %	
Sex F, %	53.6 %	46.3 %	
Corneal refractive power, D	43.29 ± 1.31	43.75 ± 1.62	0.20
Axial length of the eye, mm	26.18 ± 0.55	26.55 ± 1.48	0.55
Uncorrected visual acuity	0.04 ± 0.15	0.03 ± 0.16	0.17
Corrected visual acuity	0.88 ± 0.18	0.93 ± 0.11	0.08
Corneal thickness, μm	536.48 ± 24.06	534.73 ± 12.88	0.73
Spherical component of refraction, D	–6.94 ± 0.67	–6.95 ± 0.79	0.97
Cylindrical refraction component, D	–1.15 ± 084	–1.06 ± 0.72	0.6
Spherical equivalent, D	–7.52 ± 0.66	–7.48 ± 0.79	0.83

to standard calculations of the VisuMax laser software (Zeiss, Germany). Group 2 included 136 patients who underwent the SMILE surgery with a modified calculation of surgical parameters based on the developed mathematical model of the refractive effect of the surgery.

The demographic and clinical characteristics of patients are presented in Table 1.

The examination of patients was carried out using high-tech methods to assess the anatomical and optical parameters of the eye.

The following methods were used in order to assess the structural and functional state of the eye:

1) refractometry in vivo and under conditions of medicinal mydriasis (after 3-fold instillation of a combined drug containing 8 mg of tropicamide and 50 mg of phenylephrine hydrochloride in 1 ml of solution, with an interval of 15 minutes);

2) keratometry (RC-5000 device (Tomey, Japan));

3) distant visometry without correction and with maximum correction monocularly and binocularly (ACP 6 chart panel (Topcon, Japan), CV 5000 phoropter (Topcon, Japan)).

The thickness of the cornea was measured using Pentacam HR (Oculus, Germany); we have estimated the average thickness of the cornea, determined its thinnest point, evaluated optical biometrics data on OA-2000 (Tomey, Japan).

Examinations were performed before surgery and during the postoperative period with a frequency of 1, 5 days, 3, 6, 12 months after surgery.

The statistical analysis of the study results was carried out using the Statistica 10 computer programme (StatSoft Inc., USA). The study results were analyzed by the method of variation statistics and included the following stages: descriptive, comparative and multivariable regression analysis.

RESULTS

At the first stage of the study, a comparative analysis of refractive indices in patients with high myopia was performed before and after correction of ametropia using SMILE surgery with standard technology at all stages of follow-up. It was found that refraction differing from emmetropia by ± 0.5 D was achieved only in 51 % of cases; the remaining patients had the planned residual refractive effect, which averaged –1.96 ± 0.29 D (Fig. 3a).

To determine the key parameters involved in the formation of the refractive effect, the calculated surgery characteristics were separately evaluated (Table 2 – group 1). Based on the entire set of data, a regression model of unconditional prognosis was created, in which the spherical equivalent obtained in the long–term postoperative period was a dependent variable, and the initial characteristics of the optical system of the eye and the calculated surgery parameters were used as predictors.

The following regression model was obtained:

$$Y = 0.074 \times X_1 + 0.00038 \times X_2 + 0.0156 \times X_3 - 0.146 \times X_4 - 0.41 \times X_5 + 0.013 \times X_6,$$

where $R^2 = 0.66$; $p < 0.0001$; Y is residual refraction (D); X_1 is the initial spherical equivalent (D); X_2 is the thickness of the cornea (μm); X_3 is the thickness of the corneal valve (μm); X_4 is the diameter of the optical zone (mm); X_5 is neutral optical layer (μm) (minimum lenticular thickness); X_6 – residual thickness of the stromal bed (μm).

The percentage contribution of the predictors of the regression equation was mathematically calculated (Table 3). It shows that the greatest contribution to the formation of the refractive effect is made by the thickness of the corneal valve, the initial spherical equivalent and the diameter of the optical zone.

TABLE 2

PREOPERATIVE DESIGN PARAMETERS

Parameters	Group 1 (standard technology)	Group 2 (modified technology)	p_U
Thickness of the corneal valve	122 ± 4.22	102.94 ± 5.72	0.0001
Diameter of the corneal valve	7.73 ± 0.07	7.51 ± 0.28	0.00035
The width of the corneal access section, mm	2.35 ± 0.64	2.6 ± 0.23	0.0001
Optical zone, mm	7.0 ± 0.01	6.57 ± 0.39	0.0001
Minimum lenticular thickness (neutral optical layer), μm	15.0 ± 0	10.0 ± 0	0.0001
Maximum lenticular thickness, μm	129.48 ± 11.03	136.1 ± 16.98	0.01
Residual thickness of the cornea, μm	284.82 ± 5.44	297.69 ± 8.76	0.0001

Verification of the predicted and obtained data of patients of group 1 confirmed a high degree of predictability of the result using the developed mathematical model.

For automated application in clinical practice, this original mathematical model of the refractive effect of SMILE surgery was adapted taking into account the obtained database, where such an indicator as the residual thickness of the corneal bed was adopted as a dependent variable.

$$RST = X_1 - X_2 - (2.68 \times X_3 - 0.52 \times X_3 \times X_4 + 0.97 \times X_4 - 38.29) \times X_5 - 2.21 \times X_4 - X_6 + 10.12,$$

where RST is the residual thickness of the corneal bed (μm); X_1 is the initial thickness of the cornea (μm); X_2 is the thickness of the corneal valve (μm); X_3 is the initial corneal curvature (mm); X_4 is the diameter of the optical zone (mm); X_5 is the initial spherical equivalent (D); X_6 – neutral optical layer (μm) (minimum lenticle thickness).

Using the developed medical technology for modified calculation of the parameters of SMILE surgery (approved by the local Ethical committee of the Irkutsk Branch of the S. Fyodorov Eye Microsurgery Federal State Institution; protocol No. 6 dated June 8, 2015), 136 patients with high myopia, who made up group 2 of the study, un-

TABLE 3

PERCENTAGE CONTRIBUTION OF PREDICTORS FROM THE REGRESSION EQUATION

Predictors	Contribution (%)
Initial spherical equivalent, D	14.88 %
Corneal thickness, μm	6.86 %
Thickness of the corneal valve, μm	18.37 %
Diameter of the optical zone, mm	14.01 %
Neutral optical layer, μm	8.96 %
Residual thickness of the cornea, μm	3.93 %
Total	67.01 %

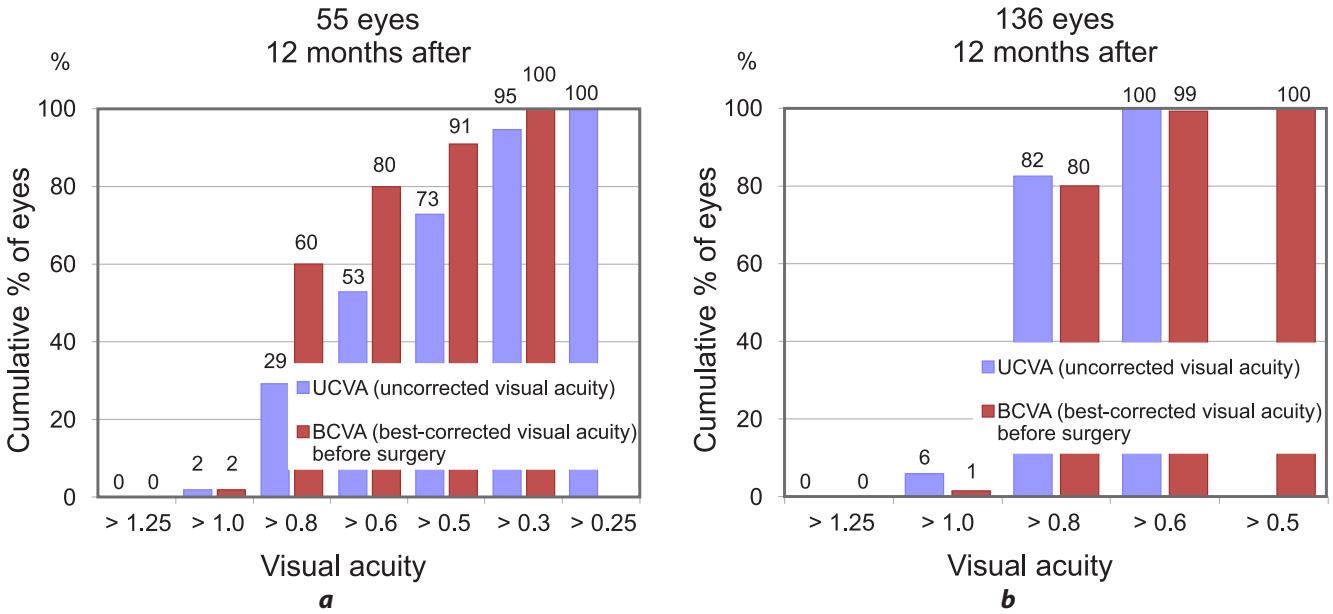

FIG. 1.

*Distribution of patients (cumulative % of eyes) with high myopia depending on visual acuity obtained 12 months after SMILE surgery using standard (**a**) and modified (**b**) technology*

derwent the surgery, the effectiveness of the treatment was analysed (Table 1 – group 2).

To confirm the effectiveness and safety of high myopia correction using the developed modified technology, a comparative analysis of the results obtained at various times of the postoperative period was carried out.

It was found that in the early postoperative period (day 1 and 5), the visual acuity of patients, who underwent SMILE surgery using standard technology, was statistically significantly lower than in patients, whose surgery was carried out using a modified calculation of surgical parameters. So, on day 1 in patients of group 1, it varied from 0.2 to 0.8, averaging 0.43 ± 0.16; in the group with a modified technology for surgery calculating – from 0.7 to 1.0 (0.90 ± 0.12; $p = 0.01$), statistically significantly exceeding the values of the comparison group. By day 5 after surgery, visual acuity improved slightly to 0.47 ± 0.16 and 0.94 ± 0.13 in groups 1 and 2, respectively ($p = 0.01$). This trend continued after 3 months of follow-up (0.47 ± 0.22 and 0.93 ± 0.11; $p = 0.01$). By year 1, the parameters of uncorrected visual acuity remained virtually unchanged and corresponded to 0.58 ± 0.19 and 0.94 ± 14, respectively ($p = 0.01$) (Fig. 1). Corrected visual acuity was 0.91 ± 0.07 and 0.97 ± 0.02, respectively ($p = 0.23$).

A comparative analysis of corrected visual acuity in the preoperative period and uncorrected visual acuity 12 months after SMILE surgery in patients with standard technology showed the loss of 1 line in 5 % of cases, 2 lines or more in 9.1 % of cases (Fig. 2a). In patients operated with modified technology, the loss of visual acuity by 1 line was noted in 4 % of cases, by 2 lines or more – only in 1.5 % (Fig. 2b). It should be noted that the excess of preoperative visual acuity parameters after SMILE surgery in group 1 was noted in 2 % of patients, in group 2 – in 6 %.

A comparative analysis of the results of changes in clinical refraction also demonstrated statistically significant differences in the two groups (Fig. 3).

Thus, on day 1 after the surgery, in patients with myopia, who underwent the standard technology surgery, residual myopic refraction was obtained, the spherical component of which varied from –0.75 to –3.0 D, averaging –1.90 ± 0.69 D, on day 5 it was – 1.86 ± 0.57 D (Fig. 3a).

A statistically significantly better refractive result was achieved in patients operated using the modified technology already on day 1. The average values were –0.08 ± 0.51 D (from –1.5 to +0.75 D; $p = 0.01$). This effect persisted at all stages of the postoperative follow-up period. By 12 months after surgery, the spherical refraction component was –1.96 ± 0.29 in group 1 and –0.24 ± 0.57 in group 2 ($p = 0.01$) (Fig. 3b). In the vast majority of cases, a qualified refractive result was achieved in patients who underwent the modified technology surgery.

In patients, who underwent the standard technology surgery, a deviation from the calculated refraction of ± 1.0 D was noted in 71 % of cases, ± 0.5 D in 51 %, i. e., deviation from the planned refraction of more than one diopter was obtained in 29 % of patients (Fig. 4a). In patients operated using a modified calculation of the surgical parameters, the deviation of more than ± 1.0 D was obtained only in 1 % of cases, the deviation from the calculated refraction of ± 0.5 D was obtained in 82 % (Fig. 4b).

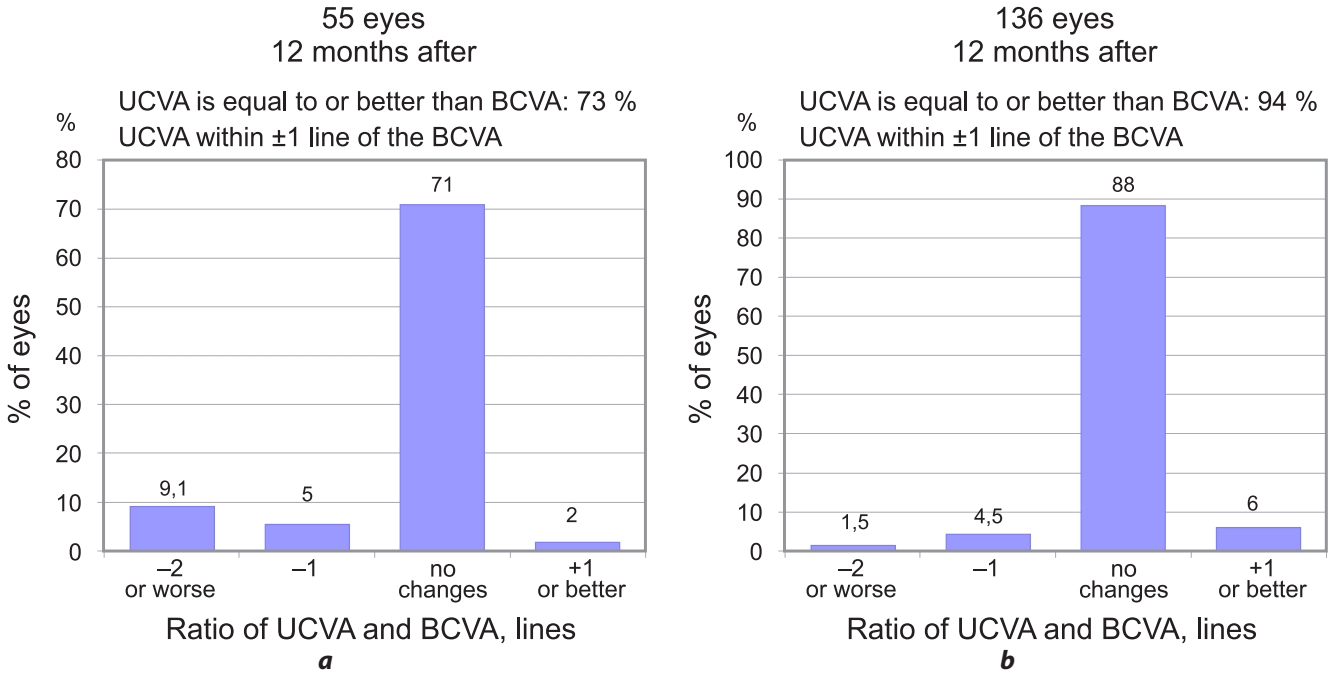

FIG. 2.

*Ratio of uncorrected and maximally corrected visual acuity in patients with high myopia after refractive SMILE surgery using standard (**a**) and modified (**b**) technologies in a long-term postoperative period*

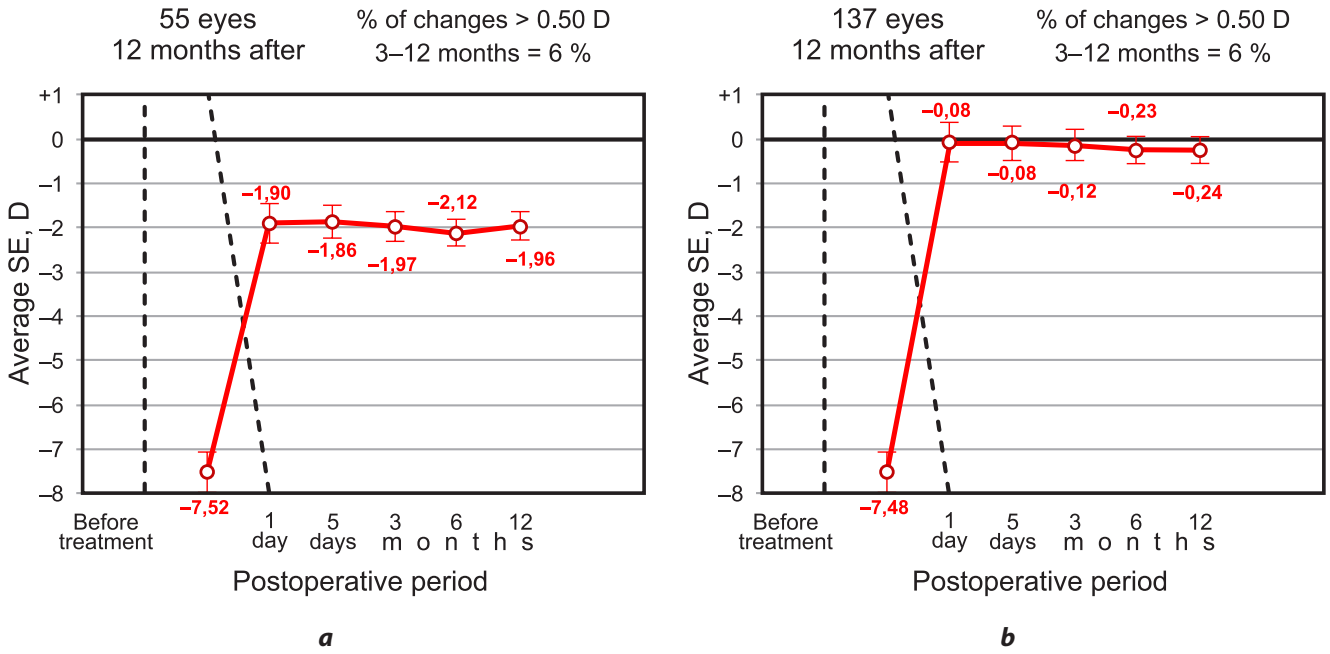

FIG. 3.

*Dynamics of changes in the spherical equivalent (SE) of refraction at different terms of postoperative period in patients after SMILE surgery using standard (**a**) and modified (**b**) technology*

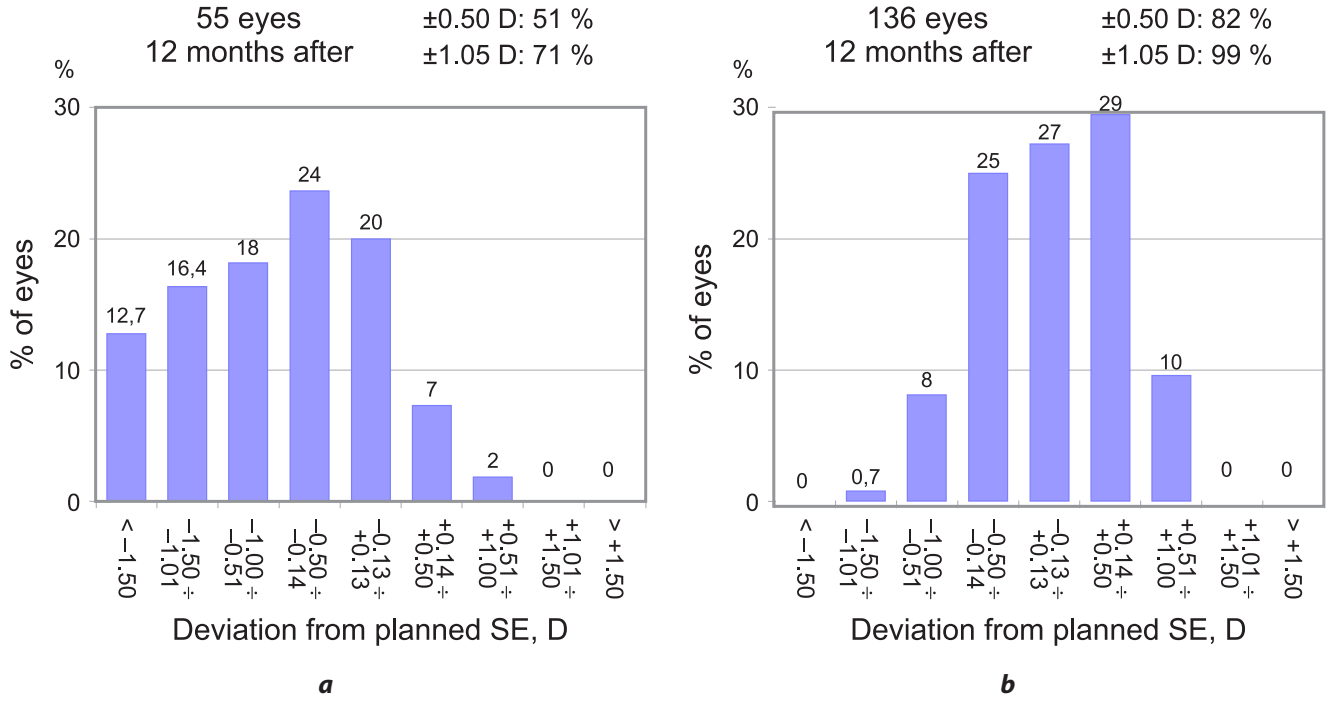

FIG. 4.

*Distribution of patients depending on the spherical equivalent obtained in the late postoperative period after SMILE surgery using standard (**a**) and modified (**b**) technology*

In patients of both groups, a statistically significant interdependence between the planned and the obtained refractive result was established. Despite comparable determination coefficients, patients in group 1 showed a significant deviation of the observed values from the regression line towards under-correction (Fig. 5a), whereas in group 2 the deviations of the predicted value from the average value were minimal (Fig. 5b).

Thus, the developed mathematical model and the identification of key parameters for the formation of the refractive effect of SMILE surgery make it possible at the stage of preoperative diagnosis to simulate surgery parameters,

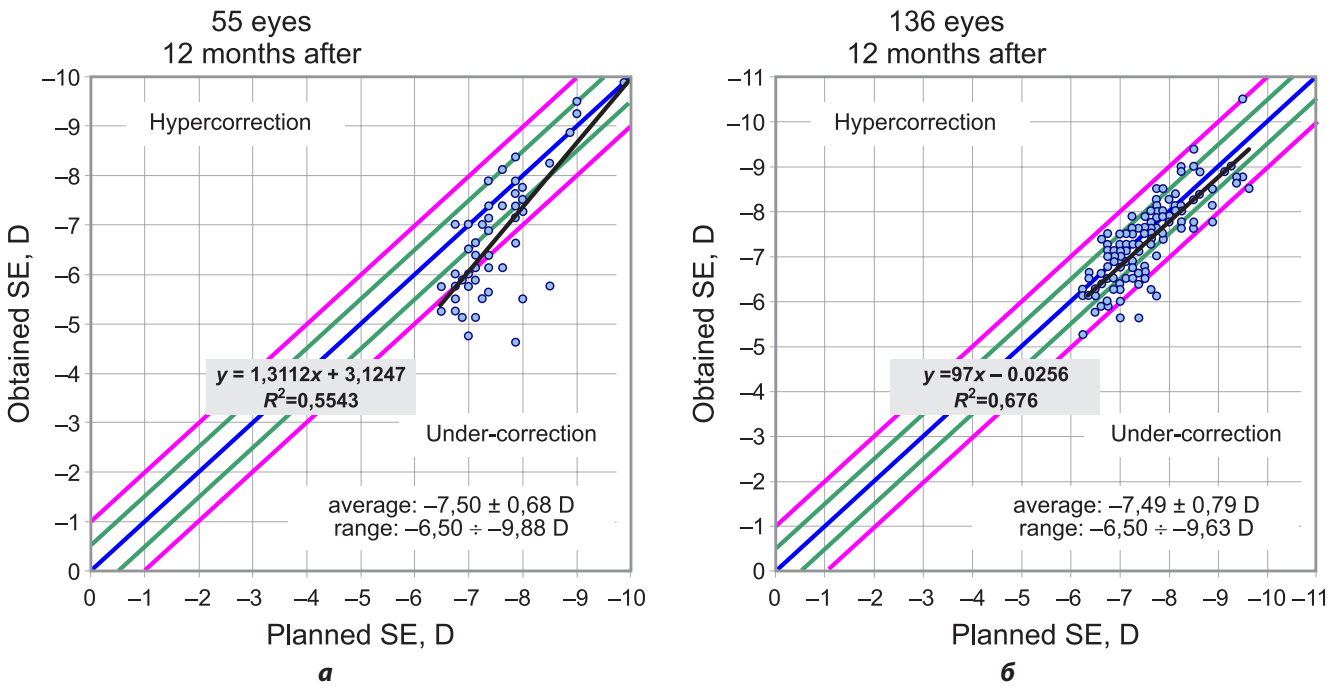

FIG. 5.

*Linear regression analysis of the relationship between the planned and obtained spherical equivalent of refraction in groups after SMILE surgery using standard (**a**) and modified (**b**) technology*

predict the refractive result, the possibility of developing additional optical effects associated with changes in the diameter of the optical zone and, in general, determine patient management tactics.

The developed technology of a modified calculation of the parameters of SMILE surgery for high myopia correction allows to obtain an optimal refractive effect in compliance with safety regulations when the structural and functional parameters of the eye are initially unfavorable for refractive surgery [10].

Conflict of interest

The authors of this article declare the absence of a conflict of interest.

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MODERN ANATOMICAL AND PHYSIOLOGICAL BASES FOR MAINTAINING THE TRANSPARENCY OF THE CORNEAL STROMA. LITERATURE REVIEW

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ABSTRACT

The article presents a literature review of the modern concept of anatomical and physiological structure and functioning of the cornea. The strict morphological structure and corneal tissue homeostasis ensure its transparency. Studying the mechanisms that regulate the constancy of the corneal tissue internal environment allows us to get closer to understanding the prospects for regenerative therapy for the corneal stroma pathology. The article discusses in detail the role and functional potential of corneal stromal cells, which are capable of reverse cytologic differentiation, which primarily ensures the maintenance of tissue homeostasis and corneal transparency. The functional activity of corneal cells can change for a number of reasons, which may be exogenous, iatrogenic (trauma, infection, etc.) or endogenous. Endogenous causes include: cell autoregulation pathologies (for example, enzyme defects); defects in transport systems leading to tissue hypoxia; disorders of the neuro-humoral regulation of trophism. The physical reason for the violation of the corneal transparency is an increase in the light scattering. The article presents five main causes of increased light scattering in the opaque cornea, and also provides an overview of the main substances – components and products of cellular synthesis of corneal stromal cells: cytokines and growth factors (complex of the signal molecule and the SDF1/CXCR4 receptor, insulin-like growth factor 1, tumor necrosis factor alpha, intercellular adhesion molecule 1, erythropoietin, neurotrophic factors, etc.). Thus, corneal opacity can be caused by a single pathogenic mechanism or be the result of a complex effect of several factors. The main processes of tissue homeostasis regulation are aimed at maintaining the unique morphological structure of the cornea.

Key words: *corneal structure, keratocyte, corneal stroma, corneal transparency*

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СОВРЕМЕННЫЕ АНАТОМО-ФИЗИОЛОГИЧЕСКИЕ ОСНОВЫ ПОДДЕРЖАНИЯ ПРОЗРАЧНОСТИ СТРОМЫ РОГОВИЦЫ. ЛИТЕРАТУРНЫЙ ОБЗОР

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РЕЗЮМЕ

Статья представляет собой литературный обзор на тему современной концепции анатомо-физиологического строения и функционирования роговицы. Строгая морфологическая структура и гомеостаз ткани роговицы обеспечивают её прозрачность. Изучение механизмов, регулирующих постоянство внутренней среды ткани роговицы, позволяет приблизиться к пониманию перспектив регенеративной терапии патологии стромы роговицы. В статье подробно рассматриваются роль и функциональный потенциал стромальных клеток роговицы, которые способны к обратной цитодифференцировке, что в первую очередь обеспечивает поддержание гомеостаза ткани и прозрачности роговицы. Функциональная активность клеток роговицы может изменяться по ряду причин, которые могут носить характер экзогенных, ятрогенных (травма, инфекции и др.) либо быть эндогенными. К эндогенным причинам относят: патологии ауторегуляции клеток (например, ферментопатии); дефекты транспортных систем, приводящих к гипоксии тканей; расстройства нервно-гуморальной регуляции трофики. Физическая причина нарушения прозрачности роговицы заключается в увеличении рассеивания света. В статье приводится пять основных причин повышенного светорассеяния в непрозрачной роговице, а также представлен обзор основных веществ – компонентов и продуктов клеточного синтеза стромальных клеток роговицы: цитокинов и факторов роста (комплекс из сигнальной молекулы и рецептора SDF1/CXCR4, инсулиноподобный фактор роста 1, фактор некроза опухоли альфа, молекула межклеточной адгезии 1, эритропоэтин, нейротрофические факторы, и др). Таким образом, помутнение роговицы может вызываться как одним патогенетическим механизмом, так и комплексным воздействием нескольких факторов. Основные процессы регуляции тканевого гомеостаза направлены на поддержание уникальной морфологической структуры роговицы.

Ключевые слова: *гомеостаз роговицы, кератоциты, строма роговицы, прозрачность роговицы*

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SECTION 1. RELEVANCE OF THE TOPIC. MODERN ANATOMICAL ASPECTS OF THE CORNEA STRUCTURE. STROMAL KERATOCYTES AND THEIR ROLE IN MAINTAINING CORNEAL TRANSPARENCY

Visual impairment due to pathological changes in the cornea is the third leading problem in ophthalmology after cataracts and glaucoma [1]. The cornea is a unique refractive vascular-free, transparent and tightly innervated connective tissue structure. Its role is to provide light transmission, protect the structures of the anterior chamber of the eye and provide 2/3 of the total refractive power of the eye. The strict morphological structure and corneal tissue homeostasis ensure its transparency [2]. 90 % of the total volume of the cornea belongs to the stromal layer, scarring (fibrosis) of which leads to blindness. According to a significant statistical study, 12.7 million people worldwide are on the waiting list for a donor cornea transplant [3].

A healthy human cornea has a central thickness of 470–620 μm, the thickness at the periphery is 650–750 μm. The stroma is a collection of tightly packed collagen fibers that form bundles (fibrils) and plates parallel to the surface of the cornea. The main cellular representatives are keratocytes and immune cells. A statistically significant tendency of a decrease of the maximum density of stroma cells from the anterior sections (on average about 40,000 cells/mm^3) to the posterior sections towards the Descemet's membrane (on average about 20,000 cells/mm^3) has been established.

Keratocytes are mitotically resting motionless cells of mesenchymal origin, which have processes-keratopodias that provide contact with neighboring keratocytes, forming a continuously connected network (Fig. 1a, b). Keratocytes perform the function of producing and maintaining the extracellular matrix, ensuring strict morphostructural and biochemical constancy and transparency of corneal tissue. Keratocytes are unique cells of the neural crest that are involved in maintaining water balance and tissue homeostasis due to the synthesis of growth factors, building protein molecules, cytokines, neuropeptides and neurotrophins, metalloproteinase inhibitors [4].

The components of the extracellular matrix (ECM) of the corneal stroma include highly organized collagen (types I, III, V, VI, XII) and glycosaminoglycans (keratocan, keratan sulfate, decorin, mimecan and lumican), which are synthesized by keratocyte cells [6]. Keratocan and lumican are important glycosaminoglycans that are highly expressed in corneal keratocytes [7] and regulate its transparency and hydration balance, organizing and maintaining the topography of collagen fibrils [8].

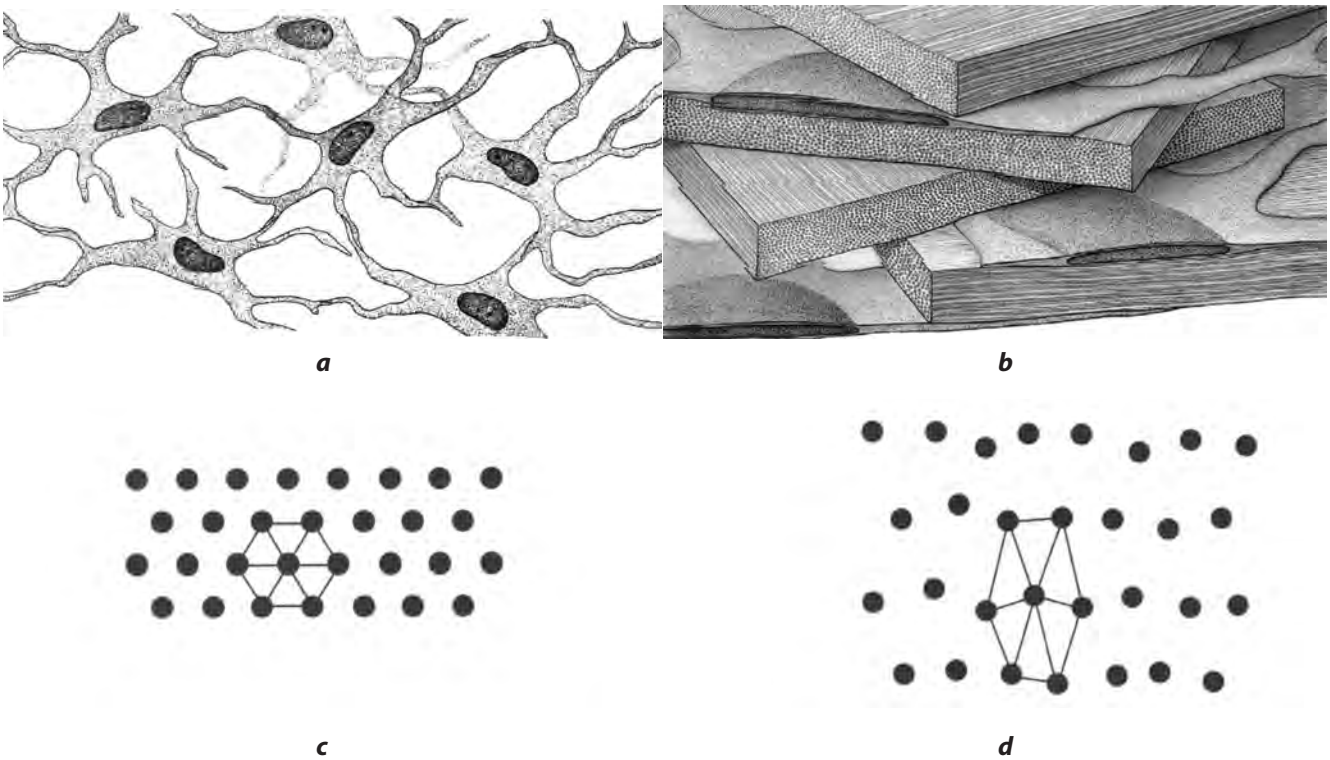


FIG. 1.

*General scheme of the corneal stroma structure: **a** – scheme of the cellular network of the corneal stroma; **b** – structure of dense fibrous connective tissue – collagen plates with thin flat cells between them which contact with neighboring cells by means of processes; **c** – scheme of orientation of collagen fibers – each fiber is located equidistant from the others; **d** – scheme of orientation of collagen fibers in an opaque cornea [5]]*

There are no blood vessels in the cornea, therefore nutrition and metabolism occur due to the vessels of the limbal network, moisture of the anterior chamber and tear fluid by osmosis and diffusion [9]. Metabolic processes are largely replenished by abundant innervation, which is represented by trophic, sensitive and vegetative nerve fibers, forming a perilimbal nerve plexus around the cornea. Entering the cornea, the nerves lose their myelin sheath and become invisible, which also ensures the transparency of the corneal tissue.

Injuries, infections and other pathological processes disrupt the organization of the stroma. Under altered conditions (with injuries, burns, surgical intervention, including the release of IL-1α, the tumor transforming growth factor β1.2 (TGF-β1.2)), part of the resting keratocytes located at the epicenter of the damage undergo apoptosis, forming an acellular zone in the area of damage [10]. Keratocytes bordering the site of injury extend new dendrite – like processes and migrate to the acellular zone before differentiating and proliferating into repair fibroblasts [11]. At the same time, keratocytes from the periphery also switch to an activated state, i.e. acquire the phenotype of motile contractile fibroblasts due to the reorganization of non-muscle myosin and smooth muscle actin α (α-SMA) into cytoskeletal stress fibers, migrating to the site of injury, proliferating and surrounding the wound in as a connected network of cells [12]. This physiological transformation of the phenotype is necessary to activate the mitotic cycle, increase functional activity and realize an active therapeutic effect – stromal wound healing. The observed increase in the size and number of organelles in cells and the acquisition of spindle-shaped cells reflects the enhanced synthetic activity of fibroblasts [13]. The fibroblast population can further differentiate into myofibroblasts characterized by α-SMA expression and an additional increase in stress fibers. Fibroblasts and myofibroblasts trigger regeneration processes in the cornea, causing rapid wound contraction due to the synthesis of a temporary matrix of opaque extracellular matrix [14].

The temporary matrix differs in composition and structure from the intact stroma. During regeneration, the extracellular matrix is synthesized by activated cells at an accelerated rate, in a less organized manner [15, 16]. A decrease in keratocan synthesis occurs in parallel with an increase in the production of decorin and chondroitin. This modified extracellular matrix promotes fibroblast migration; however, its altered biochemistry and structure can cause corneal opacity. Gradually, the temporary matrix is replaced by normal stroma components, including collagen types I and III, to restore the physiological function of the cornea. Thus, collagen expression increases in fibroblasts and reaches higher values in myofibroblasts compared to keratocytes [17]. In particular, high production of collagen I is characteristic of corneal fibroblasts and myofibroblasts, which is necessary for tissue remodeling [18].

Myofibroblasts are characterized by larger sizes compared to fibroblasts and express high levels of α-SMA, vimentin, and desmin [19, 20], which makes it possible to differentiate this cell population from others. α-SMA, vimentin and desmin are structural proteins involved in the formation of the cytoskeleton [21], a three-dimensional network inside the myofibroblast that determines the shape and mechanical support of the cell, provides movement and contractility [22]. The cell population of the corneal stroma has the ability to rapidly rebuild the cytoskeleton, which is directly related to its functional potential, since myofibroblasts acquire a high contractile ability necessary for physiological tissue remodeling. However, uncontrolled proliferation negatively affects the function of the stroma, disrupting the strictly ordered morphological structure of the tissue, thereby changing the transparency of the cornea. The question of the possibility of the reverse transition of corneal myofibroblasts to the original keratocytes under physiological conditions *in situ* is debatable and open. The possibility of reversion of myofibroblasts into fibroblasts has been shown when treating cells with low levels of fibroblast

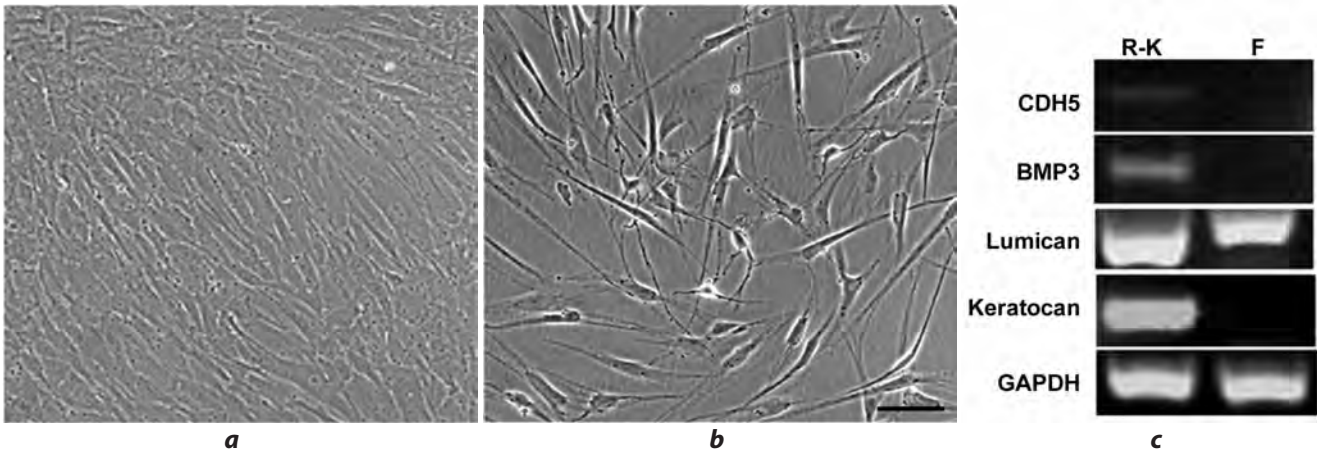


FIG. 2.

*Morphology and some markers of the expression of keratocytes and corneal stroma fibroblasts: **a** – morphology of fibroblasts (F); **b** – morphology of reversed keratocytes (R-K); **c** – expression of specific markers by reversed keratocytes and fibroblasts [14]]*

growth factor 1, 2 (FGF1, FGF2) [23]. This discovery supports the idea that corneal myofibroblasts and fibroblasts represent reversible phenotypes rather than terminally differentiated cell types.

One cell population that originated from another (Fig. 2a) under the influence of certain environmental conditions is called "reversed" population (Fig. 2b), that is, it has undergone a phenotypic transformation. Understanding the mechanisms that regulate the transformation of corneal keratocyte phenotypes is necessary for the development of tissue engineering of the corneal stroma.

Currently, numerous scientific teams are conducting research to determine the optimal conditions for the cultivation of corneal cells. There are several ways to isolate corneal stromal cells, which include mechanical (removal of epithelial and endothelial layers) and prolonged enzymatic treatment of the cornea [24, 25]. Usually, cadaver and unsuitable for transplantation human corneas are used to isolating cells. The disadvantages of the known methods are the complexity and insufficient «purity» of the cell population, since the result is a mixture of cells containing a non-homogenous population from the anterior, middle and posterior parts of the stroma, as well as an admixture of epithelial and endothelial cells of the corresponding layers of the cornea. Populations of cells derived from the stroma of bovine, pigs, rabbits and mice are used as a cell source alternative to the human eye stroma. In culture with the addition of serum, corneal stromal cells acquire the phenotype of fibroblasts, in a serum-free environment with the addition of insulin, transferrin and selenium (ITS), on the contrary, retain the phenotype of keratocytes [26], and in the presence of ascorbic acid – produce and accumulate collagen and proteoglycans, which mimics their functions in the native cornea [27].

A detailed study of the characteristics of corneal stroma cell populations is carried out in order to verify, determine the intensity and nature of the potential therapeutic effect. One of these characteristics is the determination of the phenotypic properties of a cell population, that is, the determination of specific expression markers that distinguish one cell population from another (Fig. 2b).

SECTION 2. PATHOPHYSIOLOGICAL MECHANISMS OF CORNEAL TRANSPARENCY DISORDERS

Keratocytes account for about 3–5 % of the total stromal volume [28]. Figure 3 shows the histological structure of the cornea, in whose own substance a low cell content is clearly determined. This ratio is necessary to maintain the transparency of the cornea and is explained by the peculiarities of light transmission. The cytoplasm of a mitotically resting keratocyte scatters the light beam to a less-

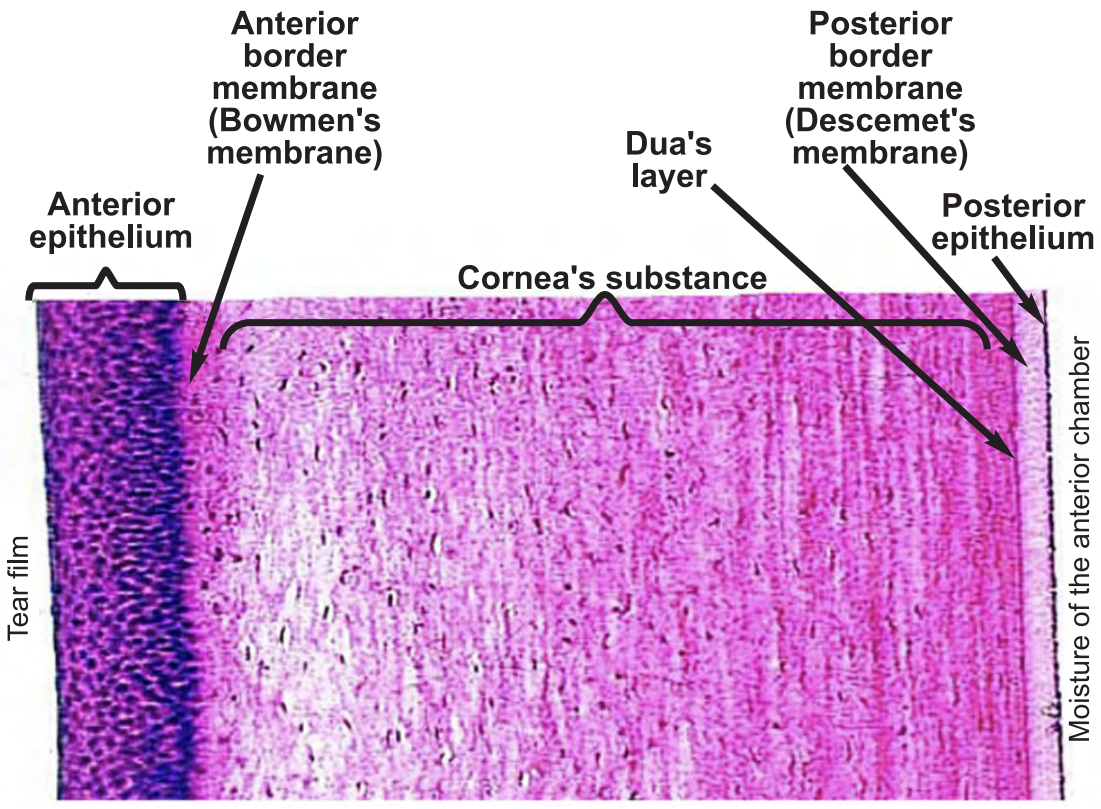


FIG. 3.
Histological structure of the cornea (hematoxylin and eosin staining) [30]

er extent due to the low content of organelles in cells [29], due to which the unique structure of the cornea determines its functional role.

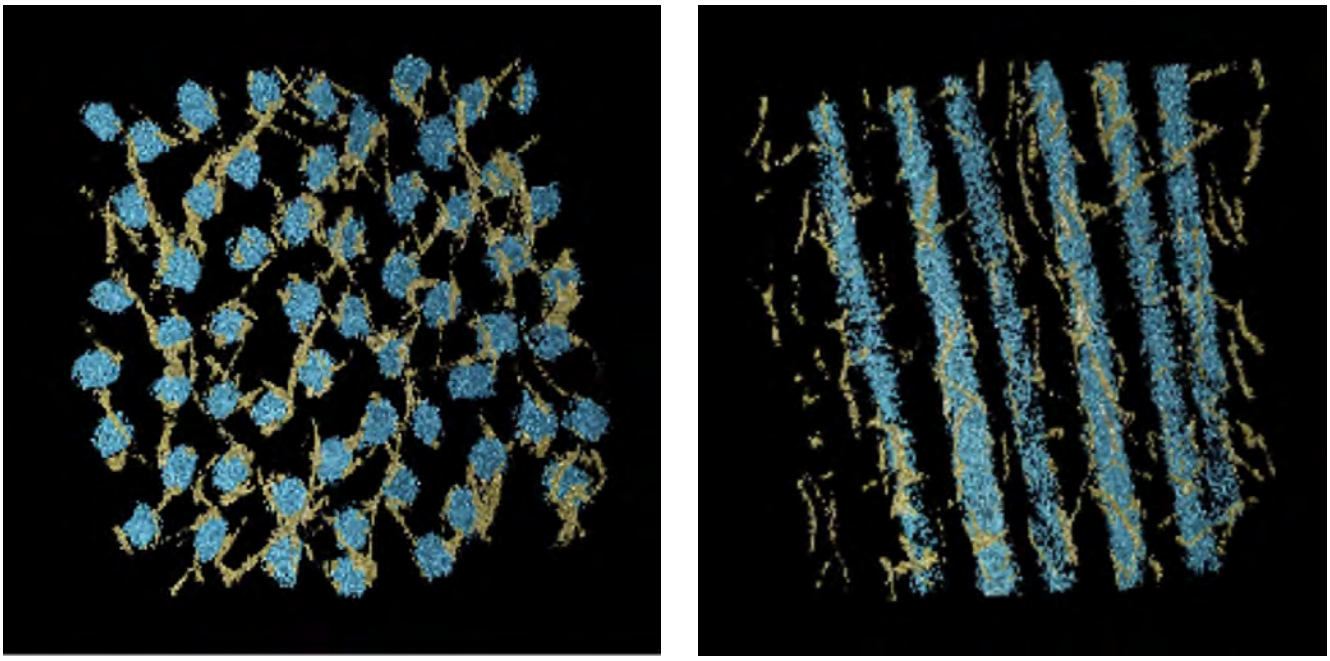
Functional activity at cell (keratocyte) level may vary for a number of reasons, which may be exogenous, iatrogenic (trauma, infections, etc.) or endogenous. Endogenous causes include: cell autoregulation disorders (for example, hereditary or acquired enzyme defects); transport systems disorders leading to tissue (cell) hypoxia; disorders of endocrine or nervous regulation of trophism [30]. By influencing the cellular microenvironment, using a wide range of biologically active substances, it is possible to influence the listed endogenous causes of impaired functional activity of the stroma population. One of the ways of such influence may be a transplantable cell population of stromal cells. Thus, the ability of donor stromal cells to repopulate recipient tissue without scarring has been proven (follow-up time is 30 years) [31].

Impaired functional activity of keratocytes has been found to result in changes in corneal transparency [32]. This fact is explained by the role of keratocytes in the synthesis of extracellular matrix components – collagen and glycosaminoglycans; regulation of the constancy of the water and electrolyte balance of the tissue directly – realizing the cellular membrane transport of substances, electrolytes and water into and out of the tissue, and indirectly – by synthesizing hydrophilic components of the extracellular matrix, which «attract» water molecules [33]. These processes are responsible for creating a strict spatially ordered structure of the corneal tissue through which light passes (the interference process). The physical reason for the disruption of the corneal transparency is an increase in the light scattering [34].

There are five main causes of increased light scattering in the opaque cornea:

1. Changes in the structure of collagen fibers, disruption of their spatial hexagonal arrangement and/or thickening of fibrils due to fusion

In a healthy transparent cornea, collagen fibers assemble into bundles called fibrils (lamellae), which are of the same size and strictly oriented in space relative to each other to minimize the scattering of the light beam. This alignment of collagen fibers in the cornea is ensured by glycosaminoglycans that line up around the fibrils (Fig. 4a, Fig. 5a) and create «bridges» between them (Fig. 4b, Fig. 5b) – equal inter-fibrillar distances [35]. Since glycosaminoglycans are hydrophilic compounds, they attract water molecules to themselves and thereby perform their function. If the position of the collagen fiber is disrupted, osmotic and electrostatic pressure forces are applied to it and return the fiber to its place (Fig. 5b, d) [36]. The strict morphological structure is also ensured by the uniform diameter of the fibrils and their ordered organization, which creates conditions for the passage of a light beam through the cornea to the retina of the eye, i.e. realizes the transparency of the corneal tissue.



a ***b***

FIG. 4.

Spatial orientation of glycosaminoglycans and lamellae: ***a*** *– glycosaminoglycans around lamellae (indicated by short thin lines);* ***b*** *– glycosaminoglycans in the shape of "bridges" between the lamellae [35]*

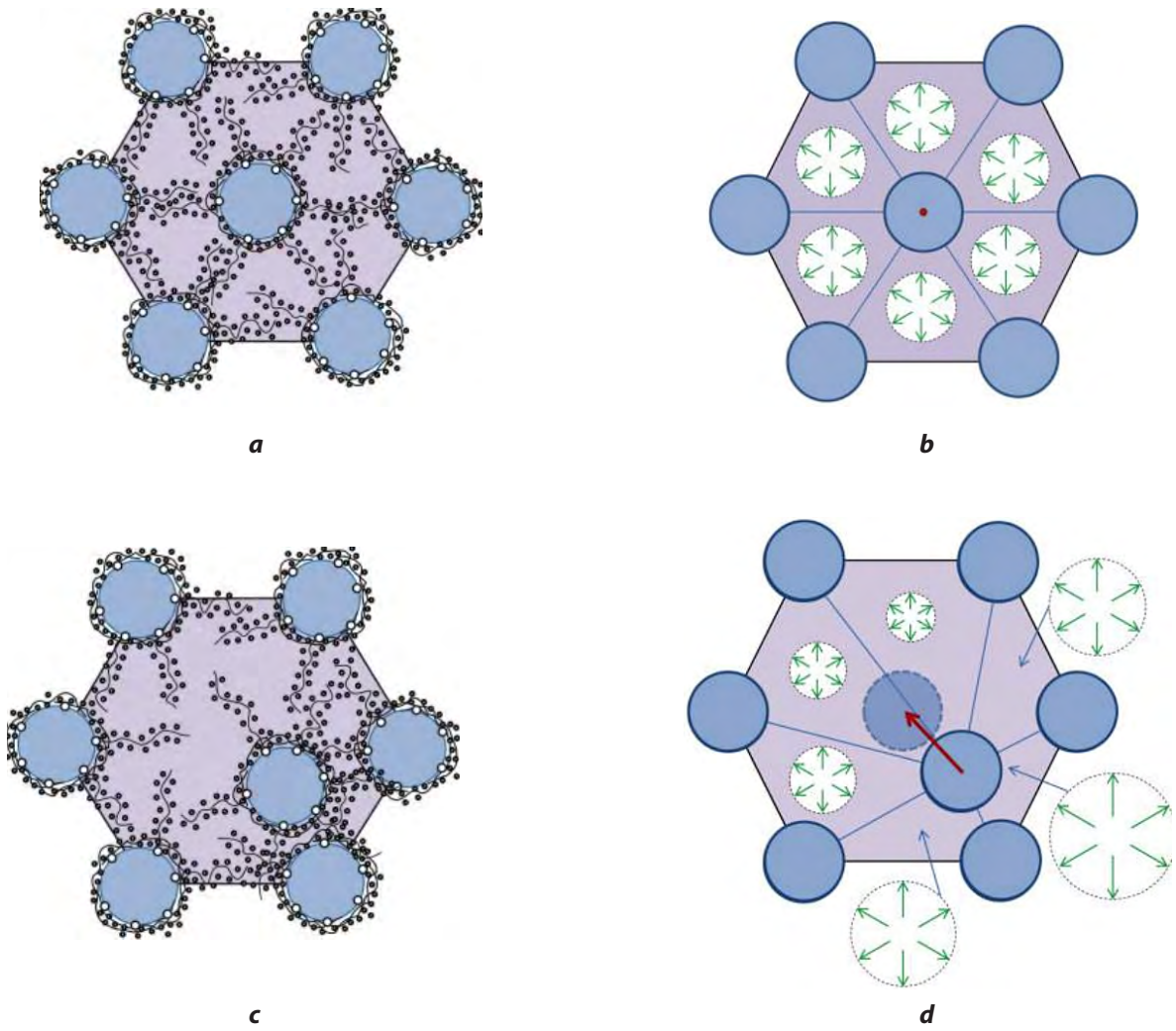


FIG. 5.

*The action of osmotic (**a**) and electrostatic (**b**) pressure at the displacement of the collagen fiber (**c**, **d**) [36]*

2. Transformation of keratocytes into fibroblasts and myofibroblasts ("activated" cells)

Keratocyte nuclei have high light scattering indicators. Therefore, the cornea would not be transparent if there were a large number of cells in the stroma, whose nuclei would not allow the light beam to pass through this connective tissue structure of the eye. The transition of resting keratocytes to a reparative phenotype is associated with profound molecular, biochemical and morphological changes. Activated stroma cells have a high degree of light scattering, thereby directly capable of causing a disruption of corneal transparency. Thus, a confirmatory fact of higher light scattering in the population of myofibroblasts compared with the population of corneal keratocytes was established [37].

3. Synthesis of fibrous opaque extracellular protein matrix by "activated" corneal cells

In response to trauma, surgical intervention (after excimer laser ablation for photorefractive keratectomy (PRK)), the influence of infectious agents (viruses/bacteria), as well as in genetically determined abnormalities and congenital corneal dystrophies, resting keratocytes become mitotically active and differentiate into fibroblasts and myofibroblasts, excessively synthesizing new proteins such as fibronectin, α-SMA, tenascin-C, collagen types III, IV, fibrillin 1 and others that disrupt the organization of fibrils, which leads to corneal opacity [38]. Thus, the main reason for the disruption of corneal transparency during activation of the stroma cell population is a change in the spatial arrangement of collagen fibrils due to the synthesis of excess protein matrix. The fibrous opaque extracellular matrix is reversible with the transition to the normal architecture of the corneal stroma during the "rejuvenation" of the keratocyte cell population, which is an important strategy for restoring tissue transparency [39].

4. Edema of the corneal stroma

Stroma edema leads to the corneal tissue opacity and can be caused by various factors: in case of disruption of the barrier structure integrity – Bowman's or Descemet's membranes – after injuries, infectious effects, acute keratoconus (hydrops), surgical interventions

and infectious lesions, corneal dystrophy. The mechanisms of "hydration" of the cornea can also include a disruption of cellular transmembrane or passive water transport in the corneal stroma and a disruption of the synthesis of protein hydrophilic components of the intercellular matrix with changes in the functional activity of the keratocyte, pathologies of the membrane-pumping function of the endothelial (for example, Fuchs syndrome) and epithelial barriers (recurrent erosion/corneal ulcer).

5. Decrease in the level of crystallin expression by keratocytes of the stroma

Crystallins are water-soluble proteins with enzymatic activity expressed by keratocytes of the stroma, epithelial cells of the cornea and lens [40], their role is in antioxidant protection and reduction of light scattering of transparent structures of the eye [41, 42]. The antioxidant role of corneal crystallins is protection from damage by peroxide compounds and free radicals, including those formed from exposure to UV radiation. The family of aldehyde dehydrogenases (ALDH3A1, -1A1, -2, -7A1, -1B1) and transketolase (TKT) belong to the corneal crystallins [43]. With a decrease in the concentration of crystallins in the cornea (TKT and ALDH1A1), there is an increase in light scattering, i.e. a decrease in its transparency is noted. The conversion of keratocytes into a reparative phenotype (fibroblasts and myofibroblasts) in the corneas of rabbits and bulls leads to loss of crystallins, which is also confirmed in cultural models [44].

Thus, corneal opacity can be caused by one of the listed pathogenetic changes, or it can be a consequence of the combined effects of several factors. The main processes of regulation of tissue homeostasis consist primarily in maintaining a strict morphological structure of the cornea.

SECTION 3. THE ROLE OF CYTOKINES AND GROWTH FACTORS IN CORNEAL STROMA REPAIR

Stromal stem cells realize the effect of tissue repair due to active interaction with the microenvironment due to the secretion of cytokines, growth factors, neuropeptides and neurotrophins [45].

The loss of functional activity of stromal keratocytes is the loss of the ability of the cornea to self–renewal. In the pathogenesis of many pathological changes of the cornea, such as post-burn, post-traumatic, ectatic conditions (including keratoconus), a special role is assigned to the loss of keratocytes caused by an increase in apoptosis [46, 47]. The inflammatory theory is one of the modern explanations of this process; modern scientists are trying to explain the decrease in the elastic properties of corneal tissue in keratoconus by the same fundamental processes at the cell level, i.e. the effect of free radicals and oxidative stress products on the tissue. It was found that stroma cells from patients with keratoconus form metabolites that indicate oxidative stress of the cell in both 2D and 3D cultures [48]. Deceleration in the progression of ectasia in a patient with keratoconus with the use of cyclosporine A, an immunomodulatory agent, was also reported [49].

The reparative potential of stromal keratocytes is feasible due to its ability to reverse transition into a less differentiated class of cells (the process of reverse cytodifferentiation). According to modern concepts, one of the reasons for the transition of keratocytes to an activated state is the effect of TGF-β on them [50]. The release of TGF-β occurs under adverse conditions, with damage to the epithelium and Bowman's membrane as a result of injury, surgery, exposure to chemical or infectious agents, or other pathological conditions.

In various organs, including the cornea, members of the TGF-β family are key regulators of fibrosis and scarring through signaling mechanisms (TGF-β/Smad-signaling) via signaling mRNA, as well as through other signaling pathways [51, 52]. The TGF-β family consists of three closely related isoforms (β1, β2 and β3) that play different roles in cell differentiation and tissue regeneration. Thus, TGF-β1 and -β2 mediate tissue fibrosis and scar formation [53, 54], unlike TGF-β3, which acts as an inhibitor of scarring processes [55]. The same mechanisms of interaction have been determined for the cornea. Thus, TGF-β1 and TGF-β2 contribute to scarring of the stroma [56], while TGF-β3 has been proven to restore corneal transparency [57].

The complex of the signaling molecule and receptor SDF1/CXCR4 (stromal cell derived factor-1) is expressed on corneal fibroblasts, participating in regeneration processes. The role of the complex is to organize the extracellular matrix, accelerate the migration of mesenchymal stem cells (MSCs) [58], increase the expression of α-SMA in fibroblasts, promoting the transition of fibroblasts to myofibroblasts, increasing tissue scarring [59]. It has been suggested that the activation of stem cell homing and secretion of growth factors through the chemokine axis SDF-1/CXCR4 [60]. The expression of the complex increases in response to an increase in the concentration of hypoxic factor HIF-1a and mechanical damage, which leads to increased migration of stem cells through chemotaxis. The antihypoxic role of the complex, the release of which occurs during radiation exposure to the tumor (immunological effect), has been established [61]. An increase in the expression of the SDF1/CXCR4 complex stimulates the effective migration of stem cells to the injury zone, recruitment of fibroblasts, and activation of endogenous repair processes [62].

Intermediate filaments are important elements of the cytoskeleton for the regulation of processes related to tissue repair. Fibronectin is a protein highly expressed on corneal fibroblasts, which is involved in the organization of the extracellular matrix during regeneration. It forms pathways-channels to accelerate migration and create a dense cellular network – the spread of cells to the site of injury [63]. Vimentin, like desmin, are intermediate protein insoluble filaments of the cy-

toskeleton of stromal keratocytes, fibroblasts and myofibroblasts [64, 65], whose role is to carry out the transition of the stroma cell population to myofibroblasts [66], as well as to accelerate the processes of proliferation and migration of fibroblasts to the site of the «wound» [67], thereby providing stroma remodeling after injuries, surgical interventions, etc. An increase in vimentin expression in stromal cells after surgery (PRK) and a decrease in the rate of fibroblast migration to the wound site in the stroma in mice with vimentin deficiency were established [68]. Fibroblasts and myofibroblasts have also been found to maintain higher levels of vimentin than keratocytes [69].

Insulin – like growth factor 1 (IGF-1) is a protein of the family of insulin-like growth factors, responsible for maintaining corneal homeostasis; regulates the formation of a communication network between keratocytes [70], proliferation and differentiation of keratocytes into fibroblasts and myofibroblasts during inflammatory processes and damage [71]. T. Sarenac et al. showed that IGF-1 increases the secretion of keratocan, lumican and cytosolic crystallin (ALDH3A1). IGF-1 reduces the likelihood of scar formation in the corneal stroma, increasing keratocyte proliferation and affecting wound healing [72].

Proinflammatory cytokine – tumor necrosis factor α (TNF-α) – and soluble intercellular adhesion molecule-1 (ICAM-1) play an important role in the regulation of inflammatory reactions in infectious and non-infectious processes (in allergic reactions) in the cornea, are expressed on corneal keratocytes and fibroblasts [73], ensure the migration of macrophages and leukocytes, regulate the processes of infiltration and activation of polymorphonuclear neutrophils in the focus of inflammation [74].

Erythropoietin (EPO) is a glycoprotein that is an active humoral factor that regulates the growth and development of various cells, tissues and organ systems. EPO not only stimulates the proliferation and differentiation of erythroid precursor cells, but also has antiapoptotic and antioxidant effects, participates in neuroprotection and angiogenesis, and increases cell survival in hypoxia. EPO expression has been shown on many cells, including corneal keratocytes of mice. The connection of this cytokine with the processes of neovasculogenesis of the eye has been established [75]. High levels of EPO have been found in vitreous samples from patients with proliferative diabetic retinopathy, however, the role of EPO in healthy cornea is largely unknown.

Neurotrophic factors (NGF, NT-3, BDNF) and tyrosine kinase receptors (TrkA, TrkB, TrkC and TrkE) are a number of compounds that are synthesized in the epithelium and stroma of the cornea, are able to influence each other, activating the processes of migration and proliferation and the ability to regulate the function of cytokine exchange inside the cornea [76].

Corneal sensitivity is provided by the ocular branch of the trigeminal nerve, which causes protective reflexes such as blinking and lacrimation. The corneal nerves depart from the ocular branch of the trigeminal nerve and provide mechanical, chemical, thermal sensitivity, as well as perform a trophic function due to the release of nutrients and trophic factors. Local and systemic conditions caused by trigeminal nerve damage (such as diabetes mellitus, dry eye syndrome, keratitis with herpes simplex virus, neurotrophic keratitis, etc.) are associated with impaired corneal innervation, decreased tear production and impaired healing of epithelial and stromal wounds. Corneal nerves express several neurotransmitters, including substance P (SP) – calcitonin gene-related peptide (CGRP), acetylcholine, cholecystokinin, norepinephrine, serotonin, neuropeptide Y (NPY), vasointestinal peptide (VIP), methencephalin, natriuretic brain peptide, vasopressin and neurotensin. It was demonstrated that SP is able to modulate the proliferation and migration of corneal cells and their adhesion. The use of SP associated with IGF-1, as well as nerve growth factor (NGF), epidermis growth factor (EGF), vascular endothelium growth factor (VEGF), semaphorins, neurotrophins 3 and 4 (NT-3, NT-4), which increase the rate of corneal healing and stimulate the adhesion of epithelial cells was demonstrated [77, 78]. Conversely, corneal stromal and epithelial cells secrete neuropeptides, neurotrophins and growth factors that affect survival, differentiation of nerve fibers and their maturation, including NGF, brain-derived neurotrophic factor (BDNF), ciliary neurotrophic factor (CNTF), neurotrophins 3, 4, 5, EGF and glial cell-derived neurotrophic factor (GDNF) [79, 80].

Thus, corneal cells and the ocular branch of the trigeminal nerve are the main participants of the interaction during corneal tissue repair at the neurotrophic level. They are able to mutually activate each other to produce cytokines, neuropeptides, neurotransmitters and growth factors to improve trophic function and accelerate corneal wound healing. Consequently, all local and systemic conditions leading to damage to the corneal sensory nerve can affect this interaction, causing a disruption of corneal repair and healing rate. New compounds capable of stimulating corneal nerve repair are under development. Among them, eye drops with nerve growth factors (including platelet-rich plasma) proved to be safe and effective for stimulating healing and improving corneal sensitivity in patients with neurotrophic keratitis.

CONCLUSION

New knowledge about the role and interaction of the extracellular matrix and the corneal stroma cell population in maintaining tissue homeostasis is important for understanding the tactics of treating many diseases and purposefully influencing the processes of repair of the connective tissue structure. In recent years, ideas about the extracellular matrix, which was previously considered only from one side – as architectonics, support for cells and tissues, have changed significantly. Numerous studies confirm that the extracellular matrix is a physiologically active participant in living tissue, which is responsible for the most important

processes of cell and tissue life. The cellular population of the corneal stroma also plays a crucial role in ensuring the physiological repair processes of the tissue. Mutually influencing each other, the connective tissue components of the stroma create a strict morphological structure that ensures the main property of the cornea of the eye – transparency.

A promising alternative way to eliminate corneal blindness is stem cell therapy, which is etiotropic in nature, due to the activation of various signaling pathways to tissue regeneration, solving two primary tasks: replenishing the lost population of keratocytes and restoring its functional role (production of extracellular matrix, synthesis of cytokines, growth factors, neuropeptides, etc.). An understanding of the modern anatomical and physiological foundations of the structure of the cornea, described in this literature review, will help to approach the study of this topic in order to determine the «application points» of potential therapeutic agents.

Due to the feedback between cellular elements and their microenvironment, which evolves during tissue development, a unique molecular composition of the extracellular matrix is formed, which has a powerful effect on biochemical and biophysical processes in cells and determines cell-matrix (epithelio-stromal) interactions. The term «corneal homeostasis» combines a whole complex of intercellular and intermolecular interactions, the study of which from the point of view of the main processes of neuro-humoral regulation is necessary for qualitative external influence on the processes of corneal tissue restoration.

Conflict of interest

The authors of this article declare the absence of a conflict of interest.

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COMPARATIVE ANALYSIS OF THE CORRECTION OF IRREGULAR POSTKERATOPLASTIC ASTIGMATISM WITH SCLERAL LENSES AND INTRASTROMAL RING IMPLANTATION

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ABSTRACT

Background. *Many patients after penetrating keratoplasty (PK) experience induced postkeratoplastic astigmatism, which is often irregular and causes an increase in corneal aberrations that reduce visual acuity and quality.*
The aim of the study. *To conduct a comparative analysis of the effect of rigid gas permeable scleral lenses and the MyoRing implantation method on clinical and functional parameters in patients with IPA.*
Material and methods. *The clinical study included 60 patients (60 eyes). The age of patients with IPA was from 25 to 42 years. All patients underwent penetrating keratoplasty. All patients were divided into two groups depending on the method for irregular postkeratoplastic astigmatism correction. Group I included 30 patients (30 eyes) who were fitted with rigid gas permeable scleral lenses. Group II consisted of 30 patients (30 eyes) who underwent implantation of the MyoRing into a penetrating corneal graft. The observation period was 1 year.*
Results. *After 12 months of observation, there was a greater increase in uncorrected visual acuity by an average of 3 lines, in corrected visual acuity – by 2 lines; a greater decrease in corneal aberrations in photo- (root mean square (RMS) of total aberrations (RMS total) by 0.30 ± 0.08 μm, RMS of higher order aberrations (RMS HOA) – by 1.01 ± 0.24 μm) and mesopic conditions (RMS total – by 0.33 ± 0.09 μm, RMS HOA – by 0.08 ± 0.03 μm) in patients wearing rigid gas permeable scleral lenses compared with patients after MyoRing implantation into a penetrating corneal graft.*
Conclusion. *Patients of group I, wearing rigid gas permeable scleral lenses, showed a greater improvement in visual acuity and a decrease in corneal aberrations in photo- and mesopic conditions compared to the patients of group II (after MyoRing implantation) at a follow-up period of 12 months.*

Key words: *irregular postkeratoplastic astigmatism, MyoRing, scleral lens*

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СРАВНИТЕЛЬНЫЙ АНАЛИЗ КОРРЕКЦИИ ИРРЕГУЛЯРНОГО ПОСТКЕРАТОПЛАСТИЧЕСКОГО АСТИГМАТИЗМА СКЛЕРАЛЬНЫМИ ЛИНЗАМИ И ИМПЛАНТАЦИЕЙ ИНТРАСТРОМАЛЬНОГО КОЛЬЦА

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РЕЗЮМЕ

Введение. *Многие пациенты после сквозной кератопластики (СКП) сталкиваются с индуцированным посткератопластическим астигматизмом, который нередко бывает иррегулярным (ИПА) и вызывает увеличение роговичных аберраций, снижающих остроту и качество зрения.*

Цель исследования*. Провести сравнительный анализ влияния жёстких газопроницаемых склеральных линз (ЖГСЛ) и метода имплантации кольца MyoRing на клинико-функциональные показатели у пациентов с ИПА.*

Материал и методы. *В клиническое исследование вошли 60 пациентов (60 глаз). Возраст пациентов с ИПА составил от 25 до 42 лет. У всех пациентов в анамнезе была выполнена СКП. Все пациенты в зависимости от метода коррекции ИПА были разделены на две группы. В I группу вошли 30 пациентов (30 глаз), которым были подобраны ЖГСЛ. II группу составили 30 пациентов (30 глаз), которым была выполнена имплантация кольца MyoRing в сквозной роговичный трансплантат. Срок наблюдения составил 1 год.*

Результаты. *Через 12 месяцев наблюдения было отмечено большее повышение некорригированной остроты зрения в среднем на 3 строки, корригированной остроты зрения – на 2 строки; большее снижение роговичных аберраций в фото- (среднеквадратичное отклонение волнового фронта (RMS, root mean square) суммарных аберраций (RMS total) на 0,30 ± 0,08 мкм, RMS аберраций высших порядков (RMS HOA, RMS of higher order aberrations) – на 1,01 ± 0,24 мкм) и мезопических условиях (RMS total – на 0,33 ± 0,09 мкм, RMS HOA – на 0,08 ± 0,03 мкм) у пациентов, носящих ЖГСЛ, по сравнению с пациентами после имплантации кольца MyoRing в сквозной роговичный трансплантат.*

Заключение. *У пациентов I группы, носящих ЖГСЛ, было отмечено большее улучшение остроты зрения и снижение роговичных аберраций в фото- и мезопических условиях по сравнению со II группой (после имплантации кольца MyoRing) при сроке наблюдения 12 месяцев.*

Ключевые слова: *иррегулярный посткератопластический астигматизм, кольцо MyoRing, склеральная линза*

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RELEVANCE

Performing penetrating keratoplasty (PK), especially in patients with keratoconus and pellucid marginal corneal degeneration, leads to induced postkeratoplastic astigmatism (PA), which is often irregular [1]. The greater the degree of irregular PA, the higher the value of corneal aberrations, especially of higher order aberrations, which reduce the acuity and quality of vision of patients and eventually lead to their dissatisfaction with the functional result of PK [2]. Thus, the correction of PA and especially its irregular form is an important task in the treatment of this category of patients. Currently, the leading position in correcting the irregular shape of PA is occupied by contact correction with rigid gas permeable scleral lenses [3]. Rigid gas permeable scleral lenses smooth out all the irregularities of the corneal graft with a tear meniscus located in the space under the lens, and at the same time correct postkeratoplastic ametropia [4]. However, rigid gas permeable scleral lenses are not suitable for all patients: restrictions on rigid gas permeable scleral lenses occur under certain workplace conditions (for example, high air temperature in production workshops). In some cases the limiting factor is the cost of these lenses, etc. The method of implantation of the MyoRing into a corneal graft that has appeared in recent years is a fairly promising direction and can be an alternative method of correcting PA, including its irregular shape, in patients who cannot wear rigid gas permeable scleral lenses [5]. After implantation of the MyoRing into the corneal graft, a so-called conditionally «additional limb» is created in it, located symmetrically with respect to the patient's visual axis, relative to which the corneal graft is evenly flattened and its sphericity and regularity increase [6]. In connection with the above, as well as with limited information in the literature on the results of implantation of the MyoRing into a corneal graft, a comparative analysis of the effect of rigid gas permeable scleral lenses and the MyoRing implantation method on clinical and functional parameters in patients with irregular postkeratoplastic astigmatism is quite relevant.

THE AIM OF THE STUDY

To conduct a comparative analysis of the effect of rigid gas permeable scleral lenses and the MyoRing implantation method on clinical and functional parameters in patients with irregular postkeratoplastic astigmatism.

MATERIAL AND METHODS

We examined 60 patients (60 eyes) aged 25 to 42 years (the average age was 33 ± 6.1 years), 37 men and 23 women. All patients had a history of PK for stage IV keratoconus 4–10 years ago. All corneal grafts were transparent. The diameter of the corneal graft was 8.0 mm in 48 eyes and 8.5 mm in 12 eyes. According to keratotopography, an irregular form of PA was noted in all patients. All patients, depending on the method of correction of postkeratoplastic ametropia, were divided into two groups.

Group I included 30 patients (30 eyes); we selected rigid gas permeable scleral lenses OKVision (Canadian company OKV-RGP Onefit Med) for them. This type of rigid gas permeable scleral lenses was made of Contamac Optimum Extra with an oxygen permeability of 100 units. The diameter of the rigid gas permeable scleral lenses was 15.6 mm, the thickness in the center was 0.22 mm. Optical coherence tomography (OCT) of the cornea was used to assess the centralization of the rigid gas permeable scleral lens, the position of its edge in different quadrants, as well as the thickness of the central, peripheral and limbal clearances.

Group II consisted of 30 patients (30 eyes) who underwent implantation of the MyoRing (Dioptex, Austria) into a penetrating corneal graft using a femtosecond laser (FSL) FemtoVisum 1 MHz (Troitsk, Russia). Surgery was performed in two stages. At stage I, an intrastromal pocket was formed within the corneal graft with a diameter of 8.0 mm at a depth of 80 % of the minimum thickness of the corneal graft using FSL. The length of the entrance tunnel incision was 1.0 mm, the width was 4.5 mm. At stage II, a MyoRing (diameter – 5.0 mm, thickness – 0.5 mm, height – 200-320 μm) was implanted into the formed intrastromal pocket through an entrance tunnel incision using special tweezers. The selection of the parameters of the whole ring was calculated based on the values of the spherical and cylindrical components of refraction [7]. The ring was centered taking into account the location of the patient's visual axis.

All patients underwent ophthalmological examination before and during rigid gas permeable scleral lens fitting, as well as before and after implantation of the MyoRing into the corneal graft. The examination included autorefractokeratometry, visometry, biomicroscopy, computer keratotopography on the TMS-4 device (Tomey, Japan), aberrometry on the Pentacam device (Oculus Optikgerate GmbH, Germany), calculation of endothelial cell density (ECD) on the Confoscan-4 device (Nidek, Japan), OCT of the corneal graft on the Casia-2 device (Tomey, Japan). Corneal aberrations in both groups were analyzed in the 3.0 mm optical zone in order to create conditions close to photopic conditions without the edge of the intrastromal ring entering the patient's field of view, as well as in the 6.0 mm zone – to create conditions close to mesopic conditions and the edge of the intrastromal ring entering the patient's field of view. The observation period was 1 year.

All studies and manipulations were conducted in compliance with the principles of the World Medical Association Declaration of Helsinki on Ethical Principles for Medical Research Involving Human Subjects.

Statistical data processing was performed in the IBM SPSS Statistics 20 software (IBM Corp., USA). The Shapiro – Wilk test was used to check the normality of the distributions of the studied parameters. The statistical significance of the differences in the studied parameters was assessed by the parametric t-test for dependent variables in connection with the normal distribution of the val-

ues of the studied parameters. When comparing the average values of the studied parameters in both groups, the t-test for independent samples was used. The average visual acuity value was recalculated logarithmically. The values of the studied parameters are presented in the form M ± SD, where M is the arithmetic mean, SD is the standard deviation. In both groups, the values of the studied parameters before the selection of the rigid gas permeable scleral lenses and implantation of the MyoRing were comparable ($p > 0.05$). During the statistical analysis, the studied parameters in both groups were compared on the next day, after 6 and 12 months, compared with the data before the selection of rigid gas permeable scleral lenses in group I and with preoperative values in group II. The values of the studied parameters were also compared between the groups after 12 months of follow-up after the stabilization of clinical and functional parameters in group II. The differences in the studied parameters were considered statistically significant at $p < 0.05$.

RESULTS

On the next day after the selection of the rigid gas permeable scleral lenses, all patients of group I noted a significant increase in visual acuity. The average value of uncorrected visual acuity (UCVA) increased 10.1 times, and corrected visual acuity (CVA) – 4.3 times (Table 1).

The spherical component of refraction (SCR) decreased by 6.10 ± 1.27 D, the cylindrical component of refraction

TABLE 1

CLINICAL AND FUNCTIONAL PARAMETERS BEFORE AND AT DIFFERENT TERMS AFTER WEARING RIGID GAS PERMEABLE SCLERAL LENSES (GROUP I; N = 30) AND INTRASTROMAL MYORING IMPLANTATION INTO THE PENETRATING CORNEAL GRAFT USING A FEMTOSECOND LASER (GROUP II; *n* = 30), M ± SD

Groups	Parameters	Before the selection of the rigid gas permeable scleral lenses (surgery)	The next day		In 6 months		In 12 months	
		M ± SD	M ± SD	*p*	M ± SD	*p*	M ± SD	*p*
Group I Rigid gas permeable scleral lenses (*n* = 30)	UCVA	0.08 ± 0.05	0.81 ± 0.12	0.0047	0.81 ± 0.12	0.0047	0.81 ± 0.12	0.0047
	CVA	0.19 ± 0.11	0.81 ± 0.14	0.0058	0.81 ± 0.14	0.0058	0.81 ± 0.14	0.0058
	$K_{av.}$, D	48.7 ± 3.9	42.6 ± 2.9	0.0066	42.6 ± 2.9	0.0066	42.6 ± 2.9	0.0066
	SRI	1.3 ± 0.3	0.4 ± 0.1	0.0096	0.4 ± 0.1	0.0096	0.4 ± 0.1	0.0096
	SAI	2.2 ± 0.9	0.5 ± 0.1	0.0083	0.5 ± 0.1	0.0083	0.5 ± 0.1	0.0083
	SCR, D	–6.25 ± 2.35	–0.15 ± 0.08	0.0061	–0.15 ± 0.08	0.0061	–0.15 ± 0.08	0.0061
	CCR, D	–5.15 ± 1.54	–0.5 ± 0.22	0.0055	–0.5 ± 0.22	0.0055	–0.5 ± 0.22	0.0055
	ECD, cells/mm^2	2106 ± 320	2112 ± 325	0.1232	2076 ± 311	0.0156	2047 ± 295	0.0146
Group II MyoRing implantation using FSL (*n* = 30)	UCVA	0.09 ± 0.05	0.26 ± 0.04	0.0352	0.44 ± 0.04	0.0152	0.53 ± 0.04	0.0152
	CVA	0.16 ± 0.10	0.48 ± 0.06	0.0362	0.59 ± 0.06	0.0163	0.71 ± 0.07	0.0145
	$K_{av.}$, D	49.26 ± 4.11	40.96 ± 2.21	0.0169	41.93 ± 3.11	0.0135	42.96 ± 3.44	0.0152
	SRI	1.49 ± 0.43	1.33 ± 0.28	0.0288	1.29 ± 0.22	0.0233	1.22 ± 0.21	0.0235
	SAI	2.69 ± 1.10	1.79 ± 0.54	0.0278	1.71 ± 0.51	0.0246	1.62 ± 0.48	0.0226
	SCR, D	–7.05 ± 2.51	1.25 ± 0.45	0.0354	0.28 ± 0.28	0.0216	–0.75 ± 0.35	0.0215
	CCR, D	–5.85 ± 1.71	–2.85 ± 1.12	0.0235	–2.36 ± 1.01	0.0274	–1.75 ± 0.85	0.0269
	ECD, cells/mm^2	2054 ± 231	2050 ± 226	0.3111	2021 ± 215	0.0215	1992 ± 203	0.0269

Note. $K_{av.}$ – the Average value of Keratometry; SRI – Surface Regularity Index; SAI – Surface Asymmetry Index; SCR – Spherical Component of Refraction; CCR – Cylindrical Component of Refraction

(CCR) – by 4.65 ± 1.32 D. According to keratotopography data the average value of keratometry ($K_{av.}$) in rigid gas permeable scleral lenses decreased by 3.6 ± 1.0 D, surface regularity index (SRI) – by 0.9 ± 0.2, surface asymmetry index (SAI) – by 1.7 ± 0.8, the spherical component of refraction – by 6.10 ± 2.27 D, the cylindrical component of refraction – by 4.65 ± 1.32 D. The data obtained remained stable throughout the entire observation period and are consistent with the literature data [7-9]. After a year of wearing of rigid gas permeable scleral lenses, the coefficient of effectiveness (Cef) was 4.3 ± 0.8, the coefficient of safety (Cs) was 4.3 ± 0.9.

When analyzing corneal aberrations measured in patients the next day after selection rigid gas permeable scleral lenses on the Pentacam device, there was a decrease in photopic conditions (in the 3 mm optical zone) of the root mean square (RMS) deviation of the wavefront of total aberrations (RMS Total) by 3.6 times, RMS of higher order aberrations (RMS HOA) – by 3.4 times, RMS of spherical aberrations (RMS SA) – by 2 times, RMS Coma – by 2.8 times, RMS Trefoil – by 4.2 times compared with the data before the selection of the rigid gas permeable scleral lenses (Table 2).

In the 6 mm optical zone, RMS Total decreased by 2.4 times, RMS HOA – by 2.5 times, RMS SA – by 1.6 times, RMS Coma – by 4.3 times, RMS Trefoil – by 4.3 times (Table 3).

The obtained results remained stable during 12 months of observation and correlated with the literature data. In 2019 V.I. Tikhonova et al. conducted a clinical study on changes in corneal aberrations after the selection of rigid gas permeable scleral lenses in 8 eyes (8 patients) after PK according to the OPD-Scan II device (Nidek, Japan). The authors noted a decrease in rigid gas permeable scleral lenses of corneal higher orders aberrations in 3 mm (RMS HOA decreased by 3.8 times, RMS Trefoil – by 2.7 times, RMS SA – by 2.9 times) and 5 mm optical zones (RMS HOA decreased by 3.0 times, RMS Trefoil – by 3.4 times, RMS SA – by 3.1 times) [8]. In 2021 A. Penbe et al., against the background of wearing rigid gas permeable scleral lenses in 35 patients (38 eyes) after PK, noted a significant decrease in higher orders aberrations, especially coma, spherical aberrations, astigmatism, which is consistent with the results of this study [9].

Corneal graft minimum thickness (CGMT) at the center, measured using OCT Casia-2 before wearing the rigid gas permeable scleral lenses, averaged 532.3 ± 33.4 μm. After 12 months in all patients, after wearing a rigid gas per-

TABLE 2

CORNEAL ABERRATIONS IN PHOTOPIC CONDITIONS (IN 3.0 MM OPTICAL ZONE) ACCORDING TO PENTACAM DATA BEFORE AND AT DIFFERENT TERMS AFTER WEARING RIGID GAS PERMEABLE SCLERAL LENSES (GROUP I; *n* = 30) AND INTRASTROMAL MYORING IMPLANTATION INTO THE PENETRATING CORNEAL GRAFT USING A FEMTOSECOND LASER (GROUP II; *n* = 30), M ± SD

Groups	Type of aberration, RMS	3.0 mm optical area						
		Before the selection of the rigid gas permeable scleral lenses (surgery)	The next day		In 6 months		In 12 months	
		M ± SD	M ± SD	*p*	M ± SD	*p*	M ± SD	*p*
Group I Rigid gas permeable scleral lenses (*n* = 30)	Total, μm	1.96 ± 0.65	0.55 ± 0.22	0.0053	0.55 ± 0.22	0.0053	0.55 ± 0.22	0.0053
	HOA, μm	3.11 ± 1.22	0.91 ± 0.32	0.0058	0.91 ± 0.32	0.0058	0.91 ± 0.32	0.0058
	SA, μm	–0.040 ± 0.030	–0.020 ± 0.005	0.0088	–0.020 ± 0.005	0.0088	–0.020 ± 0.005	0.0088
	Coma, μm	0.11 ± 0.05	0.04 ± 0.02	0.0189	0.04 ± 0.02	0.0189	0.04 ± 0.02	0.0189
	Trefoil, μm	0.21 ± 0.13	0.05 ± 0.02	0.0352	0.05 ± 0.02	0.0352	0.05 ± 0.02	0.0352
Group II MyoRing implantation using FSL (*n* = 30)	Total, μm	2.26 ± 0.73	1.42 ± 0.22	0.0123	1.34 ± 0.22	0.0144	1.15 ± 0.22	0.0164
	HOA, μm	3.17 ± 1.33	2.24 ± 0.29	0.0254	2.11 ± 0.26	0.0246	1.91 ± 0.22	0.0296
	SA, μm	–0.07 ± 0.05	–0.06 ± 0.03	0.0368	–0.05 ± 0.02	0.0346	–0.04 ± 0.02	0.0396
	Coma, μm	0.16 ± 0.05	0.12 ± 0.04	0.0189	0.11 ± 0.03	0.0189	0.09 ± 0.03	0.0189
	Trefoil, μm	0.25 ± 0.12	0.16 ± 0.05	0.0362	0.14 ± 0.05	0.0363	0.12 ± 0.04	0.0396

TABLE 3

CORNEAL ABERRATIONS IN PHOTOPIC CONDITIONS (IN 6.0 MM OPTICAL ZONE) ACCORDING TO PENTACAM DATA BEFORE AND AT DIFFERENT TERMS AFTER WEARING RIGID GAS PERMEABLE SCLERAL LENSES (GROUP I; *n* = 30) AND INTRASTROMAL MYORING IMPLANTATION INTO THE PENETRATING CORNEAL GRAFT USING A FEMTOSECOND LASER (GROUP II; *n* = 30), M ± SD

Groups	Type of aberration, RMS	3.0 mm optical area						
		Before the selection of the rigid gas permeable scleral lenses (surgery)	The next day		In 6 months		In 12 months	
		M ± SD	M ± SD	*p*	M ± SD	*p*	M ± SD	*p*
Group I Rigid gas permeable scleral lenses (*n* = 30)	Total, μm	3.16 ± 1.23	1.33 ± 0.45	0.0063	1.33 ± 0.45	0.0063	1.33 ± 0.45	0.0063
	HOA, μm	4.12 ± 1.31	1.66 ± 0.39	0.0095	1.66 ± 0.39	0.0095	1.66 ± 0.39	0.0095
	SA, μm	0.41 ± 0.18	0.25 ± 0.04	0.0046	0.25 ± 0.04	0.0046	0.25 ± 0.04	0.0046
	Coma, μm	0.99 ± 0.51	0.23 ± 0.04	0.0236	0.23 ± 0.04	0.0236	0.23 ± 0.04	0.0236
	Trefoil, μm	0.95 ± 0.43	0.22 ± 0.03	0.0214	0.22 ± 0.03	0.0214	0.22 ± 0.03	0.0214
Group II MyoRing implantation using FSL (*n* = 30)	Total, μm	3.3 ± 1.23	2.03 ± 0.72	0.0163	1.93 ± 0.69	0.0165	1.8 ± 0.65	0.0142
	HOA, μm	4.82 ± 1.55	2.83 ± 0.86	0.0136	2.64 ± 0.79	0.0145	2.44 ± 0.62	0.0185
	SA, μm	0.33 ± 0.19	0.45 ± 0.21	0.0156	0.49 ± 0.22	0.0167	0.55 ± 0.24	0.0187
	Coma, μm	0.94 ± 0.42	0.95 ± 0.44	0.0288	0.99 ± 0.46	0.0294	1.03 ± 0.49	0.0268
	Trefoil, μm	0.99 ± 0.44	1.02 ± 0.45	0.0356	1.09 ± 0.48	0.0368	1.13 ± 0.49	0.0389

meable scleral lens for 8 hours it was removed and CGMT was measured at the center and was 543.5 ± 36.2 μm. Thus, the increase in CGMT in the center did not exceed 2.1 %, which indicated the absence of clinically significant corneal transplant edema. According to the literature, an increase in the minimum thickness of the cornea in the center of less than 4.0 % after removal of the rigid gas permeable scleral lens, as in this clinical study, corresponds to the physiological norm, since it does not exceed the increase in corneal thickness in physiological edema after sleep [10, 11]. In 2016 J. Vincent et al. in 15 patients (in 15 eyes), after removal of the rigid gas permeable scleral lenses, obtained an increase in the minimum thickness of the cornea in the center, not exceeding 2 % of the minimum thickness of the cornea before wearing the rigid gas permeable scleral lenses [12].

After 12 months of follow-up, according to Confoscan-4, there was a decrease in ECD by 2.8 %, which did not exceed the physiological loss.

In group II, after intrastromal implantation of the MyoRing into the corneal graft, intra- and postoperative complications were not observed in any of the patients. During biomicroscopy, a transparent penetrating corneal graft was visualized on the 1st day after surgery in all patients; the MyoRing was centered relative to the visual axis (Fig. 1).

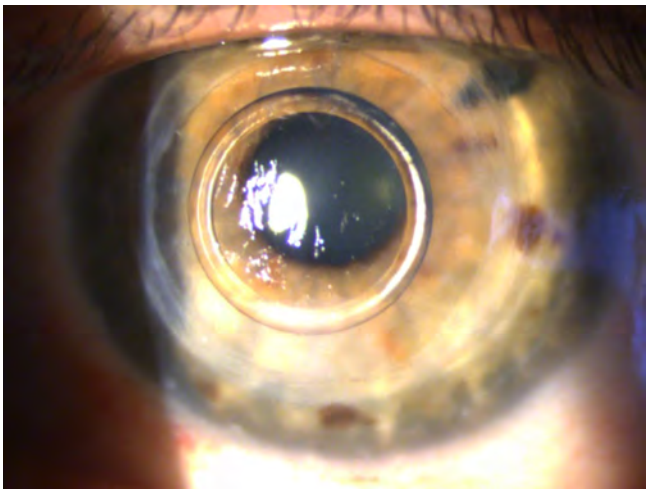

FIG. 1.

Biomicroscopy of a corneal graft on the first day after MyoRing implantation using femtosecond laser

On the first day after the surgery, a high refractive result was noted. UCVA increased by 0.17 ± 0.02, CVA – by 0.32 ± 0.04. SCR decreased by 8.3 ± 2, D, CCR – by 3.0 ± 0.5 D. 6 months after the surgery, UCVA increased by 0.18 ± 0.04, CVA – by 0.11 ± 0.04, SCR increased by 0.97 ± 0.17 D, CCR decreased by 0.49 ± 0.11 D. 12 months after the surgery, UCVA increased by 0.09 ± 0.04, CVA – by 0.12 ± 0.05, SCR increased by another 0.47 ± 0.07 D, CCR decreased by 0.61 ± 0.16 D. 1 year after the surgery, the Cef was 3.3 ± 0.6, Cs – 4.4 ± 0.6.

When analyzing the keratotopographic parameters obtained on the first day after surgery, there was a decrease in the values of $K_{av.}$ by 8.3 ± 1.9 D, SRI – by 0.16 ± 0.05, SAI – by 0.9 ± 0.2. 6 months after the surgery, the $K_{av.}$ increased by 0.97 ± 0.31 D, SRI decreased by 0.04 ± 0.02, SAI – by 0.08 ± 0.03. 12 months after the surgery, the $K_{av.}$ increased by 1.03 ± 0.33 D, SRI decreased by 0.07 ± 0.02, SAI – by 0.09 ± 0.03.

After 12 months of follow-up, patients in both groups showed a statistically significant difference in the values of UCVA, CVA, SRI, SAI, CCR ($p < 0.05$); there was no difference in $K_{av.}$ and SCR ($p > 0.05$). In group I, there was a greater increase in UCVA by an average of 3 lines, CVA – by 2 lines, a decrease in SRI by 3.3 times, SAI – by 1.7 times, CCR – by 0.55 ± 0.12 D compared with group II.

When analyzing corneal aberrations measured in patients the day after surgery under photopic conditions, RMS Total decreased 1.6 times, RMS HOA – 1.4 times, RMS SA – 1.2 times, RMS Coma – 1.3 times, RMS Trefoil – 1.6 times. 6 months after the operation, RMS Total decreased by 0.08 ± 0.04 μm, RMS HOA – by 0.13 ± 0.04 μm, RMS SA – by 0.01 ± 0.01 μm, RMS Coma – by 0.01 ± 0.01 μm, RMS Trefoil – by 0.02 ± 0.01 μm. 12 months after the operation, RMS Total decreased by 0.19 ± 0.04 μm, RMS HOA – by 0.2 ± 0.04 μm, RMS SA – by 0.01 ± 0.01 μm, RMS Coma – by 0.02 ± 0.01 μm, RMS Trefoil – by 0.02 ± 0.01 μm.

After 12 months of follow-up, patients in both groups showed a statistically significant difference in corneal aberrations in photopic conditions according to RMS Total, RMS HOA, RMS Trefoil ($p < 0.05$); difference in RMS SA, RMS Coms was absent ($p > 0.05$). In group I, compared with group II, there was a greater decrease in RMS Total by an average of 0.30 ± 0.08 μm, RMS HOA – by 1.01 ± 0.24 μm, RMS Trefoil – 0.03 ± 0.02 μm.

In the 6 mm optical zone, RMS Total decreased by 1.37 ± 0.41 μm, RMS HOA – by 2.18 ± 0.59 μm, RMS SA – by 1.6 times, RMS Coma – 4.3 times, RMS Trefoil – 1.6 times. The results obtained remained stable during 12 months of follow-up.

After 12 months of study under mesopic conditions, a statistically significant difference was revealed in all studied corneal aberrations in both groups. In group I, compared with group II, there was a greater decrease in RMS Total by an average of 0.33 ± 0.09 μm, RMS HOA by 0.08 ± 0.03 μm, RMS SA by 0.39 ± 0.11 μm, RMS Coma – by 0.27 ± 0.05 μm, RMS Trefoil – by 0.4 ± 0.1 μm.

The correct position of the MyoRing was confirmed by the corneal OCT data (Fig. 2).

The next day after surgery, the average value of CGMT in the center according to the OCT of the cornea increased by 19 ± 4 μm, which is associated with edema of the corneal graft due to irrigation of the intrastromal pocket. The CGMT at the center reached preoperative values in group II patients within a month after surgery and did not change further.

The loss of ECD one year after implantation of the MyoRing into a penetrating corneal graft using FSL was 3.0 %, which did not exceed the physiological loss. There was no statistically significant difference in the decrease in ECD between the two groups after 12 months of follow-up ($p < 0.05$).

Thus, a greater increase in visual acuity (UCVA and CVA) in the rigid gas permeable scleral lenses compared with intrastromal implantation of the MyoRing into the corneal graft is confirmed by a more pronounced decrease in CCR, SRI and SAI, total corneal aberrations and highest order corneal aberrations.

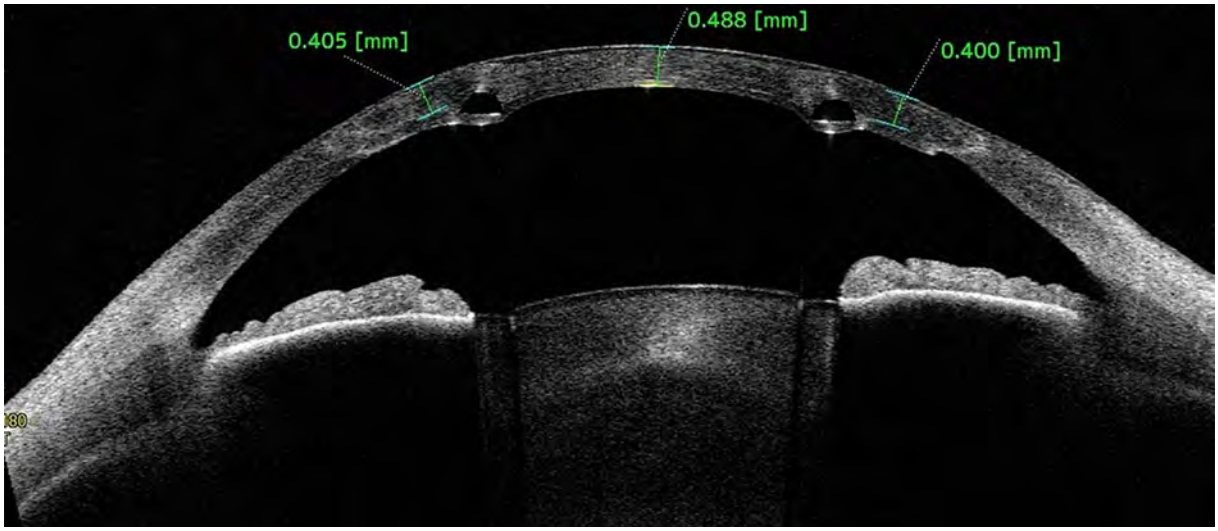

FIG. 2.

Optical coherence tomography of the corneal graft after MyoRing implantation into the penetrating corneal graft using femtosecond laser: the profile of the MyoRing is visualized being located at a depth of 80 % of the minimum thickness of the penetrating corneal graft

CONCLUSION

A comparative analysis of the effect of rigid gas-permeable scleral lenses and the MyoRing implantation method on clinical and functional parameters in patients with irregular postkeratoplastic astigmatism with a follow–up period of 12 months showed: a greater increase in UCVA – by an average of 3 lines, CVA – by 2 lines, a greater decrease in SRI – by 3.3 times, SAI – by 1.7 times, CCR – by 0.55 ± 0.12 D, corneal aberrations in photopic conditions (RMS Total – by 0.30 ± 0.08 μm, RMS HOA – by 1.01 ± 0.24 μm) and mesopic conditions (RMS Total – by 0.33 ± 0.09 μm, RMS HOA – by 0.08 ± 0.03 μm) in patients wearing rigid gas permeable scleral lenses compared to patients implanted with MyoRing in the corneal graft.

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Conflict of interest

The authors of this article declare the absence of a conflict of interest.

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PREVENTIVE MEDICINE

INTERSYSTEM INTERACTIONS OF PHYSIOLOGICAL SYSTEM INDICATORS IN STUDENTS WITH DIFFERENT TYPES OF AUTONOMIC REGULATION

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ABSTRACT

Studying not only individual students indicators of students, but complex intersystem interactions that reflect the specifics of adaptive capabilities is of scientific interest.
The aim of the study. *To reveal the features of intersystem interactions of indicators of the blood vessels functional state, morphological and neurodynamic characteristics of students with different types of autonomic regulation, living in the Khanty-Mansiysk Autonomous Okrug – Yugra.*
Methods. *429 first–fourth-year students (348 girls, 80 boys) of the Surgut State Pedagogical University were examined. The initial type of autonomic regulation was determined by the average duration of electrocardiogram RR intervals. Anthropometry parameters (body length and weight) and body composition were assessed using Tanita BC-601 device (Tanita, Japan), vascular wall elasticity – using AngioScan-01 (AngioScan-Electronics Ltd, Russia), neurodynamic indicators – using NS-PsychoTest device (Neurosoft, Russia). Statistical analysis was carried out using Statistica 7.0 software (StatSoft Inc., USA). We used the nonparametric Mann – Whitney U-test to evaluate differences and the nonparametric Pearson R-test to analyze correlations.*
Results. *An increase in the stiffness of the vascular wall of large and small arteries in girls is accompanied by an increase in parasympathetic activity. In young men, an increase in the stiffness of large arteries raises with an increase in sympathoadrenal activity, while a compensatory increase in the stiffness of small muscular arteries is noted with an increase in parasympathetic activity. In persons with the sympathicotonic type of autonomic regulation, an excess content of fat mass, an increased frequency of visceral obesity, and a deficiency in water content were registered. With the predominance of the parasympathetic component contribution to the regulation of cardiac rhythm, an increase in the processes of inhibition in the central nervous system was noted. In young men, reduced sensorimotor reactions are consistent with sympathetic activation in the regulation of heart rate. Correlation analysis made it possible to establish some features in the interaction of the vascular, morphological and central nervous systems.*
Conclusion. *Differences in indicators of functional systems (physical development, blood flow, neurodynamic characteristics) in the examined groups of students are shown, taking into account gender and autonomic regulation type. Features of intersystem interactions of indicators of physiological systems of students' bodies with different types of autonomic regulation were revealed.*

Key words: *type of neurovegetative regulation, functional state of the vascular system, anthropometry, body composition, neurodynamics, intersystem interactions, students, Northern region*

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ОСОБЕННОСТИ МЕЖСИСТЕМНЫХ ВЗАИМОДЕЙСТВИЙ ПОКАЗАТЕЛЕЙ ФИЗИОЛОГИЧЕСКИХ СИСТЕМ ОРГАНИЗМА СТУДЕНТОВ С РАЗНЫМ ТИПОМ ВЕГЕТАТИВНОЙ РЕГУЛЯЦИИ

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РЕЗЮМЕ

Научный интерес представляет изучение не отдельных показателей организма обучающихся, а комплексных межсистемных взаимодействий, отражающих специфику адаптационных возможностей.

Цель исследования. *Выявить особенности межсистемных взаимодействий показателей функционального состояния сосудов, морфологических и нейродинамических характеристик студентов с разным типом вегетативной регуляции, проживающих в Ханты-Мансийском автономном округе – Югре.*

Методы. *Обследовано 429 студентов 1–4-х курсов (девушки – 348, юноши – 80) БУ «Сургутский государственный педагогический университет». Исходный тип вегетативной регуляции определяли по средней продолжительности RR-интервалов электрокардиограммы. Оценивали показатели антропометрии (длину и массу тела) и компонентного состава тела (прибор Tanita BC-601 (Tanita, Япония)), эластичность сосудистой стенки (прибор «АнгиоСкан-01» (ООО «АнгиоСкан-Электроникс», Россия)); нейродинамические показатели (прибор «НС-ПсихоТест» (Нейрософт, Россия)). Статистический анализ проведён в среде Statistica 7.0 (StatSoft Inc., США). Использовали непараметрический U-критерий Манна – Уитни для оценки различий и непараметрический R-критерий Пирсона для анализа корреляционных связей.*

Результаты. *Увеличение жёсткости сосудистой стенки крупных и мелких артерий у девушек сопровождается ростом парасимпатической активности; у юношей увеличение жёсткости крупных артерий возрастает с увеличением симпатоадреналовой активности, при этом отмечается компенсаторное увеличение жёсткости мелких мышечных артерий с ростом парасимпатической активности. У лиц симпатикотонического типа вегетативной регуляции отмечены избыточное содержание жировой массы и повышенная частота висцерального ожирения, дефицит содержания воды. С преобладанием вклада парасимпатического компонента в регуляцию кардиоритма отмечено увеличение процессов торможения в центральной нервной системе. У юношей сниженные сенсомоторные реакции согласованы с симпатической активацией в регуляции кардиоритма. Корреляционный анализ позволил установить некоторые особенности во взаимодействии сосудистой, морфологической и центральной нервной системы.*

Заключение. *Показаны отличия по показателям функциональных систем (физического развития, сосудистого русла, нейродинамических характеристик) в обследованных группах студентов с учётом пола и типа вегетативной регуляции. Выявлены особенности межсистемных взаимодействий показателей физиологических систем организма студентов с разным типом вегетативной регуляции.*

Ключевые слова: *тип нейровегетативной регуляции, функциональное состояние сосудистой системы, антропометрия, компонентный состав тела, нейродинамика, межсистемные взаимодействия, студенты, Северный регион*

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OBJECTIVES

Modern physiological research is aimed not only at analyzing individual average values of parameters of functional systems, but also at an integrative assessment of the functioning of the body. In this regard, along with the individual components of the systems, it seems relevant to study the interrelationships between them [1]. From our point of view, it is interesting to study the relationship between the functioning of the cardiovascular system and other body systems. Many modern authors single out this system as the most sensitive to unfavorable climatic and ecological factors in the northern territories, including the Khanty-Mansiysk Autonomous Okrug – Yugra [2–4].

Students as a special cohort exposed to a complex of specific social and environmental factors is of special scientific interest [5]. It is known that in modern conditions, studying at a university is associated with a high level of mental and physical stress, which often leads to a strain on the adaptive capabilities of the body of students.

According to E.M. Kazin et al. [6], if a specific functional system does not cope with its task, the functional performance support system is activated, while the costs of functional reserves increase. Having different adaptive potential, students' bodies react differently to external impacts, and as a result, the "cost" of adaptation is determined by the state of the body's regulatory systems, primarily the central nervous system (CNS) and autonomic regulation. The variability of heart rate indicators acts as a universal indicator of the functional state of the multilevel links of the regulatory systems of the human body, they are the first to activate and adapt to environmental factors.

An increasing number of authors are inclined to the need to take into account the type of autonomic regulation in the analysis and interpretation of heart rate variability (HRV) indicators [4, 7, 8]. The establishment of the type of autonomic regulation is extremely important in the individual assessment of HRV parameters and is especially important when choosing the type and intensity of exercise, identifying the risks of developing pathology of the cardiovascular system.

It is known about the influence of the type of autonomic regulation on the functioning of individual systems [9], including in extreme conditions of the Northern territories [10], however, the features of intersystem interactions of the functional state of blood vessels, morphological and neurodynamic parameters of students with different types of autonomic regulation have not been sufficiently studied [11]. Recent publications [3, 4, 9–11] indicate the influence of many factors (the level and duration of stress influences, the season of the year, gender and age, type of professional and educational activities, etc.) on the nature and strength of intersystem interactions. In this regard, we believe it is relevant to identify the features of intersystem interactions in students living in the conditions of the northern region.

THE AIM OF THE STUDY

To reveal the features of intersystem interactions of functional state of arterial vessels, morphological and neurodynamic characteristics of students with different types of autonomic regulation, living in the Khanty-Mansiysk Autonomous Okrug – Yugra.

MATERIALS AND METHODS

The examination was carried out on the basis of the Scientific Laboratory "Biological Foundations of Educational Safety", Surgut State Pedagogical University. A total of 429 first–fourth-year students aged 17–21 years were examined. All the examined students were representatives of non-indigenous nationalities. They were born and permanently (or more than 10 years) reside on the territory of the Khanty-Mansiysk Autonomous Okrug – Yugra (a territory equalled to the conditions of the Far North).

The total sample is differentiated by gender and type of autonomic regulation: girls (n = 348) – sympathotonics (n = 107), normotonics (n = 210) and vagotonics (n = 31); boys (n = 80) – sympathotonics (n = 8), normotonics (n = 49), vagotonics (n = 23). The study included only those girls who were in the follicular phase of the menstrual cycle. The exclusion criteria were acute infectious diseases or exacerbation of chronic pathology.

The initial type of autonomic regulation was determined by the electrocardiogram (ECG) indicator – the average duration of RR intervals (RRNN). ECG recording was performed under standardized conditions in a supine position (5 min), with calm breathing, in the second standard lead. HRV recordings different from sinus rhythm were excluded from the analysis. The normotonic type corresponded to the range of RRNN values of 750–980 ms (60–80 bpm), RRNN values above this range characterize the parasympathetic type, below this range – sympathicotonic type of autonomic regulation.

The functional state of the vascular system was assessed by the level of blood pressure (BP) measured using a standard technique using an electronic tonometer from A&D Medical (Japan), model – UA-777, and the following parameters were recorded: SBP – systolic blood pressure (mmHg); DBP – diastolic blood pressure (mmHg). To assess the elasticity of the arterial vascular wall, the AngioScan-01 diagnostic complex (AngioScan-Electronics LLC, Russia; certificate of conformity No. РОСС RU.ИМ25.Д06096) was used: according to the photoplesiogram, the stiffness index (SI, m/s) was analyzed, reflecting the average velocity of pulse waves propagation through the aorta and its branches; reflection index (RI, standard units), characterizing the tone of small muscular arteries; augmentation index, standardized for a fixed pulse rate of 75 bpm (AIp75, %); pressure gain indicator, depending on the total peripheral vascular resistance and elastic resistance of the vascular wall; aging index (AGI, standard units), evaluating the shapes of pulse waves of volume; ejection duration

in the cardiac cycle (ED, ms); stress index (standard units), characterizing heart rate variability.

Absolute anthropometric parameters – length (cm) and body weight (BW; kg) – were measured using standardized methods, using a medical stadiometer and scales. Next, the body mass index (BMI; kg/m^2) was calculated. The assessment of body composition: body fat mass (FM; %), visceral fat (VF; standard units) and body water percentage (BWP; %), was carried out using Tanita BC-601, body composition analyzer scales (Tanita, Japan; certificate of conformity No. POCC JP.ME77.B08130) in modification for screening purposes.

The study of neurodynamic parameters was carried out using NS-Psychotest hardware and software complex (NeuroSoft, Russia; certificate of conformity No. POCC RU. *И*М18.Д00567) according to the following methods: "Simple visual-motor reaction" (SVMR; ms), reflecting the speed of sensorimotor response to a similar stimulus and the level of CNS activation; "Complex visual-motor reaction (choice reaction)" (CVMR, ms), characterizing the mobility of nervous processes and the level of differentiating inhibition; "Tap test", diagnosing the strength of nerve processes. Along with this, the values of the standard deviation (SD) of the SVMR and CVMR (SVMR SD and CVMR SD) reflecting the stability of the sensorimotor response were recorded.

The statistical analysis of the results was carried out using the Statistica 7.0 application software package (StatSoft Inc., USA). A descriptive statistical analysis of the data was performed. The assessment of the normality of the distribution of the studied indicators was carried out using the Shapiro – Wilk test. Some quantitative features did not correspond to the law of normal distribution, so these results are presented in the form of median (Me) and interquartile range – the 25th and 75th percentiles (Q_{25}; Q_{75}). The differences were analyzed using the nonparametric Mann – Whitney U-test by pairwise comparison of the studied groups. Nonparametric Spearman's test was used to evaluate correlations. The critical level of statistical significance was $p < 0.017$ for all calculations.

The study was carried out during the intersessional period.

Ethical review

The fundamental principle of the study was the absence of risk to the health of students, compliance with humane and ethical standards that meet the requirements of the World Medical Association Declaration of Helsinki (ed. 2013). Written informed consent was obtained from each participant of the study before performing the procedures. The study protocol was approved by the Bioethics Committee of the Surgut State Pedagogical University (Protocol No. 31 dated September 7, 2022).

RESULTS

The average values of indicators characterizing the state of the cardiovascular system (AD, SI, AIp75, RI, AGI) indicate the optimal functional state of the examined students of both genders. The results obtained, taking into account gender and type of autonomic regulation, are presented in Table 1.

The blood pressure indicators of young men and women corresponded to the age norm. The value of SI (m/s) in young men exceeded the values of this indicator in young women, which indicates a more pronounced decrease in the elasticity of large resistive arterial vessels in male students. The value of AIp75 (%), characterizing the elasticity of large-caliber arterial vessels, showed a different connection with the type of autonomic regulation: in young women, it was the largest in the group of sympathicotonics, the smallest in the group of vagotonics, and in young men, on the contrary. Indicators of elasticity of small arteries (RI, standard units) in young women showed a statistically significant increase from the sympathicotonic group to the vagotonic group; in young men, the same trend was found, but not statistically confirmed. In young men, the average augmentation index decreased from the group of sympathotonics to vagotonics, which have the most optimal average index of elasticity of large arteries. The values of the aging index (AGI, standard units) are due to changes in blood pressure and heart rate (HR). The established values of this indicator fell within the range of reference values (–0.93 ± 0.25) for all study groups. Statistically significant differences in the values of AIp75, RI and AGI in young men have not been established. The average stress index in the groups of young men and women with pronounced sympathicotonia was statistically significantly higher than this indicator in the groups of normotonics and vagotonics (2 and 4 times, respectively), which indicates a pronounced stress-induced fatigue of the body of these students.

The features of neurovegetative regulation determine the nature of adaptive reactions of the body, which is reflected, among other things, on metabolic parameters. The indicators of morphological development of students with different types of autonomic regulation are presented in Table 2.

It was found that in young men and women with pronounced sympathicotonia body weight (kg) and fat mass (%) indicators were higher than the values of similar indicators in other groups. Statistically significant intersex differences between the normotonic groups were found by BMI (kg/m^2). An increase in BWP (%) was noted in young men and women of the examination groups from sympathotonics to vagotonics.

Generalized characteristics of neurodynamic characteristics of students with different types of autonomic regulation are presented in Table 3.

A decrease in sensorimotor activity was found in young women with an increase in vagal tonic activity, which is reflected in an increase in the average reaction time. The results obtained characterize a decrease in the speed of processing sensory information and the formation of a motor response to a stimulus, lower mobility and CNS excitability of vagotonic girls compared with sympathiconics (on average by 4–7 %). Among the examined young men, the optimal type of sensorimo-

TABLE 1

INDICATORS OF THE ARTERIAL WALLS IN STUDENTS WITH DIFFERENT TYPES OF AUTONOMIC REGULATION, ME (Q_{25}; Q_{75})

Indicators	Gender	Type of autonomic regulation			*P*
		sympathicotonia	normotonia	vagotonia	
SBP, mmHg	♀	111.0 (105.0; 121.0)	108.0 (101.0; 115.0)	110.0 (103.0; 117.0)	$p_1 = 0.003$
	♂	117.0 (110.0; 124.0)	118.0 (110.0; 124.0)	119.0 (108.0; 125.0)	–
*p**		–	< 0.001	0.01	
DBP, mmHg	♀	72.0 (67.0; 79.0)	70.0 (64.0; 75.0)	65.0 (60.0; 70.0)	$p_1 < 0.001$ $p_2 = 0.003$ $p_3 < 0.001$
	♂	72.5 (66.0; 76.0)	75.0 (70.0; 80.0)	71.0 (66.0; 74.0)	$p_2 < 0.001$
*p**		–	< 0.001	0.006	
SI, m/s	♀	7.2 (6.8; 7.7)	7.0 (6.7; 7.4)	6.9 (6.5; 7.7)	–
	♂	7.3 (7.0; 8.0)	7.5 (6.8; 7.8)	7.5 (7.0; 7.7)	–
*p**		–	< 0.001	–	
AIp75, %	♀	–12.0 (–17.3; –3.4)	–11.4 (–18.8; –5.3)	–3.7 (–11.7; –1.0)	$p_1 < 0.001$ $p_2 < 0.001$ $p_3 < 0.001$
	♂	–17.0 (–22.3; –11.7)	–18.6 (–23.2; –6.1)	–25.7 (–35.9; –15.7)	–
*p**		–	0.009	< 0.001	
AGI, standard units	♀	–0.9 (–1.1; –0.8)	–0.9 (–1.0; –0.8)	–0.8 (–1.0; –0.6)	–
	♂	–1.1 (–1.2; –1.0)	–0.8 (–1.1; –0.9)	–1.0 (–1.2; –0.9)	–
*p**		–	–	0.003	
RI, standard units	♀	19.3 (14.4; 24.8)	26.2 (19.8; 32.1)	34.1 (24.1; 41.3)	$p_1 < 0.001$ $p_2 = 0.003$ $p_3 < 0.001$
	♂	16.1 (13.7; 21.8)	20.2 (14.2; 23.4)	23.5 (20.5; 30.3)	–
*p**		–	< 0.001	0.01	
ED, ms	♀	263.0 (250.0; 275.0)	274.0 (262.0; 288.0)	287.0 (268.0; 300.0)	$p_1 < 0.001$ $p_3 < 0.001$
	♂	270.0 (243.0; 282.5)	264.0 (251.0; 272.0)	276.0 (268.0; 288.0)	$p_2 < 0.001$
*p**		–	< 0.001	–	
Stress index, standard units	♀	206.0 (132.0; 337.0)	96.0 (63.0; 180.0)	46.0 (31.0; 75.0)	$p_1 < 0.001$ $p_2 < 0.001$ $p_3 < 0.001$
	♂	235.0 (179.5; 362.5)	125.0 (83.0; 182.0)	49.0 (30.0; 87.0)	$p_2 < 0.001$
*p**		-	–	–	

Note. ♀ – women; ♂ – men; statistically significant differences between groups: p_1 – sympathicotonics and normotonics; p_2 – normotonics and vagotonics; p_3 – sympathicotonics and vagotonics; *p** – statistically significant differences between genders.

TABLE 2

INDICATORS OF MORPHOLOGICAL DEVELOPMENT OF STUDENTS WITH DIFFERENT TYPES OF AUTONOMIC REGULATION, ME (Q_{25}; Q_{75})

Indicators	Gender	Type of autonomic regulation			*p*
		sympathicotonia	normotonia	vagotonia	
BMI, kg/m^2	♀	21.9 (19.2; 26.1)	21.8 (20.0; 24.3)	20.2 (19.3; 22.6)	–
	♂	22.7 (21.2; 26.3)	23.1 (20.9; 26.3)	23.0 (21.5; 24.7)	–
*p**		–	0.008	–	
BW, kg	♀	61.0 (50.9; 69.4)	59.5 (53.0; 66.4)	56.2 (52.0; 65.0)	–
	♂	75.2 (60.7; 82.8)	73.0 (65.9; 80.5)	73.4 (63.6; 77.0)	–
*p**		0.01	< 0.001	< 0.001	
FM, %	♀	28.1 (21.1; 36.2)	27.8 (22.7; 33.1)	26.2 (21.4; 29.8)	–
	♂	26.1 (17.3; 35.0)	16.5 (11.8; 21.2)	16.3 (13.2; 19.2)	–
*p**		–	< 0.001	< 0.001	
BWP, %	♀	53.3 (47.7; 58.4)	53.6 (49.8; 57.1)	54.9 (52.3; 58.0)	–
	♂	54.2* (48.0; 61.1)	60.9 (56.5; 66.0)	63.4 (59.1; 66.8)	$p_3 = 0.01$
*p**		–	< 0.001	< 0.001	
VF, standard units	♀	1.0 (1.0; 4.0)	1.0 (1.0; 3.0)	1.0 (1.0; 2.0)	–
	♂	3.5 (1.5; 5.5)	1.0 (1.0; 4.0)	1.0 (1.0; 2.0)	–
*p**		–	–	–	

Note. ♀ – women; ♂ – men; statistically significant differences between groups: p_1 – sympathicotonics and normotonics; p_2 – normotonics and vagotonics; p_3 – sympathicotonics and vagotonics; *p** – statistically significant differences between genders.

tor response was revealed in normotonics, while the indicators of sympathicotonics and vagotonics had similar severity. It is important to note that the variability indicators (SVMR SD and CVMR SD), reflecting the homeostatic level of sensorimotor response, corresponded to the range of normative values in all study groups. In the groups of examined young women, we did not find a statistically significant effect of the type of autonomic regulation on the performance of the motor component of activity (according to the tap test). In young men, optimal indicators were noted in normotonics, while sympathicotonics and vagotonics found similar manifestations of sensorimotor performance. Statistically significant differences between genders in most of the analyzed characteristics were revealed in the group of normotonics. In general, the most adequate indicators of sensorimotor response of normotonic young men can be noted in comparison with other studied groups.

In physiology, the principle is increasingly being introduced, the meaning of which is that the optimal result of the system's performance under the same type of conditions is achieved by a variety of states that are characterized by certain quantitative combinations of parameters. One of the ways to characterize physiological systems can be a correlation analysis to identify the structure of the connection between individual indicators in various adaptive states [12]. The results of our correlation intersystem analysis of groups of students with different types of vegetative response indicate pronounced differences between them. The correlation matrix uniting students of both genders is presented in Table 4, it reflects statistically significant connections between the indicators.

According to the classical concepts of systemic physiology of I.I. Schmalthausen, R. Settler, generalized in the work of A.A. Pozdnyakova [13], a living organism is considered as a complex hierarchically subordinated dynamic struc-

TABLE 3

NEURODYNAMIC INDICATORS OF STUDENTS WITH DIFFERENT TYPES OF AUTONOMIC REGULATION, ME (Q_{25}; Q_{75})

Indicators	Gender	Type of autonomic regulation			*p*
		sympathicotonia	normotonia	vagotonia	
SVMR, ms	♀	230.3 (209.4; 257.0)	236.8 (221.6; 264.5)	246.0 (214.3; 267.7)	$p_1 = 0.01$
	♂	235.6 (209.4; 243.1)	221.6 (205.6; 238.1)	234.0 (204.3; 243.1)	–
*p**		–	< 0.001	0.004	
SVMR SD, ms	♀	56.7 (44.9; 70.2)	60.7 (48.2; 78.0)	56.6 (42.9; 75.9)	–
	♂	51.6 (39.2; 61.3)	58.4 (46.9; 72.9)	57.2 (38.0; 77.5)	–
*p**		–	–	–	
CVMR, ms	♀	347.6 (323.0; 388.6)	354.1 (327.4; 384.4)	360.0 (340.5; 389.7)	–
	♂	357.1 (349.5; 376.8)	328.1 (310.3; 357.8)	333.3 (325.0; 366.9)	–
*p**		–	< 0.001	–	
CVMR SD, ms	♀	86.8 (76.3; 113.5)	86.6 (72.4; 101.2)	85.4 (66.8; 119.8)	–
	♂	88.1 (68.1; 128.3)	78.2 (68.7; 92.1)	79.2 (70.4; 97.7)	–
*p**		–	0.003	–	
TT (average rate), standard units	♀	6.7 (6.1; 7.1)	6.7 (6.3; 7.1)	6.9 (6.3; 7.3)	–
	♂	6.9 (6.4; 7.2)	7.2 (6.7; 7.7)	6.8 (6.5; 8.0)	–
*p**		–	< 0.001	–	
TT (number of taps), standard units	♀	200.0 (182.0; 213.0)	199.0 (188.0; 213.0)	204.5 (188.0; 217.0)	–
	♂	204.5 (190.5; 215.0)	214.0 (200.0; 229.0)	204.0 (195.0; 238.0)	–
*p**		–	< 0.001	–	

Note. ♀ – women; ♂ – men; statistically significant differences between groups: p_1 – sympathicotonics and normotonics; p_2 – normotonics and vagotonics; p_3 – sympathicotonics and vagotonics; *p** – statistically significant differences between genders; TT – tap test.

ture in which the functioning of one system depends on the work of other body systems, the correlation between organs is considered as a network dynamic interaction. The absence of correlations in this context is considered as isolation of the system (organ) in a functional sense. Based on this position, we believe that the functional diversity of the network interactions of the system determines its greater dynamic stability in the implementation of the adaptive effect. Interpreting the obtained results of the correlation analysis, it can be noted that they were the most variable in normotonics: they had 19 intersystem connections of weak and moderate strength (at $p < 0.05$) between nine indicators characterizing the elasticity of the arterial vessel wall and five morphological indicators; 10 connections between indicators of neurodynamics and morphological features of the examined and three connections neurodynamics with an aging index indicator. In all groups, a direct relationship was established between blood pressure and height and weight index, body weight and visceral fat index. The indices of elasticity of the arterial vessel wall were generally inversely related to the parameters of morphological development. Indicators of neurodynamics, indicating an increase in the performance of sensorimotor response, found a direct relationship with body weight and an inverse relationship with the value of the fat mass component.

TABLE 4

CORRELATION MATRIX OF INTERSYSTEM INTERACTIONS BETWEEN THE STUDIED INDICATORS IN STUDENTS WITH DIFFERENT TYPES OF AUTONOMIC REGULATION

Indicators	BMI, kg/m²	BW, kg	FM, %	BWP, %	VF, standard units	SVMR, ms	SVMR SD, ms	CVMR, ms	CVMR SD, ms	TT (average rate), standard units	TT (number of taps), standard units
Sympathotonics (*n* = 126)											
SBP, mmHg	0.37	0.36	0.33	–0.35	0.36	0.22	–	–	–	–	–
DBP, mmHg	0.27	0.28	0.25	–0.30	0.29	0.22	0.19	–	–	–	–
AIp75, %	–0.22	–0.29	–0.19	0.15	–0.20	–	–	–	–	–	–
Normotonics (*n* = 259)											
SBP, mmHg	0.34	0.36	–	–	0.27	–	–	–	–	–	–
DBP, mmHg	0.25	0.23	–	–	0.20	–	–	–	–	–	–
SI, m/s	–	–	–0.15	0.15	–	–	–	–	–	–	–
AIp75, %	–0.14	–0.26	–	–	–0.16	–	–	–	–	–	–
AGI, standard units	–	–	–	–		–	–	–0.12	–	0.14	0.14
RI, standard units	–	–0.20	–	–		–	–	–	–	–	–
ED, ms	0.28	0.17	0.29	–0.26	0.23	–	–	–	–	–	–
Stress index, standard units	–0.16	–	–0.13	–	–	–	–	–	–	–	–
BW, kg	–	–	–	–	–	–	–	–0.15	–0.15	0.13	0.13
FM, %	–	–	–	–	–	0.23	–	–	–	–0.16	–0.16
BWP, %	–	–	–	–	–	–0.22	–	–	–	0.15	0.15
Vagotonics (*n* = 54)											
SBP, mmHg	0.31	0.41	–	–	–	–	–	–	–	0.32	0.32
DBP, mmHg	0.41	0.49	–	–	–	–	–	–	–	–	–
SI, m/s	–	–	–0.43	0.39	–	–	–	–	–	0.35	0.35
AIp75, %	–0.31	–0.55	0.49	–0.47	–	–	–	–	–	–	–
AGI, standard units	–	–	0.41	–0.39	–	–	–	–	–	–0.34	–0.34
RI, standard units	–	–0.28	0.34	–0.32	–	–	–	–	–	–	–
ED, ms	–	–	0.34	–0.30	–	–	–	–	–	–	–
BW, kg	–	–	–	–	–	–	–	–0.31	–	–	–

Sympathicotonics revealed the least intersystem connections, they had the unidirectionality of correlations of weak and moderate strength between blood pressure, augmentation index, morphological development indicators and SVMR indicators.

In the group of vagotonics, moderate strength connections with morphological development and elasticity of arterial vessels were noted. At the same time, it is possible to note a more pronounced connection between the elasticity of arterial vessels with the water and fat mass components of the body. Six of the seven identified connections of neurodynamic characteristics are related to vascular tone indicators.

DISCUSSION OF RESULTS

According to the study [14, 15], the values of elasticity of the vascular wall of the arteries act as prognostic indicators in the development of arterial hypertension, which is especially relevant in the conditions of the northern region [2, 3]. An increase in arterial stiffness leads to an increase in central blood pressure and an increase in afterload on the heart. It contributes to the restriction of coronary blood flow, which leads to a decrease in the contractility of the myocardium. Analyzing the indicators characterizing the functional state of the arterial vascular wall in students with different types of autonomic regulation, we assume the existence of several mechanisms to maintain an optimal blood pressure level. An increase in the stiffness of the vascular wall of large and small arteries, expressed in an increase in the augmentation index and the resistance index in groups of young women, is noted with an increase in parasympathetic activity; in young men, an increase in the stiffness of large arteries increases with an increase in sympathoadrenal activity, while there is a compensatory increase in the stiffness of small muscular arteries with an increase in parasympathetic activity.

From our point of view, in the group of young women with a high level of sympathetic activation of the central mechanisms of regulation of the cardiovascular system, the hyperkinetic type of blood circulation is insufficiently compensated by the elasticity of large arteries, which causes a decrease in the tone of small muscular arteries, despite increased sympathetic activity. In the group of young women with high parasympathetic activity, a decrease in the elastic properties of large arteries leads to an increase in afterload on the myocardium, which reduces peripheral blood flow, that requires an increase in peripheral arterial tone. The combined effect of these mechanisms further increases the afterload on the heart and excessive stimulation of the mechanisms of regulation of vascular tone can contribute to depletion of nitrogen monoamine production. This allows us to make an assumption about the more pronounced risks of cardiovascular disorders in young women with high parasympathetic activity.

In the group of young men with a high level of sympathetic activation of the central mechanisms of regulation of the cardiovascular system, a decrease in the elasticity of large arteries and an increase in afterload on the heart are partially compensated by a decrease in the tone of small peripheral arteries, despite the sympathetic activation of vascular tone regulation. From our point of view, this imbalance of regulation indicates an increased risk of cardiovascular disorders in this group of examined students. In the group of young men with high parasympathetic activity, the high elasticity of the vascular wall of arterial vessels does not provide adequate peripheral blood flow, which requires an increase in the tone of small arteries to maintain it. The resulting constant stimulation of nitrogen monoamine production can lead to depletion of the mechanisms of its formation.

The absence of statistically significant differences in the rate of pulse wave propagation (SI) characterizes the preservation of the elastic properties of the aorta, characteristic of young people. With increasing parasympathetic activity and decreasing average HR, ejection duration compensatory increases.

Thus, an analysis of the features of the regulatory mechanisms of the cardiovascular system revealed that in groups of young men and women with a high level of sympathetic activation of the central mechanisms of regulation of the cardiovascular system, the risks of disorders in this system are associated with dysregulation of vascular tone (decreased tone of small arteries against the background of increased activity of the sympathetic nervous system). The predominance of parasympathetic influence in the regulation of heart rhythm against the background of high elasticity of large arterial vessels and increased tone of small arterial vessels allows us to conclude that there are higher needs for capillary blood flow of organs and tissues. The noted imbalance in the regulation of blood circulation suggests that in groups of young men and women with high parasympathetic activity, the risks of impaired blood flow are associated with a possible depletion of the mechanisms of production of nitrogen monoamine as one of the leading factors in the development of arterial hypertension.

A decrease in heart rate variability and, consequently, an increase in stress index are associated with a high risk of cardiovascular pathology. The absence of people with pronounced manifestations of cardiac pathology in the examination cohort, moderately high stress index indicators in the groups of sympathotonics, probably indicate the presence of a genetically determined stress-induced response of the latter's body.

The results of the assessment of the anthropometric indicators of students obtained by us indicate the optimal physical development of the majority of the examined students. According to a study by the team of authors presented in [16], the body mass index currently is an objective tool for obesity screening. The average BMI values fit into the medial values of the reference norm range, which states the dominance of mesosomatotypes in the cohort of the study. Modern scientific studies [17] characterizing the average anthropometric indicators of students state that the height of young men

is 170–177 cm, young women – 160–165 cm; the average body weight of young men is 68–72 kg, young women – 55–57 kg. However, the results of the assessment of the physical development of students in the Ural region, presented in our previous work [18], allowed us to establish statistically higher BMI and body weight due to a higher content of body fat, visceral obesity and a statistically significantly lower body water percentage in students in Surgut compared with students in more southern territories. I.V. Averyanova [19] showed that among men born in the North, there is also a prevalence of persons with a hypersthenic type of constitution, overweight and signs of obesity. The results obtained are consistent with the global and all-Russian trend of a higher prevalence of overweight in a cohort with high social and economic status and income [20].

The revealed intersex features of the body composition of the examined students reflect the general population trend [21] of a higher fat content in the body of young women, which is consistent with the studies of a number of other authors. Studying age-related changes in BMI and fat mass component of both genders, C. Palomino-Devia et al. [22] state that with age, an increase in BMI in young women is combined with an increase in fat mass ($p < 0.05$); in young men – with a decrease in fat mass ($p < 0.05$) and an increase in the muscular component of the body.

The most optimal body composition was noted in the groups of vagotonics and normotonics. The average content of fat and water components of the body of students belonging to these groups corresponded to the normative values. According to our data, the predominance of sympathoadrenal activity in ensuring the regulation of the rhythm of cardiac activity of the body is characterized by an excessive content of fat mass and visceral obesity, water insufficiency in students, which is confirmed by other modern studies [23]. Physiologists note the need to take into account the characteristics of body composition of the examined in the prediction and prevention of various nosologies. In particular, overweight and obesity are the leading risk factors for the development of cardiovascular [24] and endocrine pathologies [25]. Excess body fat causes the formation of extensive inflammatory processes in the body, leading to cancer and the progression of many types of tumors [26], increases cancer mortality by up to 20 % [27]. It was found that with an increase in the fat component, both young men and women experience a decrease in the water level in the body [28]. Water plays a crucial role in ensuring the normal functioning and maintenance of homeostasis, and is also an indirect indicator of preload of the heart. The deficient content of the liquid component of blood plasma causes an increase in the load on the contractile function of the myocardium due to a violation of the rheological properties of blood.

Our results indicate that with the predominance of parasympathetic regulation of heart rate, inhibitory processes in the central nervous system increase, there is a decrease in activating effects, expressed in average indicators of a simple sensorimotor reaction and in conditions of sensory differentiation of the visual stimulus. This pattern is most clearly seen in groups of female students. In the group of sympathicotonic male students, the variability of the average values of SVMR and CVMR reflects the least favorable level of performance in conditions of both simple and complex sensory load, which reflects the reduced activation of the central nervous system and attention switching of the latter compared with young men of other types of autonomic regulation. The indicators of variability of the average indicators of SVMR and CVMR (SD) reflect the well-established relatively stable manifestations of sensorimotor reactions of students of different sexes and with different types of autonomic regulation.

Relatively high neuromuscular performance in terms of the tap test in young women is characteristic of vagotonics, in young men – normotonics. Large values of the upper quartile of the tap test indicators in normotonic young women and vagotonic young men allow us to note their relatively better performance (endurance) compared to sympathicotonics of both genders. Intersex differences are clearly manifested in the results of neurodynamic testing in normotonics. Young men are characterized by a more effective sensorimotor response and a more advanced mechanism of differentiation inhibition of conditioned reflex activity, which is consistent with the literature data [29].

The results of a comprehensive study of intersystem connections of blood flow indicators, morphotype and neurodynamics obtained using correlation analysis revealed statistically significant weak and moderate strength connections between the studied parameters. Direct and inverse connections, along with the elements of the system, make up its structure; their analysis expands the understanding of the mechanisms of subordination, redistribution, reactivity between the individual components of the links of homeostasis. As R.M. Bayevsky notes [30] that almost all functional systems of the body participate in adaptation, one of the leading roles in it belongs to the cardiovascular system, which ensures the vital activity of the body at an optimal homeostatic level and is the most sensitive indicator of the productivity of adaptive reactions. In this regard, there is a special group of sympathicotonic students, in which the number and severity of correlations are less variable, which indicates greater stiffness (compared with groups of vagotonics and normotonics) of the regulation mechanisms in achieving a positive adaptation result.

CONCLUSION

In the majority of the examined students of the pedagogical university of the Northern region, the indicators of the cardiovascular system, body composition and neurodynamic processes correspond to the reference values.

The intersex differences in the mechanisms of maintaining optimal blood pressure, body composition and neurodynamic performance in students of different

types of autonomic regulation were revealed. For individuals with a high level of sympathetic activation, the risks of disorders are associated with dysregulation of vascular tone; for individuals with a predominance of parasympathetic activity – with a possible depletion of the mechanisms of nitrogen monoamine production. In persons of the sympathicotonic type of autonomic regulation, body composition is due to an excessive content of fat mass and visceral obesity, water insufficiency. In the implementation of neurodynamic reactions, it is noted that with the predominance of parasympathetic regulation of heart rate, inhibitory processes in the central nervous system increase. In young men, reduced sensorimotor reactions are also consistent with sympathetic activation in the regulation of heart rate.

The correlation analysis between the parameters of the morphofunctional state in students with different types of heart rate regulation made it possible to establish some features in the interaction of physiological systems: vascular, morphological and central nervous systems.

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Conflict of interest

The authors of this article declare the absence of a conflict of interest.

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PHARMACOLOGY AND PHARMACY

STUDY OF ANTI-INFLAMMATORY AND ANTINOCICEPTIVE PROPERTIES OF NEW DERIVATIVES OF CONDENSED 3-AMINOTHIENO[2,3-b]PYRIDINES AND 1,4-DIHYDROPYRIDINES

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ABSTRACT

Background. *α-cyanothioacetamide derivatives are promising targets for the search for effective and safe antinociceptive agents with antipyretic and antiexudative activity.*

The aim. *To conduct in vivo experimental study of anti-inflammatory and analgesic effects of new thienopyridines and 1,4-dihydropyridines derivatives.*

Materials and methods. *The synthesized cyanothioacetamide derivatives were subjected to virtual bioscreening using Swiss Target Prediction online service. 140 laboratory rats were randomly distributed into intact and control (dextran edema) groups, reference groups (acetylsalicylic acid and nimesulide) and ten experimental groups for the investigated derivatives of thieno[2,3-b]pyridine and 1,4-dihydropyridine. The anti-inflammatory activity of the compounds at a dose of 5 mg/kg was evaluated by modeling acute dextran edema of rat paw. Determination of analgesic activity was carried out in the hotplate analgesic assay on 130 rats in comparison with sodium metamizole.*

Results. *1,4-dihydropyridines AZ331 and AZ420, as well as thienopyridine derivative AZ023 were determined to have strong anti-inflammatory activity (2.5 times more effective than nimesulide and 2.2 times more effective than acetylsalicylic acid). Compounds AZ023, AZ331 and AZ383 showed pronounced analgesic activity. The time of stay on the heated plate for rats of experimental groups that were fed with AZ331 and AZ383 for prophylactic purpose was respectively 9.56 and 9.93 times more than the same index in the reference group. The animals receiving AZ023 were characterized by an increase in the latent reaction time up to 241.2 seconds, which is 14.53 times higher than that in the rats received sodium metamizole.*

Conclusion. *New thienopyridine and 1,4-dihydropyridine derivatives with high anti-inflammatory and analgesic activity were synthesized and studied; they were recognized as promising targets for further preclinical studies.*

Key words: *condensed thienopyridines, 1,4-dihydropyridines, antiexudative properties, analgesic activity, anti-inflammatory properties, dextran edema*

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ИССЛЕДОВАНИЕ ПРОТИВОВОСПАЛИТЕЛЬНЫХ И АНТИНОЦИЦЕПТИВНЫХ СВОЙСТВ НОВЫХ ПРОИЗВОДНЫХ КОНДЕНСИРОВАННЫХ 3-АМИНОТИЕНО[2,3-b]ПИРИДИНОВ И 1,4-ДИГИДРОПИРИДИНОВ

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РЕЗЮМЕ

Обоснование. *Перспективными объектами для поиска эффективных и безопасных антиноцицептивных средств с жаропонижающей и антиэкссудативной активностью являются производные α-цианотиоацетамида. Цель исследования. Изучение противовоспалительной и болеутоляющей активности новых производных тиенопиридина и 1,4-дигидропиридина в эксперименте in vivo.*

Методы. *Синтезированные производные цианотиоацетамида подвергались виртуальному биоскринингу с использованием программного сервиса Swiss Target Prediction. 140 лабораторных крыс рандомно распределялись на интактную и контрольную («декстрановый отёк») группы, референтные группы (ацетилсалициловая кислота и нимесулид) и на десять опытных групп по исследуемым производным тиено[2,3-b]пиридина и 1,4-дигидропиридина. Противовоспалительная активность соединений в дозе 5 мг/кг оценивалась при моделировании острого «декстранового отёка» лапы крыс. Определение анальгетической активности проводилось в тесте горячей пластины на 130 крысах в сравнении с метамизолом натрия.*

Результаты. *Установлено, что 1,4-дигидропиридины AZ331 и AZ420, а также производное тиенопиридина AZ023 обладают отчётливо выраженной противовоспалительной активностью (в 2,5 раза эффективнее нимесулида и в 2,2 раза – ацетилсалициловой кислоты).*

Отчётливо выраженную анальгетическую активность проявили соединения AZ023, AZ331 и AZ383. Время пребывания на разогретой пластине крыс экспериментальных групп, получавших с профилактической целью AZ331 и AZ383 соответственно в 9,56 и 9,93 раза больше аналогичного показателя в референтной группе. Животные, получавшие AZ023, характеризовались увеличением латентного времени реакции до 241,2 секунды, что выше такового в 14,53 раза у крыс, которым вводили метамизол натрия.

Заключение. *Синтезированы и исследованы новые производные тиенопиридина и 1,4-дигидропиридина с установленной высокой противовоспалительной и болеутоляющей активностью, перспективные для дальнейших доклинических исследований.*

Ключевые слова: *конденсированные тиенопиридины, 1,4-дигидропиридины, антиэкссудативные свойства, анальгетическая активность, противовоспалительные свойства, декстрановый отёк*

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OBJECTIVES

The problem of the effectiveness and safety of the use of non-steroidal anti-inflammatory drugs (NSAIDs) in clinical practice is very relevant today. Statistical studies of the last decade indicate that on all continents more than 30 million people take NSAIDs constantly to eliminate the manifestations of pain, fever and inflammatory syndromes. About 300 million patients use this group of medicines periodically. Moreover, up to 200 million people of them purchase drugs without a prescription [1–3].

According to the results of the analysis of almost 4,000 spontaneous reports received by Federal Service for Surveillance in Healthcare of Russia (Roszdravnadzor) for the period 2008–2017, to record information about adverse reactions that occurred when using NSAIDs, it is reported that the most common reactions were registered to acetylsalicylic acid, diclofenac, ibuprofen and ketorolac. Disorders of the immune system, skin, subcutaneous tissues and digestive tract were predominant in the list of 6,500 reports. Such reactions to angioedema, urticaria (hives), erosive gastritis, skin rash and increased blood pressure are also characterized by a high frequency [4]. It is important to note that not in all patients the use of NSAIDs is accompanied by the desired relief of pain and elimination of signs of swelling of inflammatory genesis in diseases of various etiologies [5].

The low safety profile of NSAIDs and antipyretic analgesics common in clinical practice, as well as an extensive list of contraindications to their use, emphasize the importance of a targeted search for new highly effective and safe medicines. In this regard, the search for newly synthesized effective and safe painkillers and anti-inflammatory medicine currently remains relevant [6–9].

In the last decade, new organic compounds from a number of cyanothioacetamide derivatives have been of particular interest to scientists of chemical, biological, pharmaceutical and medical profiles, since cyanothioacetamide is an easily accessible and multifunctional reagent with several nucleophilic and electrophilic centers. Cyanothioacetamide easily reacts by condensation and cyclization with a wide range of reagents. This circumstance causes a significant variety of possible products of such reactions – sulfur- and nitrogen-containing heterocyclic compounds, which in many cases are structural fragments of natural molecules; a large number of biologically active compounds have been found among them [10–12].

Some α-cyanothioacetamide derivatives are promising targets for the search for effective and safe antinociceptive agents with antipyretic and antiexudative activity [13–18].

An important feature of cyanothioacetamide derivatives is the results of a study of their acute oral toxicity *in vivo*, indicating their low toxicity (toxicity classes 4–5) [19].

At the preparatory stage, before designing an experiment to determine samples of heterocyclic compounds, the most interesting in terms of their ability to bind to probable biotargets for the pharmacocorrection of pain, inflammatory or febrile syndromes, a virtual bioscreening of 340 new cyanothioacetamide derivatives synthesized by us in the ChemEx Research Laboratory of Lugansk State University named after Vladimir Dahl was carried out. At the same time, the following information resources were used: Online SMILES Translator and Structure File Generator of the U.S. National Cancer Institute, OPSIN: Open Parser for Systematic IUPAC nomenclature of the University of Cambridge, Center for Molecular Informatics [20, 21].

As a result, ten samples of new heterocyclic compounds containing 3-aminothieno[2,3-b]pyridine and 1,4-dihydropyridine fragments were selected, potentially capable of interacting with receptors and enzymes involved in the functioning of the antinociceptive system. These are samples with laboratory codes: AZ023, AZ169, AZ213, AZ257, AZ331, AZ420, AZ383, AZ729, AU04271 and AU04288. The structure and chemical formulas of these heterocyclic compounds are shown in Figure 1.

According to the results of virtual bioscreening, the biotargets for these samples are arachidonate-5-lipoxygenase, cyclooxygenase-2, phospholipase *A2*, phosphodiesterase, prostanoid, somatostatin, adenosine and cannabinoid receptors. Having planned a series of pharmacological studies in the in *vivo* experiments to study their analgesic and anti-inflammatory effects, the test of «dextran edema» of rat paw was selected among the recommended various classical pharmacological tests.

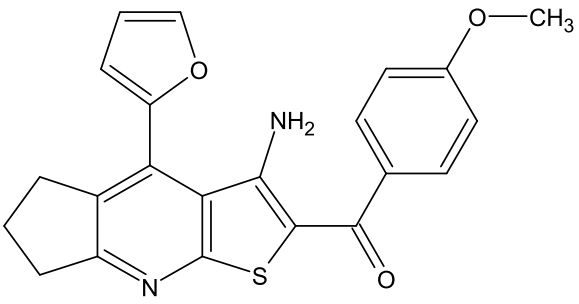
THE AIM OF THE STUDY

The study of anti-inflammatory and antinociceptive properties in *in vivo* experiments of new derivatives of 3-aminothieno[2,3-b]pyridine and 1,4-dihydropyridine in an experiment.

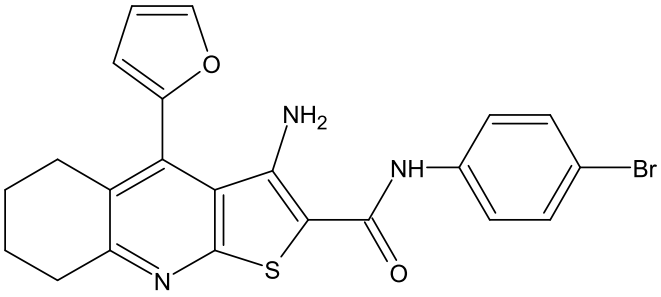
METHODS

The experiment was carried out on 140 white mongrel male rats weighing 250–280 g, obtained from the vivarium of the Lugansk State Medical University named after St. Luke in the autumn-winter period in the laboratory of the Department of Fundamental and Clinical Pharmacology. Laboratory animals were randomly (using the "envelope" method) divided into groups consisting of 10 rats.

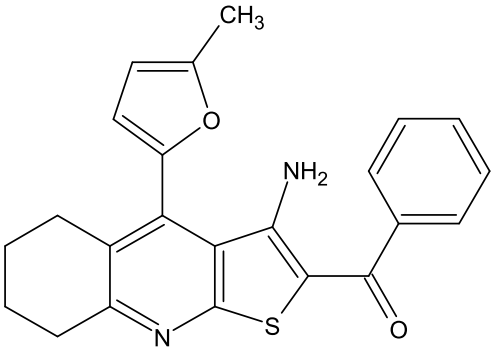
The animals were divided into intact, control (rats injected with 2 ml of 0.9 % sodium chloride solution intragastrically before the modelling process), two reference groups (receiving acetylsalicylic acid from Uralbiopharm OJSC at a dose of 50 mg/kg and nimesulide from Berezovsky Pharmaceutical Plant CJSC at a dose of 5 mg/kg) and 10 experimental groups according to the number of new derivatives of condensed



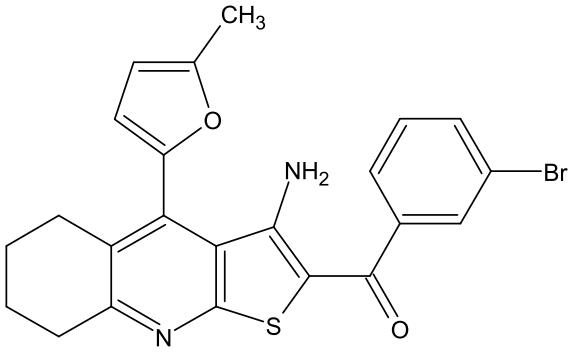
AU04288
[3-amino-4-(2-furyl)-6,7-dihydro-5*H*-cyclopenta[*b*]thieno[3,2-*e*]pyridin-2-yl](4-methoxyphenyl)methanone



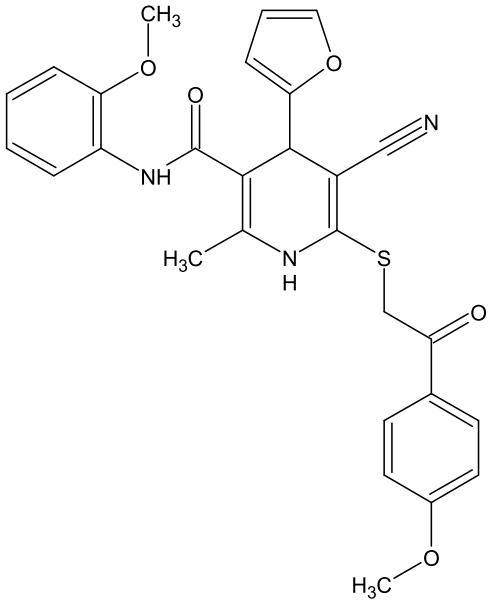
AU04272
3-amino-*N*-(4-bromophenyl)-4-(2-furyl)-5,6,7,8-tetrahydrothieno[2,3-*b*]quinoline-2-carboxamide



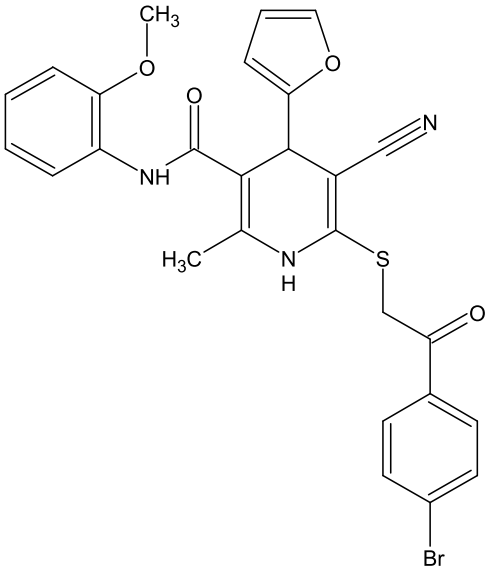
AZ023
[3-amino-4-(5-methyl-2-furyl)-5,6,7,8-tetrahydrothieno[2,3-*b*]quinolin-2-yl](phenyl)methanone



AZ729
[3-amino-4-(5-methyl-2-furyl)-5,6,7,8-tetrahydrothieno[2,3-*b*]quinolin-2-yl](3-bromophenyl)methanone

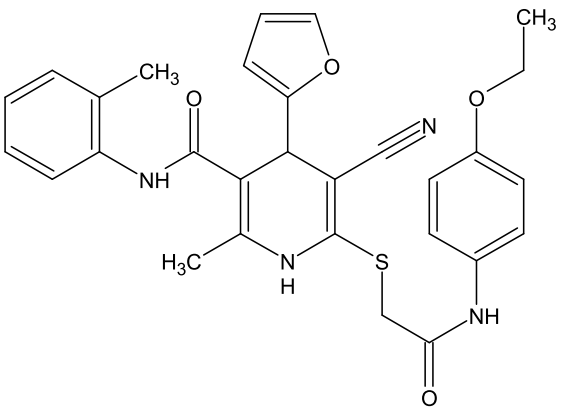


AZ331
5-cyano-4-(2-furyl)-*N*-(2-methoxyphenyl)-6-{[2-(4-methoxyphenyl)-2-oxoethyl]thio}-2-methyl-1,4-dihydropyridine-3-carboxamide

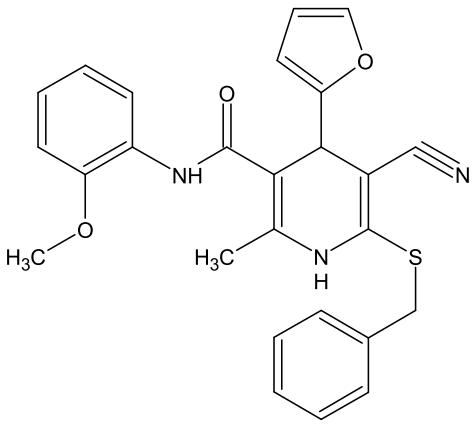


AZ257
6-{[2-(4-bromophenyl)-2-oxoethyl]thio}-5-cyano-4-(2-furyl)-*N*-(2-methoxyphenyl)-2-methyl-1,4-dihydropyridine-3-carboxamide

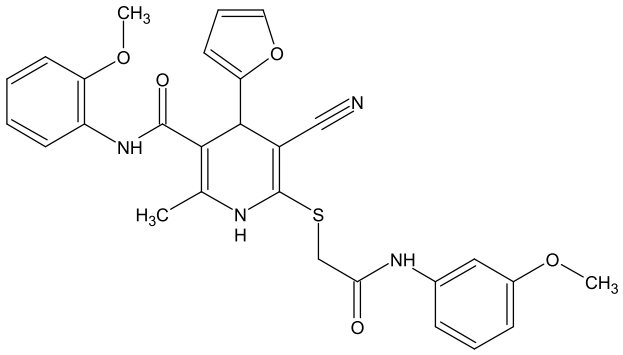
FIG. 1.
Structural formulas and names according to the IUPAC nomenclature for the studied 3-aminothieno[2,3-b]pyridines and 1,4-dihydropyridines



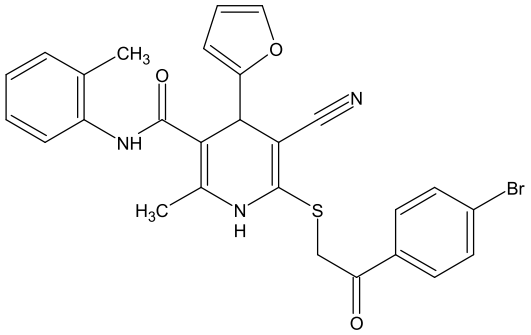
AZ383
5-cyano-6-({2-[(4-ethoxyphenyl)amino]-2-oxoethyl}thio)-4-(2-furyl)-2-methyl-*N*-(2-methylphenyl)-1,4-dihydropyridine-3-carboxamide



AZ169
6-(benzylthio)-5-cyano-4-(2-furyl)-*N*-(2-methoxyphenyl)-2-methyl-1,4-dihydropyridine-3-carboxamide



AZ420
5-cyano-4-(2-furyl)-*N*-(2-methoxyphenyl)-6-({2-[(3-methoxyphenyl)amino]-2-oxoethyl}thio)-2-methyl-1,4-dihydropyridine-3-carboxamide



AZ213
6-{[2-(4-bromophenyl)-2-oxoethyl]thio}-5-cyano-4-(2-furyl)-2-methyl-*N*-(2-methylphenyl)-1,4-dihydropyridine-3-carboxamide

FIG. 1. (contined)
Structural formulas and names according to the IUPAC nomenclature for the studied 3-aminothieno[2,3-b]pyridines and 1,4-dihydropyridines

3-aminothieno[2,3-b]pyridines and 1,4-dihydropyridine being studied. Rats with standard signs of inflammation formed during the modelling process were included in the experiment.

The anti-inflammatory properties of the new heterocyclic compounds synthesized by us were evaluated using modeling acute "dextran edema", formed after subplantar administration of a 6 % dextran solution with a volume of 0.1 ml into the right hind paw (Fig. 2, 3). The studied compounds were administered through a gastric tube at a dose of 5 mg/kg 1.5 hours before the induction of edema.

Registration and quantitative measurement of the swelling of the injected extremity in animals of all experimental groups were carried out oncometrically by changing the circumference of the right hind extremity 1 and 3 hours after the induction of inflammation, according to the method of A.F. Leshchinsky and the guidelines for preclinical studies [22, 23]. The data were compared with similar values of the symmetrical limb and with indicators in rats of the intact group. The animals of all groups were monitored for two weeks.

In parallel, an experiment was carried out on other 130 white mongrel male rats weighing 250–280 g, which were similarly divided into intact, control (rats injected with 2 ml of 0.9 % sodium chloride solution intragastrically before modeling the test), reference (animals receiving sodium metamizole intragastrically at 7 mg/kg) and 10 experimental groups (according to the number of samples of cyanothioacetamide derivatives). The studied compounds with laboratory codes AZ023, AZ169, AZ213, AZ257, AZ331, AZ420, AZ383, AZ729, AU04271, AU04288

FIG. 2.
Modeling dextran edema of the paw

FIG. 3.
Swelling of the right limb of the rat of control group an hour after subplantar administration of dextran solution

were administered intragastrically at a dose of 5 mg/kg 1.5 hours before the study.

Analgesic activity was determined in the hotplate analgesic assay based on behavioral reactions controlled by supraspinal structures in response to pain irritation. The animals were placed on a metal plate heated up to aprox. 52 °C (50–55 °C) and surrounded by a cylinder. We recorded the time from the moment we placed the animal on a hot surface to the appearance of a behavioral response to nociceptive stimulation in the form of jumps, jerks and licking of the hind legs. A statistically significant increase in the latent reaction period after administration of a biologically active compound was considered a criterion for the analgesic effect.

The studies were conducted in accordance with the Order of the Ministry of Health of the Russian Federation dated April 1, 2016 No. 199n "On approval of the rules of good laboratory practice". Throughout the entire research period, the animals were monitored with free access to water and food, which corresponds to GOST 33044-2014 "Principles of good laboratory practice" (approved by Order of the Federal Agency for Technical Regulation and Metrology No. 1700-st, dated November 20, 2014).

The study was approved by the Commission on Bioethics of the Lugansk State Medical University named after St. Luke (Protocol No. 6 dated November 1, 2021).

Statistical processing of the obtained data was carried out on the basis of [24], according to well-known formulas and methods of mathematical statistics characterizing quantitative variability. When processing experimental data, the following were determined: the arithmetic mean of the circumference of the extremity *a*; the variance of values s^2 around the arithmetic mean; the standard deviation s; the standard error of the arithmetic mean *m*. The uniformity of the experimental data obtained was estimated by the coefficient of variation *V*.

The statistical significance of the differences between the samples and the comparison preparations was determined using the Student's t-test with a critical value of the Student's t-test equal to 2,101, the level of statistical significance $\alpha = 0.05$ and the number of degrees of freedom $f = 18$ in the online calculator "Calculation of the Student's t-test when comparing averages"[1].

STUDY RESULTS

During statistical processing of data obtained in the reference (comparison drugs) and experimental (new condensed derivatives of thienopyridine and 1,4-dihydropyridine) groups, the values of the difference in circumference of the limbs were calculated 1 and 3 hours after the induction of inflammation. They are shown in Tables 1 and 2.

It should be noted that in the conditions of the conducted experiments, the value of the indicator p is inverse, i. e. the larger the p, the smaller the differences in the circumference of the limb when exposed to the test samples; therefore, a sample with a higher p value is more effective.

When comparing the circumference of the paws of animals of the intact group, the sizes of the right and left hind limbs differed slightly (based on the data presented in Tables 1 and 2). Subplantar injection of a 6 % dextran solution to laboratory rats of the control group contributed to the appearance of pronounced edema. At the same time, 1 hour after injection of dextran solution, the circumference of the right hind paw exceeded the corresponding value of the symmetrical left paw by 43.5 % (Table 1). Three hours after the reproduction of the inflammatory reaction to "dextran edema" in the modelling, the circumference of the right paw of the control group rats was 43.9 %

[1] https://medstatistic.ru/calculators/averagestudent.html

TABLE 1

STATISTICAL CHARACTERISTICS OF CHANGES IN THE CIRCUMFERENCE OF THE LIMBS OF RATS AFTER THE FORMATION OF DEXTRAN EDEMA 1 HOUR AFTER INDUCING INFLAMMATION

Groups of animals	Circumference of the limb (a), mm		Difference (δ)	
	left	right	mm	%
Intact	$a = 24,3$; $s^2 = 0,90$; $s = 0,95$; $m = 0,32$; $V = 3,9$ %	$a = 25,1$; $s^2 = 0,98$; $s = 0,99$; $m = 0,33$; $V = 3,9$ %	0.80	3.30
Control	$a = 24,6$; $s^2 = 0,71$; $s = 0,84$; $m = 0,24$; $V = 3,4$ %	$a = 35,3$; $s^2 = 25,8$; $s = 5,08$; $m = 1,69$; $V = 14,4$ %	10.7	43.5
	1. Student's test $t = 6.27$. The differences are statistically significant ($p = 0.000008$). 2. Student's test $t = 5.92$. The differences are statistically significant ($p = 0.000017$).			
	Comparison drugs			
Acetylsalicylic acid	$a = 27,6$; $s^2 = 0,63$; $s = 0,25$; $m = 0,08$; $V = 9,1$ %	$a = 34,5$; $s^2 = 1,29$; $s = 0,36$; $m = 0,12$; $V = 10,4$ %	6.90	25.0
	1. Student's test $t = 47.8$. The differences are statistically significant ($p < 0.00001$). 2. Student's test $t = 26.8$. The differences are statistically significant ($p < 0.00001$).			
Nimesulide	$a = 25,5$; $s^2 = 0,25$; $s = 0,16$; $m = 0.05$; $V = 6.3$ %	$a = 33,4$; $s^2 = 0,26$; $s = 0,16$; $m = 0,05$; $V = 4,8$ %	7.90	31.0
	1. Student's test $t = 111.7$. The differences are statistically significant ($p < 0.00001$). 2. Student's test $t = 24.9$. The differences are statistically significant ($p < 0.00001$).			
	New studied derivatives of condensed 3-aminothieno[2,3-b]pyridines and 1,4-dihydropyridine			
AZ383	$a = 27,8$; $s^2 = 12,40$; $s = 3,52$; $m = 1,17$; $V = 12,7$ %	$a = 32,0$; $s^2 = 10,20$; $s = 3,20$; $m = 1,07$; $V = 10,0$ %	4.20	15.1
	1. Student's test $t = 2.65$. The differences are statistically significant ($p = 0.016875$). 2. Student's test $t = 6.16$. The differences are statistically significant ($p = 0.00001$).			
AZ023	$a = 25,9$; $s^2 = 2,54$; $s = 1,60$; $m = 0,53$; $V = 6,20$ %	$a = 28,9$; $s^2 = 4,10$; $s = 2,02$; $m = 0,67$; $V = 7,0$ %	3.00	11.6
	1. Student's test $t = 3.51$. The differences are statistically significant ($p = 0.002675$). 2. Student's test $t = 5.09$. The differences are statistically significant ($p = 0.000091$).			

TABLE 1 (*continued*)

AZ420	$a = 28,3$; $s^2 = 6,45$; $s = 2,54$; $m = 0,85$; $V = 9,0$ %	$a = 31,6$; $s^2 = 5,38$; $s = 2,32$; $m = 0,77$; $V = 7,3$ %	3.30	11.7
	1. Student's test $t = 2.88$. The differences are statistically significant ($p = 0.010453$). 2. Student's test $t = 7.76$. The differences are statistically significant ($p = 0.000001$).			
AZ257	$a = 29,3$; $s^2 = 6,90$; $s = 2,63$; $m = 0,88$; $V = 9,0$ %	$a = 34,3$; $s^2 = 5,34$; $s = 2,31$; $m = 0,77$; $V = 6,7$ %	5.00	17.1
	1. Student's test $t = 4.28$. The differences are statistically significant ($p = 0.000511$). 2. Student's test $t = 11.0$. The differences are statistically significant ($p < 0.00001$).			
AZ213	$a = 31,3$; $s^2 = 16,0$; $s = 4,00$; $m = 1,33$; $V = 12,8$ %	$a = 38,5$; $s^2 = 6,94$; $s = 2,63$; $m = 0,88$; $V = 6,8$ %	7.20	23.0
	1. Student's test $t = 4.51$. The differences are statistically significant ($p = 0.000306$). 2. Student's test $t = 14.26$. The differences are statistically significant ($p < 0.00001$).			
AZ331	$a = 28,8$; $s^2 = 4,18$; $s = 2,04$; $m = 0,68$; $V = 7,1$ %	$a = 32,9$; $s^2 = 2,77$; $s = 1,66$; $m = 0,55$; $V = 5,1$ %	4.10	14.2
	1. Student's test $t = 4.69$. The differences are statistically significant ($p = 0.000212$). 2. Student's test $t = 12.16$. The differences are statistically significant ($p < 0.00001$).			
AZ729	$a = 27,7$; $s^2 = 5,79$; $s = 2,40$; $m = 0,80$; $V = 8,7$ %	$a = 34,4$; $s^2 = 5,82$; $s = 2,41$; $m = 0,80$; $V = 7,0$ %	6.70	24.2
	1. Student's test $t = 5.92$. The differences are statistically significant ($p = 0.000017$). 2. Student's test $t = 10.75$. The differences are statistically significant ($p < 0.00001$).			
AZ169	$a = 26,5$; $s^2 = 4,72$; $s = 2,17$; $m = 0,72$; $V = 8,2$ %	$a = 32,4$; $s^2 = 16,0$; $s = 4,00$; $m = 1,33$; $V = 12,4$ %	5.90	22.3
	1. Student's test $t = 3.90$. The differences are statistically significant ($p = 0.001149$). 2. Student's test $t = 5.33$. The differences are statistically significant ($p = 0.000056$).			
AU04271	$a = 28,4$; $s^2 = 4,26$; $s = 2,06$; $m = 0,69$; V = 7,3 %	$a = 37,4$; $s^2 = 5,60$; $s = 2,36$; $m = 0,79$; $V = 6,3$ %	9.00	31.7
	1. Student's test $t = 8.58$. The differences are statistically significant ($p < 0.00001$). 2. Student's test $t = 14.37$. The differences are statistically significant ($p < 0.00001$).			
AU04288	$a = 25,1$; $s^2 = 0,98$; $s = 0,99$; $m = 0,33$; $V = 3,9$%	$a = 32,3$; $s^2 = 18,7$; $s = 4,32$; $m = 1,44$; $V = 13,4$ %	7.20	28.7
	1. Student's test $t = 4.86$. The differences are statistically significant ($p = 0.000143$). 2. Student's test $t = 4.87$. The differences are statistically significant ($p = 0.000143$).			

Note. 1 – in comparison with the indicators of the symmetrical limb; 2 – in comparison with the indicators in the intact group.

TABLE 2

STATISTICAL CHARACTERISTICS OF CHANGES IN THE CIRCUMFERENCE OF THE EXTREMITIES OF RATS AFTER THE FORMATION OF DEXTRAN EDEMA 3 HOURS AFTER INDUCING INFLAMMATION

Groups of animals	Circumference of the limb (*a*), mm		Difference (δ)	
	left	right	mm	%
Intact	$a = 24,3$; $s^2 = 0,90$; $s = 0,95$; $m = 0,32$; $V = 3,9$ %	$a = 25,1$; $s^2 = 0,98$; $s = 0,99$; $m = 0,33$; $V = 3,9$ %	0.80	3.30
Control	$a = 24,6$; $s^2 = 0,71$; $s = 0,84$; $m = 0,28$; $V = 3,4$ %	$a = 35,4$; $s^2 = 30,7$; $s = 5,54$; $m = 1,85$; $V = 15,6$ %	10.8	43.9
	1. Student's test $t = 5.77$. The differences are statistically significant ($p = 0.000023$). 2. Student's test $t = 5.48$. The differences are statistically significant ($p = 0.000041$).			
	Comparison drugs			
Acetylsalicylic acid	$a = 27,6$; $s^2 = 0,63$; $s = 0,25$; $m = 0,083$; $V = 9,1$ %	$a = 30,9$; $s^2 = 0,26$; $s = 0,16$; $m = 0,05$; $V = 5,3$ %	3.30	12.0
	1. Student's test $t = 34.1$. The differences are statistically significant ($p < 0.00001$). 2. Student's test $t = 17.38$. The differences are statistically significant ($p < 0.00001$).			
Nimesulide	$a = 25,5$; $s^2 = 0,25$; $s = 0,16$; $m = 0,053$; $V = 6,3$ %	$a = 30,9$; $s^2 = 0,16$; $s = 0,12$; $m = 0,04$; $V = 4,0$ %	5.40	21.2
	1. Student's test $t = 81.3$. The differences are statistically significant ($p < 0.00001$). 2. Student's test $t = 17.45$. The differences are statistically significant ($p < 0.00001$).			
	New studied derivatives of condensed 3-aminothieno[2,3-b]pyridines and 1,4-dihydropyridine			
AZ383	$a = 27,8$; $s^2 = 12,4$; $s = 3,52$; $m = 1,17$; $V = 12,7$ %	$a = 30,9$; $s^2 = 6,99$; $s = 2,64$; $m = 0,88$; $V = 8,6$ %	3.10	11.2
	1. Student's test $t = 2.12$. The differences are statistically significant ($p = 0.049262$). 2. Student's test $t = 6.17$. The differences are statistically significant ($p = 0.000010$).			
AZ023	$a = 25,9$; $s^2 = 2,54$; $s = 1,59$; $m = 0,53$; $V = 6,16$ %	$a = 27,8$; $s^2 = 2,40$; $s = 1,55$; $m = 0,52$; $V = 5,6$ %	1.90	7.30
	1. Student's test $t = 2.56$. The differences are statistically significant ($p = 0.020332$). 2. Student's test $t = 4.38$. The differences are statistically significant ($p = 0.000405$).			

TABLE 2 (*continued*)

AZ420	$a = 28,3$; $s^2 = 6,45$; $s = 2,54$; $m = 0,85$; $V = 9,0$ %	$a = 31,1$; $s^2 = 2,10$; $s = 1,44$; $m = 0,48$; $V = 4,7$ %	2.80	9.90
	1. Student's test $t = 2.87$. The differences are statistically significant ($p = 0.010653$). 2. Student's test $t = 10.3$. The differences are statistically significant ($p < 0.00001$).			
AZ331	$a = 28,8$; $s^2 = 4,18$; $s = 2,04$; $m = 0,68$; $V = 7,1$ %	$a = 30,8$; $s^2 = 0,84$; $s = 0,91$; $m = 0,30$; $V = 3,0$ %	2.00	6.90
	1. Student's test $t = 2.69$. The differences are statistically significant ($p = 0.015465$). 2. Student's test $t = 12.78$. The differences are statistically significant ($p < 0.00001$).			
AZ257	$a = 29,3$; $s^2 = 6,90$; $s = 2,62$; $m = 0,87$; $V = 9,0$ %	$a = 34,7$; $s^2 = 8,90$; $s = 2,98$; $m = 0,99$; $V = 8,6$ %	5.40	18.4
	1. Student's test $t = 4.10$. The differences are statistically significant ($p = 0.000751$). 2. Student's test $t = 9.20$. The differences are statistically significant ($p < 0.00001$).			
AZ213	$a = 31,3$; $s^2 = 16,0$; $s = 4,0$; $m = 1,33$; $V = 12,8$ %	$a = 36,9$; $s^2 = 15,9$; $s = 3,98$; $m = 1,33$; $V = 10,8$ %	5.60	17.9
	1. Student's test $t = 2.98$. The differences are statistically significant ($p = 0.008454$). 2. Student's test $t = 8.61$. The differences are statistically significant ($p < 0.00001$).			
AZ169	$a = 26,5$; $s^2 = 4,72$; $s = 2,17$; $m = 0,72$; $V = 8,2$ %	$a = 30,2$; $s^2 = 7,51$; $s = 2,74$; $m = 0,91$; $V = 9,1$ %	3.70	14.0
	1. Student's test $t = 3.19$. The differences are statistically significant ($p = 0.005378$). 2. Student's test $t = 5.27$. The differences are statistically significant ($p = 0.000063$).			
AZ729	$a = 27,7$; $s^2 = 5,79$; $s = 2,40$; $m = 0,80$; $V = 8,7$ %	$a = 34,5$; $s^2 = 2,72$; $s = 1,65$; $m = 0,55$; $V = 4,8$ %	7.30	24.5
	1. Student's test $t = 7.00$. The differences are statistically significant ($p = 0.000002$). 2. Student's test $t = 16.06$. The differences are statistically significant ($p < 0.00001$).			
AU04271	$a = 29,4$; $s^2 = 13,38$; $s = 3,7$; $m = 1,23$; $V = 12,4$ %	$a = 38,1$; $s^2 = 4,54$; $s = 2,13$; $m = 0,71$; $V = 5,6$ %	8.70	29.6
	1. Student's test $t = 6.13$. The differences are statistically significant ($p = 0.000011$). 2. Student's test $t = 16.6$. The differences are statistically significant ($p < 0.00001$).			
AU04288	$a = 25,1$; $s^2 = 0,98$; $s = 0,99$; $m = 0,33$; $V = 4,0$ %	$a = 33,5$; $s^2 = 20,50$; $s = 4,5$; $m = 1,5$; $V = 13,5$ %	8.40	33.5
	1. Student's test $t = 5.47$. The differences are statistically significant ($p = 0.000042$). 2. Student's test $t = 5.47$. The differences are statistically significant ($p = 0.000042$).			

Note. 1 – in comparison with the indicators of the symmetrical limb; 2 – in comparison with the indicators in the intact group.

bigger than the same value of the left one. In other words, by this time of observation, pronounced edema, redness, soreness and dysfunction of the distal part of the free hind limb were registered (Fig. 3).

Non-steroidal anti-inflammatory drugs used as referents in comparison groups showed the expected anti-inflammatory activity in experiments.

Thus, preliminary (90 minutes before modeling an acute inflammatory reaction) intragastric administration of acetylsalicylic acid to rats of the corresponding group contributed to the formation of a less pronounced edema of the injected right paw (the difference in the circumference of the right and left limbs was 25 % at an early stage of observation).

It is important to note the following: 3 hours after administration of dextran solution in rats of the reference group treated with acetylsalicylic acid, the difference in the circumference of the hind paws is halved, amounting to almost 12 %. This highlights the fact that antiexudative activity increases over time.

Nimesulide, administered intragastrally to rats of the second reference group, has moderate antiedemic properties. So, 1 hour after modeling the inflammatory reaction, the difference in the circumference of the injected and non-injected limbs was 31 %. After another 2 hours, the signs of swelling decrease significantly, and the difference in the circumference of the paws of animals in this group is 21.2 % (Table 2).

When comparing the indicators of anti-inflammatory activity of new synthesized derivatives of thienopyridine and 1,4-dihydropyridine, it was found that derivatives of condensed 3-aminothieno[2,3-b]pyridines with laboratory codes AU04271 and AU04288 demonstrate anti-inflammatory properties almost identical to nimesulide at an early stage of the experiment.

Antiedemic activity similar to acetylsalicylic acid under the conditions of this pharmacological test is shown by three new studied heterocyclic compounds of 1,4-dihydrothiopyridine derivatives with codes AZ729, AZ213 and AZ169, administered intragastrally for prophylactic purposes. The difference between the circumference of the right and left hind limbs of the rats of these experimental groups 1 hour after modeling dextran edema was 24.2, 23.0 and 22.3 %, respectively.

The remaining 5 out of 10 new studied derivatives of condensed 3-aminothieno[2,3-b]pyridines and 1,4-dihydropyridine are able to reduce the development of dextran edema more effectively than classical NSAIDs used by us as reference drugs in the early stages of follow-up.

Thus, the difference between the circumference of the injected right and non-injected left paws of rats of experimental groups receiving derivatives of 1,4-dihydropyridian with laboratory codes AZ257 and AZ383 through a gastric tube, 1 hour after the modelling of an acute inflammatory reaction, is at the level of 17.1 % and 15.1 %, respectively. This is almost 2 times less than after the use of nimesulide.

As shown in Table 1, the following three samples have significantly more pronounced anti-inflammatory properties according to the experimental results: derivatives of 1,4-dihydropyridine with the codes AZ331 and AZ420 and a condensed derivative of thienopyridine with the code AZ023. The difference in the circumference of the distal limbs of the animals of these experimental groups at the one-hour term of the experiment is 14.2, 11.7 and 11.6 %, respectively. This is more than 2.5 times less than the indicator registered in the comparison group after the administration of nimesulide, and 2.2 times less than the indicator registered after the use of acetylsalicylic acid. At the same time, when walking around the cage, the rats of these experimental groups showed no signs of severe pain that was present in rats of the control group without pharmacocorrection.

Observation in the dynamics of the experiment showed (Table 2) that 3 hours after administration of dextran solution, 7 out of 10 studied heterocyclic compounds have anti-inflammatory properties in the spectrum of their pharmacodynamic effects, which exceed those of nimesulide. The most pronounced ability to prevent the development of edema in this experimental test was shown by samples of new heterocyclic compounds with laboratory codes AZ331, AZ420 and AZ023. By this time of observation, the circumference difference between the injected and non-injected limbs ranges from 9.9 to 6.9 %.

The results of an experiment conducted on a pharmacological model to study antinociceptive properties showed that the average value of time on the surface of a heated plate in animals of the control group without pharmacocorrection was 8.6 seconds The use of sodium metamizole led to an increase in the latent reaction period by almost 2 times – up to 16.6 seconds In animals of the experimental groups, under the conditions of the experiment, it was recorded that the studied samples with laboratory codes AZ169, AU04271, AU04288 did not show antinociceptive activity, since the time on the surface of a heated plate before characteristic jumping and licking of paws ranged from 5.3 to 9.0 seconds in rats of these groups.

Moderate analgesic activity exceeding one and a half or more times that of the comparison drug metamizole sodium was detected by new heterocyclic compounds with laboratory codes AZ257, AZ729 and AZ213 in this test.

According to the results of the conducted studies, the new biologically active compound with the code AZ420 increases the latent reaction time to 127.9 seconds, which is 7.7 times more than after the use of sodium metamizole.

Three samples showed pronounced analgesic activity – compounds with the codes AZ023, AZ331 and AZ383. Moreover, the time rats of the experimental groups treated with AZ331 and AZ383 for preventive purposes spend on the heated plate was 158.8 seconds and 164.9 seconds on average for the groups, which is 9.56 and 9.93 times more than the same indicator in the reference group, respectively. Animals treated with condensed thienopyridine with the code AZ023 were characterized by an increase in the latent reaction time to 241.2 seconds, which is 14.53 times higher than that in rats injected with sodium metamizole.

CONCLUSIONS

When conducting pharmacological studies *in vivo* and modelling dextran edema of rat paw for ten new derivatives of condensed 3-aminothieno[2,3-b]pyridines and 1,4-dihydropyridines with potential analgesic activity, it was found that four samples with the following laboratory codes have the most pronounced antiexudative properties at a dose of 5 mg/kg: AZ023 (3-amino-4-(5-methyl-2-furyl)-5,6,7,8-tetrahydrothieno[2,3-*b*]quinolin-2-yl](phenyl) methanone); AZ331 (5-cyano-4-(2-furyl)-N-(2-methoxyphenyl)-6-{[2-(4-methoxyphenyl)-2-oxoethyl]thio}-2-methyl-1,4-dihydropyridine-3-carboxamide), AZ420 (5-cyano-4-(2-furyl)-N-(2-methoxyphenyl)-6-({2-[(3- methoxyphenyl) amino]-2-oxoethyl}thio)-2-methyl-1,4-dihydropyridine-3-carboxamide) and AZ383 (3-amino-4-(5-methyl-2-furyl)-5,6,7,8-tetrahydrothieno[2,3-b]quinolin-2-yl](phenyl)methanone). They are 2.5 times more effective than nimesulide in terms of antiexudative properties, and 2.2 times more effective than acetylsalicylic acid.

Three new studied heterocyclic compounds of 1,4-dihydrothiopyridine derivatives with the codes AZ729, AZ213 and AZ169 show antiedemic activity similar to acetylsalicylic acid.

In addition, a hot plate test performed on white mongrel rats showed the presence of analgesic activity in seven studied derivatives of condensed 3-aminothieno[2,3-b] pyridines. Among them, the compound AZ023 [3-amino-4-(5-methyl-2-furyl)-5,6,7,8-tetrahydrothieno[2,3-*b*]quinolin-2-yl](phenyl)methanone] is 14.53 times more effective than sodium metamizole.

DISCUSSION OF THE STUDY RESULT

Literature data indicate that partially cyanothioacetamide derivatives have a variety of pharmacological properties. For example, 1,4-dihydropyridine-3-carbonitriles are hepatoprotectors, pyrido-1,3,5-thiadiazines have antiviral effects against Powassan virus and tick-borne encephalitis virus, and also demonstrate an analeptic effect and adaptogenic effect. Hexahydroquinoline derivatives are known to be active against HIV. In addition, saturated nicotinonitriles exhibit an inhibitory effect against autotaxin. A hybrid molecule combining thiophene and hexahydroquinoline fragments inhibits the formation of β-amyloid peptide and thus prevents the formation of amyloid plaques, a factor concomitant with a number of serious diseases such as Alzheimer's disease, hemodialysis amyloidosis, lysozyme amyloidosis.

In the last decade, the polypharmacological approach in the industry of creating new medicines has become particularly relevant. It involves moving away from the concept of "one drug – one target – one disease" and creating and/or using a single pharmaceutical product that can simultaneously bind several protein targets or act on different biochemical routes [25]. The strategy of the polypharmacological approach is to create hybrid or multimodal compounds. The latter consist of residues of two or more pharmacophore subunits covalently bound by a flexible spacer. This combination allows the molecule to interact with several protein targets at once, which sometimes gives a synergistic effect. Therefore, the use of hybrid bioactive molecules allows for the combined therapy of multifactorial diseases using a single drug.

The results obtained in our experimental study on white rats allowed us to identify new heterocyclic compounds with pronounced anti-inflammatory and analgesic properties. This may be due to the fact that a sample with the laboratory code AZ331, selected according to virtual bioscreening data from an extensive library of new organic compounds synthesized in the ChemEx laboratory, can bind to phospholipase *A2* and arachidonate-5-lipoxygenase. A new dihydropyridine derivative with the laboratory code AZ383 can also potentially bind to arachidonate-5-lipoxygenase and cyclooxygenase-2.

The 1,4-dihydropyridine derivative with the laboratory code AZ420 can affect the activity of serine threonine protein kinase, phospholipase *A2*, arachidonate-5-lipoxygenase. The heterocyclic compound – condensed thienopyridine with the laboratory code AZ023 – according to the results of virtual bioscreening is potentially capable of binding to prostanoid receptors of types *EP1*, *EP2* and *EP4*, *CB1* type cannabinoid receptors and arachidonate-5-lipoxygenase.

In terms of discussion, we assume that it is common for the leading samples in the experimental study of proto-inflammatory and analgesic activity to consider their effect on the activity of the enzyme arachidonate-5-lipoxygenase as a biomishen for these new derivatives of thienopyridines and dihydropyridines according to the results of virtual bioscreening. At the same time, the ability of individual samples to bind to cyclooxygenase-2 and phospholipase*A2* only enhances their potential anti-inflammatory activity.

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Conflict of interest

The authors of this article declare the absence of a conflict of interest.

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SURGERY

PSEUDOMEMBRANOUS COLITIS COMPLICATED BY TOXIC MEGACOLON IN ONCOLOGICAL PATIENTS

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ABSTRACT

In recent years, information on the increase in the incidence of infection associated with Clostridioides difficile (CDI) has appeared in the literature. It is known that C. difficile which causes pseudomembranous colitis (PMC) most often affects debilitated patients who receive treatment for the main pathology for a long time. That is why PMC is most common in cancer patients receiving long-term and aggressive anticancer treatment, which is often accompanied by the use of several courses of antibiotics. The result of the irrational use of antibiotics, incorrect PMC therapy may be the formation of toxic megacolon, intestinal perforation, sepsis, which in turn is fraught with a fatal outcome. It is this state of affairs that aroused our interest in the study of this topic.
The steady increase in the incidence of Clostridioides difficile infection makes it particularly relevant to study CDI problem in relation to cancer patients, since they most often have a wide range of risk factors for developing clostridial infection.
The article presents an overview of domestic and foreign sources describing this pathology, discusses epidemiology, pathogenesis, clinical picture and current understanding of the CDI treatment. At the end of the review, we present a case of successful treatment of pseudomembranous colitis after stoma closure, which was complicated by the development of toxic megacolon. Colproctectomy was performed as part of the complex treatment of this pathology. The patient received respiratory, renal replacement, hepatoprotective, antibiotic and antifungal therapy and other treatments.

Key words: *pseudomembranous colitis, colorectal cancer, toxic megacolon, colectomy, C. difficile*

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ПСЕВДОМЕМБРАНОЗНЫЙ КОЛИТ, ОСЛОЖНЁННЫЙ ТОКСИЧЕСКИМ МЕГАКОЛОНОМ, У ПАЦИЕНТОВ ОНКОЛОГИЧЕСКОГО ПРОФИЛЯ

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РЕЗЮМЕ

В последние годы в литературе появляется информация о росте заболеваемости Clostridioides difficile-ассоциированной инфекции (CDI, Clostridioides difficile infection). Известно, что наиболее часто C. difficile, вызывающая псевдомембранозный колит (ПМК), поражает ослабленных пациентов, долго получающих лечение основной патологии. Именно поэтому ПМК наиболее часто встречается у пациентов онкологического профиля, получающих длительное и агрессивное противоопухолевое лечение, нередко сопровождающееся применением нескольких курсов антибиотиков. Следствием нерационального применения антибиотиков, некорректной терапии ПМК может быть формирование токсического мегаколона, перфорации кишечника, сепсиса, что в свою очередь чревато летальным исходом. Именно такое положение вещей вызвало наш интерес к изучению данной темы.

Неуклонный рост заболеваемости инфекцией Clostridioides difficile повсеместно делает особенно актуальным изучение проблемы CDI в мировом сообществе применительно к больным онкологического профиля, так как именно у них наиболее часто имеется широкий спектр факторов риска развития клостридиальной инфекции.

В статье представлен обзор отечественных и зарубежных источников описывающих данную патологию. Освещаются эпидемиология, патогенез, клиническая картина и современное представление о лечении CDI. По окончании обзора нами представлен случай успешного лечения псевдомембранозного колита после закрытия стомы, осложнившегося развитием токсического мегаколона. В рамках комплексного лечения данной патологии была выполнена операция колпроктэктомии. Пациент получал респираторную, почечно-заместительную, гепатопротективную, антибиотико-и противогрибковую терапию и другое лечение.

Ключевые слова: *псевдомембранозный колит, колоректальный рак, токсический мегаколон, колэктомия, C. difficile*

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LIST OF ABBREVIATIONS

C. difficile	–	*Clostridioides difficile*
CDI	–	*Clostridioides difficile infection*
TcdA	–	*Clostridium difficile* toxin A
TcdB	–	*Clostridium difficile* toxin B
GDH	–	glutamate dehydrogenase
RRT	–	renal replacement therapy
ALV	–	artificial lung ventilation
MIC	–	minimum inhibitory concentration
PMC	–	pseudomembranous colitis
PCR	–	polymerase chain reaction
HSCT	–	hematopoietic stem cell transplantation

INTRODUCTION

Clostridioides difficile infection (CDI) is currently the leading detectable cause of nosocomial diarrhea associated with antibacterial therapy [1]. *C. difficile* toxin B was isolated from stool in more than 95 % of cases of pseudomembranous colitis (PMC) and in 15–25 % of cases of antibiotic-related diarrhea [2]. After the first episode of clostridial colitis, the risk of recurrence may be exponential [3, 4]. Despite modern treatment options for *C. difficile* infection, there is an increase in morbidity and mortality associated with PMC [5]. Mortality from PMC against the background of *C. difficile* ranges from 6 % to 30 % [6]. The observations of K. Neemann et al. [7] show that in patients with malignant neoplasms, the incidence of PMC is 7 %, and about 8 % of these infected people develop a severe form of the disease. Fulminant *C. difficile* leads to toxic megacolon, has a mortality rate of almost 50 %, and in some cases, it is found to be resistant to treatment with drugs such as metronidazole, vancomycin, fidaxomicin [8]. According to R.L. Harries et al., based on an analysis of 13,728 surgical manuals, PMC is a potentially life-threatening complication of ileostomy closure surgery. The frequency of such a complication can reach 4 % [9]. In this article, we would like to review a clinical case demonstrating the successful treatment of PMC, which developed after the closure of the stoma and was complicated by the development of toxic megacolon, which makes the above observation interesting. According to the Department of coloproctology of the Irkutsk Regional Cancer Center (head of the department – Medvednikov A.A., Cand. Sc. (Med)), in 2020–2022, the incidence of PMC developed due to closure of various types of stomas was 3.2 %, sigmoid colectomy – 3.3 %, right hemicolectomy – 2.5 %.

Clostridioides difficile is a toxin–producing spore-forming gram-positive obligate anaerobe found everywhere in nature, most often in soil. Initially, the pathogen was named *Bacillus difficilis* because of its morphology and difficulty of cultivation [10]. Spores protect the microorganism from the damaging effects of temperature, oxygen, chemicals and disinfectants, radiation, which plays an important role in the spread of CDI.

This bacterium was first described by I.C. Hall and E. O'Toole in 1935 as a part of the normal microflora of newborns [11]. In 1970 J.G. Bartlett et al. determined the leading role of toxin A, secreted by *C. difficile,* in the pathogenesis of clindamycin-associated enterocolitis in Syrian hamsters [12]; later, this toxin was isolated from stool samples of patients with diarrhea. By 1978, *C. difficile* was clearly identified as a pathogenic agent of PMC [13].

C. difficile lives in the human intestine and in the environment in the form of spores. The sources of infection are sick people and asymptomatic bacterial carriers. According to the observations of E.J. Kuipers and C.M. Sura-

TABLE 1

RISK FACTORS FOR THE DEVELOPMENT OF PSEUDOMEMBRANOUS COLITIS

Factors affecting the normal intestinal microflora	Contact of patients with *C. difficile*	Patient-related factors
• antibiotic therapy – especially 2nd and 3rd generation cephalosporins, clindamycin, fluoroquinolones, even vancomycin; • antisecretory treatment with proton pump inhibitors and H2 blockers – contributes to a change in the biocenosis of the gastrointestinal tract; • the use of loperamide exacerbates the course of PMC.	• prolonged stay in a medical facility, including hospitals and nursing homes, prolonged contact in the ward with an infected patient; • long-term use of nasogastric tubes and enemas.	• older age – over 60–65 years old; • nutritional status – depletion, low initial albumin level; • immunodeficiency conditions, immunosuppression (glucocorticoid therapy, cytostatics, monoclonal antibodies); • abdominal surgery, organ transplantation; • severe concomitant pathology – CKD, diabetes mellitus, chronic inflammatory bowel diseases, COPD, malignant neoplasms.

Note. CKD is a chronic kidney disease; COPD is a chronic obstructive pulmonary disease.

wicz (2008), it turned out that up to 57 % of elderly people in nursing homes, 84 % of newborns, and 15 % of healthy adults were carriers of the microorganism [14]. *C. difficile* carriage reaches 16–35 % in inpatient patients, and the percentage is proportional to the length of hospital stay and increases when exposed to antibiotics [15]. The main ways of transmission of clostridial infection are fecal-oral transmission from person to person, through environmental pollution, through household items and the hands of medical staff. The risk factors for the development of pseudomembranous colitis are presented in Table 1.

PATHOGENESIS

The pathogenesis of CDI is complex and so far, it has not been sufficiently studied. It is known that the clinical picture of the disease is caused only by toxigenic strains of *C. difficile* [16]. However, pseudomembranous colitis is not formed in all cases. The reason for this is both the protective qualities of the intestinal microbiota and the response of the immune system. With the development of an imbalance of microorganisms and violation of the integrity of the mucous membrane of the colon, *C. difficile* colonizes the intestine, proliferates in it, forming vegetative toxin-producing forms. Their synthesis is encoded by the corresponding genes, and this aspect underlies the molecular biological methods of CDI diagnosis [17].

C. difficile causes multiple changes in the wall of the colon: total neutrophil infiltration; circulatory disorders in the form of dilation, vascular congestion and submucosal edema; obturating thrombi without signs of organization in the vessels of the submucosal layer, including superficial damage to the mucous membrane with the formation of "pseudomembranes" – exudative plaques. In the absence of effective specific therapy directed against *C. difficile*, the infection continues to progress further and contribute to the formation of extensive inflammatory changes [18]. It is generally believed that the combination of chronic diseases and antibiotic therapy in hospital patients affects the normal microbiota of the colon, increases susceptibility to colonization and production of *C. difficile* toxins, which increases the risk of PMC from 2 to 16 times [19].

C. difficile can produce toxins such as A (TcdA), B (TcdB) and binary AB, which contribute to the development of PMC:

- toxin A (enterotoxin) – disrupts the barrier function of the intestinal mucosa, stimulates guanylate cyclase, increases the secretion of fluid into the intestinal lumen and promotes the development of diarrhea, is produced 3–4 times more often than toxin B;
- toxin B (cytotoxin) – stronger than the toxin A by thousands of times, has a pronounced cytopathogenic effect by inhibiting the processes of protein synthesis in enterocytes and colonocytes, determines the severity of infection and the clinical picture of PMC [20];
- binary toxin of the NAP1/BI/027 ribotype (hospital infection in Quebec and clinics in the USA since 2003) – forms a complex on the membrane of an intestinal cell consisting of ADP-ribosyl transferase and a receptor, which subsequently penetrates into the enterocyte by receptor-mediated endocytosis and endosomal exchange and contributes to disruption of cell functioning through ADP-ribosylation of globular actin, which leads to disorganization of the cytoskeleton and subsequent cell death [12, 13]. This toxin also enhances the adhesion and colonization ability of *C. difficile* by inducing the synthesis of microtubules at the base of cell protrusions, which contributes to easier attachment to colonocytes [14, 15, 21].

The appearance of a binary toxin is associated with a mutation in the gene for the regulator-repressor of *C. difficile* toxin production, which leads to increased production of toxins A and B. In this case, toxins A and B are produced 16 and 23 times more, respectively. M.C. McEllistrem et al. show a tendency to a more severe course of the disease in patients whose feces contain a binary toxin [21].

Glutamate dehydrogenase is an enzyme that converts glutamate to α-ketoglutarate, produced by *C. difficile* in relatively large amounts compared to toxins A and B [22]. Although glutamate dehydrogenase (GDH) tests are sensitive, they are not as specific to PMC because this enzyme is produced by both toxigenic and non-toxigenic strains of the microorganism.

The patient's immune status is an important determining factor in the development of the disease. As the observations of J.K. Shim et al. show, bacterial carriers without clinical manifestations have higher concentrations of serum antibodies to toxin A than symptomatic patients and are less prone to the development of PMC [23]. For patients who develop PMC, it is characteristic that a higher level of antibodies to the toxin is associated with a shorter duration of the disease and a reduced risk of recurrence [24].

CLINICAL PICTURE

PMC clinical picture varies from asymptomatic colonization to fulminant toxic megacolon requiring surgical intervention.

In 2013, the classification of PMC by the severity of the disease, proposed by the American Gastroenterological Association (AGA), was published [25]. The classification is presented in Table 2.

With untimely diagnosis and lack of treatment, the disease progresses, that can lead to complications. A rare but life-threatening complication of the disease – toxic megacolon – that is defined as segmental or complete distension of the colon more than 6 cm in the presence of signs of colitis and systemic intoxication. Toxic megacolon syndrome occurs in 0.4–3 % of cases, with concomitant mortality from 38 to 80 % [26, 27]. The development of toxic megacolon in combination with shock, sepsis, and intestinal perforation is characterized as a severe form of clostrid-

TABLE 2

CLASSIFICATION OF PSEUDOMEMBRANOUS COLITIS BY THE GRAVITY OF THE DISEASE

Mild course	Moderate severity course	Severe course	Severe complicated course
• diarrhea* • minor abdominal pain	• diarrhea • body temperature increase up to febrile values	• diarrhea • abdominal pain of a spastic nature • fever to hectic values • hypoalbuminemia < 30 g/l, leukocytosis (over 15×10^9 leukocytes in peripheral blood) • Abdominal tenderness during palpation of the abdomen	• watery diarrhea with blood Plus one of the symptoms: • hypotension with or without vasopressors • fever ≥ 38.5 °C • signs of intestinal obstruction (acute nausea, vomiting, sudden cessation of diarrhea, bloating or radiographic signs of impaired passage through GIT), multiple organ failure • changes in mental status • leukocytes ≥ 35×10^9 or < 2×10^9 cells in peripheral blood, serum lactate level > 2.2 mmol/L

Note. * – according to the definition of the World Health Organization, this is loose stool corresponding to the 5^{th}–7^{th} types of the Bristol stool scale, occurring 3 or more times a day; GIT – gastrointestinal tract.

ial infection. For the first time, toxic dilation of the colon as a complication of PMC was described by C.H. Brown et al. more than forty years ago [28]. According to some data [29], the use of antiperistaltic drugs, for example, loperamide, in patients with PMC is associated with the development of toxic megacolon, probably because these drugs delay the release of the toxin.

PMC recurs after treatment in 3.7 to 64.0 % of cases [30]. A recurrent form of the disease is indicated when the clinical picture occurs less than 8 weeks after the end of therapy. Risk factors for recurrence of infection include: repeated administration of one or more antibiotics; age over 65 years; severity of the underlying disease; low albumin concentration; stay in the intensive care unit or in the hospital for more than two weeks [31, 32]. The recurrence rate of PMC is especially high among cancer patients, as shown by M.S. Chung et al. [33] (20.4 % vs. 9.5 %, respectively; $p = 0.005$), and cancer is an independent risk factor for recurrence. Cases of the occurrence of PMC in *patients with malignant neoplasms* against the background of postoperative chemoradiotherapy without the use of antibiotics in the anamnesis have been described [34, 35].

Studies show that the mortality rate directly associated with PMC in cancer patients was higher than in cancer patients without PMC (9.3 % vs. 7.4 %, respectively; $p < 0.0001$). PMC is also associated with longer hospital stays in cancer patients than in uninfected individuals (9 days vs. 4 days, respectively; $p < 0.0001$) [36].

DIAGNOSTICS

Rapid and accurate diagnosis of PMC is necessary not only for individual patient management, but also for the prevention of nosocomial transmission of infection. According to the recommendations of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) of 2009, all patients with diarrhea admitted to any hospital should be tested for *C. difficile* regardless of age, previous antibiotic use, concomitant pathology and its treatment [16]. The diagnosis of PMC is based on the clinical picture and laboratory data presented in Table 2, but the detection of toxin A and/or B in feces is fundamental.

Currently, various laboratory tests are available to detect *C. difficile*:

- detection of *C. difficile* products (GDH, toxins A and/or B) – methods of immunochromatographic, enzyme immunoassay, immunochemiluminescence assays, polymerase chain reaction (PCR);
- analysis of cytotoxicity of cell cultures and cultural methods of isolation of toxin-producing strains of *C. difficile* – reference standard methods for the diagnosis of PMC;
- molecular genetic tests – PCR, ribotyping, pulsed-field gel electrophoresis, multilocus assay and determination of the multilocus sequence.

The material for the diagnosis of clostridial infection is samples of fresh lumen feces of patients with diarrhea in an amount of about 10–15 ml. It is also possible to examine smears or tissue of the mucous membrane of the colon obtained during colonoscopy or during surgery.

Fibrocolonoscopy determines diffuse hyperemia and swelling of the mucous membrane of the colon, characteristic yellowish-white fibrinous plaques with a diameter of up to 20 mm or more, pseudomembranes – fused plaques – more often in the left half of the colon (Fig. 1, 2). It should be borne in mind that performing fibrocolonoscopy with a pathological change in the intestine can lead to perforation of the colon.

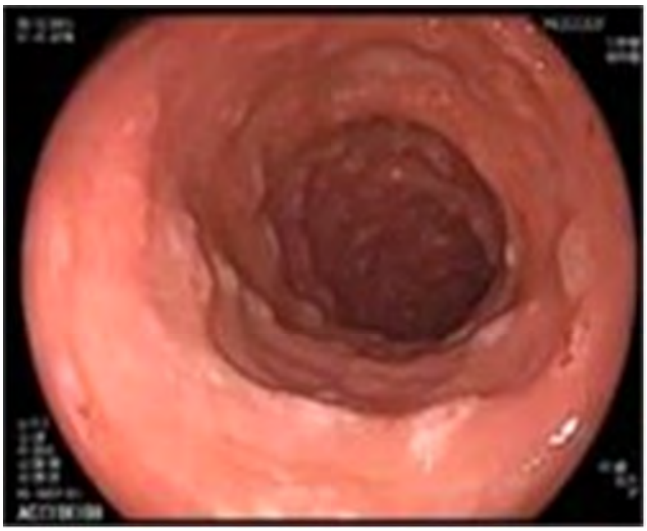

FIG. 1.
Endoscopic picture of pseudomembranous colitis (photo from the Dicom archive of Irkutsk Regional Cancer Center): the folds are smoothed; diffuse hyperemia of the mucosa with thickening of the intestinal wall; vascular pattern is blurred; characteristic whitish yellowish fibrinous plagues 2–5 mm in diameter

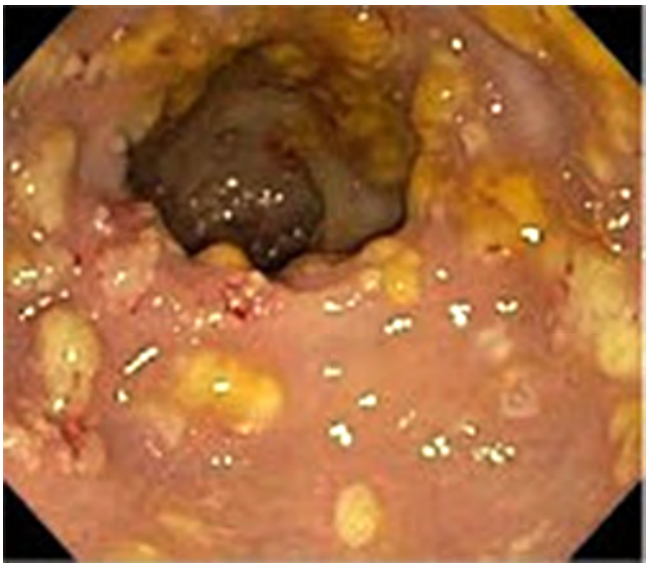

FIG. 2.
Endoscopic picture of pseudomembranous colitis (photo from the Dicom archive of Irkutsk Regional Cancer Center): intestinal mucosa is swollen, loose, hyperemic, capillary pattern is blurred; many yellowish white plagues 3–5 mm in diameter, which are tightly fixed to the mucous membrane

Ultrasound examination shows increased peristalsis of the small intestine and thickening of the colon wall.

Methods of radiological examination used to diagnose CDI:

1. Plain abdominal radiography: edematous colon with areas of thickening of the intestinal wall, haustration disorders are detected. In 30–35 % of patients, radiographic signs of small and large intestinal obstruction are determined.

2. Irrigoradiography: rounded "filling defects" – pseudomembranes – are revealed.

3. Computed tomography of the abdominal cavity can be informative to confirm the diagnosis of PMC. Typical signs are [37] thickening of the intestinal wall, narrowing of the intestinal lumen, effusion in the abdominal cavity, accordion sign (alternating edematous haustral folds separated by transverse mucous ridges filled with oral contrast material imitating an accordion), target sign (alternation of three concentric rings – high, low and high). Also, computed tomography of the abdominal cavity and pelvic organs can be used to determine the severity of the disease, identify toxic megacolon, intestinal obstruction or perforation of the intestinal wall.

TREATMENT

The treatment scheme for PMC, according to the Clinical Guidelines of the National Association of Specialists on Healthcare-Associated Infection Control and the Association of Proctologists of Russia (2017), is presented in Table 3.

Asymptomatic carriers of *C. difficile* have a relatively low risk of developing PMC [23], treatment is not recommended.

Antibiotic therapy is a cornerstone of the treatment of pseudomembranous colitis. R. Morales Chamorro et al. emphasize the importance of early detection of PMC in patients receiving chemotherapy and the initiation of antibacterial therapy immediately after diagnosis [38]. Some studies [39] show that 15–23 % of patients with PMC had spontaneous disappearance of symptoms within 48–72 hours after discontinuation of the antibiotic, as well as continued use of systemic antibiotics was associated with refractory treatment of PMC. However, in practice, it is almost impossible to cancel antibiotic therapy against the background of an infectious process.

The most commonly used antibiotics in PMC are metronidazole and vancomycin, including in cancer patients. These drugs are used either as monotherapy or in combination, depending on the severity of PMC, premorbid background, disease refractoriness and recurrence [40]. The usual dose of metronidazole is 500 mg 3 times a day for 10–14 days. Metronidazole has the same efficacy as vancomycin for the treatment of mild and moderate forms of PMC.

In a retrospective analysis conducted by S.R. Parmar et al. [41] in patients with hematological malignancies, including those who underwent hematopoietic stem cell transplantation (HSCT), patients were divided into 3 groups depending on the treatment received: metronidazole only; vancomycin only; combination therapy. The response rate was 53.7 %, 50 %, 38.5 % with metronidazole monotherapy, vancomycin monotherapy and combination therapy, respectively (p = 0.55). K. Tsuchida et al. [42] report a case involving

a 74-year-old patient with sigmoid colon cancer who received cefepime for the treatment of febrile neutropenia, who subsequently developed pseudomembranous colitis. Against the background of vancomycin enemas, the symptoms of PMC regressed.

The minimum inhibitory concentration (MIC) of vancomycin required to inhibit 90 % of strains (MIC_{90}) is 0.75–2.0 μg/ml. Vancomycin is used orally in the dose of 125 to 500 mg 4 times a day. In *vitro*, the MIC_{90} of metronidazole for *C. difficile* ranges from 0.20 to 2.0 μg/ml (median is 1 μg/ml). After administration by healthy volunteers, metronidazole is completely absorbed from the gastrointestinal tract and is not detected in feces. However, the concentration of this drug in feces is significantly higher if the stool is watery or unformed than if it is solid. This occurs as a result of an increase in the time of passage of the drug through the digestive tube, leading to incomplete absorption or excretion of the drug through the inflamed mucous membrane of the colon [43]. Intravenous administration of metronidazole should reach the luminal surface of the colon in therapeutic concentrations, which depends on the biliary secretion of the drug into the small intestine [43].

Oral vancomycin cannot reach areas of the colon that are not continuous segments of GIT, for example, in ascending ileostomy, obstructive colon resection (Hartmann surgery) or colostomy. If PMC is detected in a disconnected segment of the colon, it is recommended to administer vancomycin with an enema, which guarantees that the drug reaches the affected area [44]. Enemas with vancomycin are used – 500 mg in 100–500 ml of 0.9 % sodium chloride every 6 hours; the volume of the solution depends on the length of the treated segment. The duration of rectal administration of vancomycin is determined by the clinical course of PMC.

Tigecycline is a glycylcycline derivative of tetracycline and has bacteriostatic properties due to inhibition of protein translation in bacteria. It shows broad antimicrobial activity against gram-negative and gram-positive organisms, including *C. difficile*. Due to its properties, it is able to overcome the main mechanisms of resistance of microorganisms to tetracyclines.

Rifaximin is a semi-synthetic derivative of rifampicin and is characterized by a wide range of antimicrobial properties. The drug has a bactericidal effect by inhibiting DNA-dependent RNA polymerase of bacteria. Rifaximin is active against most gram-negative and gram-positive bacteria, anaerobes and aerobes and has in *vitro* activity against *C. difficile* [45].

Fecal microbiota transplantation means that the feces of a healthy person are transplanted into a patient, used in extreme cases when other treatment methods are ineffective [46]. Fecal transplantation has shown its effectiveness in cancer patients with recurrent PMC. Observations describe clinical cases in which two recipient patients underwent fecal microbiota transplantation after HSCT after unsuccessful standard antibacterial therapy. The first patient underwent two fecal transplants within 6 months, and the second patient was cured within 48 hours after one transplant [47, 48].

In recent years, several new specific therapeutic agents against C. difficile have appeared on the market. Fidaxomycin is an antibiotic from the group of macrolides that has a bactericidal effect and inhibits the synthesis of bacterial RNA. The drug is characterized by little or no systemic absorption after oral administration and a narrow spectrum of activity against gram-positive aerobic and anaerobic bacteria, including *C. difficile,* since 2011 it has been approved for the treatment of PMC in the USA. *The* dosage of fidaxomycin is 200 mg 2 times per day for 10 days. In terms of its effectiveness in *in vitro* studies, fidaxomycin was more active than vancomycin in PMC [49]. Human monoclonal antibody – bezlotoxumab – was approved by the U.S. Food and Drug Administration (FDA) in 2016. Bezlotoxumab binds to two very similar sites in the TcdB CROPs domain, thereby blocking the binding of the toxin to carbohydrate receptors. The interaction between the antibody and TcdB prevents intoxication. Given the specificity of these antibodies, it is not surprising that they have minimal adverse effects on the mi-

TABLE 3

TREATMENT SCHEME FOR PSEUDOMEMBRANOSIC COLITIS

Mild and moderate course	Severe course	Severe complicated course
• metronidazole (500 mg orally 3 times a day for 10 days); • in the absence of clinical effect in 5–7 days the drug is replaced with vancomycin (125 mg 4 times a day *per os for* 10 days).	• vancomycin (125 mg orally 4 times a day for 10 days)	• vancomycin orally (500 mg 4 times a day) in combination with metronidazole (500 mg 3 times a day intravenously); • if it is impossible to administer the drug orally, vancomycin is prescribed rectally (500 mg), diluted in 500 ml of 0.9% sodium chloride solution and administered as enemas 4 times a day; • symptomatic therapy

crobiota. Bezlotoxumab is a successful history of monoclonal antibody therapy, but this approach is not without limitations, which is not least due to production difficulties and, as a result, high cost [50, 51].

The development and clinical trials of vaccines against *C. difficile* remain promising. This is evidenced by the large number of registered clinical trials on ClinicalTrials.gov: these include the currently completed studies *NCT01887912, NCT02316470, NCT02561195, NCT040026009*, and those studies still in progress – *NCT05805826*. The studied drugs are based on the action of detoxified recombinant forms of *C. difficile* toxins and enter the body parenterally. It is likely that vaccination can become an effective method of prevention in certain groups of high-risk.

Symptomatic treatment of PMC involves restoration of the water-electrolyte balance with balanced crystalloid solutions, drug prevention of venous thromboembolism (these patients are at high risk), correction of protein-energy deficiency, detoxification therapy, correction of anemia.

If a toxic megacolon is detected in a patient with PMC and there are no signs of improvement in the condition against the background of conservative therapy, surgical intervention is indicated. A number of publications [52–55] investigating the level of postoperative mortality in toxic megacolon against the background of PMC confirm that surgery is indicated in severe cases and that subtotal colectomy is the operation of choice. P.A. Lipsett et al. [52] studied the medical histories of 13 patients in one institution who underwent surgery for PMC, which was only 0.39 % of the total number of patients with PMC who were observed in this hospital over a 6-year period of time. The overall mortality rate in this study was 38 %: 100 % of patients who underwent colon resection died, whereas in the subtotal colectomy group, the mortality rate was only 14 %. Similarly, K. Koss et al. [53] examined the medical histories of 14 patients who underwent surgery for PMC and found that the overall mortality rate was 35 %, with 11 % in the subtotal colectomy group and 80 % in the colon resection group. Patients diagnosed with *C. difficile*-associated colitis before surgery demonstrated a statistically significant survival advantage (85.7 % vs. 33.3 %) [53]. One study demonstrated a tendency to decrease mortality in patients with complicated PMC who underwent colectomy, compared with those who did not undergo the surgery [56]. Postoperative mortality was higher after total colectomy among patients with preoperative acute renal failure, the need for vasopressors and respiratory failure requiring artificial lung ventilation (ALV) [57]. It is worth noting that in recent years, taking into account the development of surgical tactics and perioperative management of patients, the mortality rate in left (right) hemicolectomy has decreased and equaled that of colproctectomy (30.1 % each; $p > 0.99$) [58, 59]. Despite this, the updated recommendations of the World Society of Emergency Surgery (WSES) in 2019 retained colproctectomy as the main choice of surgical intervention [57, 60].

Negative prognostic risk factors for death in patients undergoing colectomy include the development of shock determined by the need for vasopressors, an increase in lactate levels (≥ 5 mmol/L), a change in mental status, multiple organ failure, as well as the need for lung ventilation [61]. This indicates that early surgical treatment before the development of shock and multiple organ dysfunction leads to improved survival. Currently, there are no clear criteria defining the threshold for surgical intervention. However, the more negative prognostic factors a patient has, the earlier the issue of surgical consultation and surgical treatment should be considered.

PREVENTION

The existing primary and secondary prevention of *C. difficile* is multifaceted and includes various measures. Primary prevention is a set of measures aimed at preventing the influence of risk factors on the body: vaccination; optimal work and rest regime; high–quality nutrition; physical activity; environmental protection, etc. Secondary prevention includes a list of measures aimed at eliminating risk factors that, under conditions of stress, decreased immunity, excessive loads on the body, can lead to the onset, exacerbation and recurrence of the disease. The most effective method of secondary prevention is considered to be medical examination as a comprehensive method of early detection of diseases, dynamic monitoring, targeted treatment, as well as rational consistent recovery [62].

Patients with suspected clostridial infection should be placed in a separate room or in a room where patients with already confirmed CDI are located.

A.B. Zafar et al. [63] showed that the strict application of periodic educational measures, environmental disinfection and strict hand washing were associated with a decrease in detected cases of PMC from 155 to 67 per year in medical institutions.

CASE STUDY

In March 2013, patient L. was diagnosed with sigmoid colon cancer pT2aN0M0G2 of the 2nd stage of the 2nd clinical group, confirmed histologically (moderately differentiated adenocarcinoma). Of the concomitant diseases, it should be noted the presence of coronary heart disease, congestive heart failure stage 1, exogenous constitutional obesity 1st degree, cerebral atherosclerosis.

On 25.03.2013, the patient was admitted to Irkutsk Regional Cancer Center for the surgical treatment of the oncological process. On 01.04.2013, left hemicolectomy and anterior rectal resection were performed. In the postoperative period, he received intravenously ceftazidime 1 g 3 times/day. On 04.04.2013, the patient was diagnosed with colorectal anastomosis failure; relaparotomy, resection of colorectal anastomosis, terminal transverse colostomy in the left mesogastric zone were performed. After repeated surgery, ceftazidime was canceled, meropenem was prescribed intravenously 1 g 3 times/day extended in-

fusion for 7 days. The patient was discharged in a satisfactory condition, and did not receive adjuvant chemoradiotherapy for the next six months.

On 18.10.2013, the patient was admitted for inpatient treatment at the coloproctology department of Irkutsk Regional Cancer Center in order to restore intestinal continuity.

On 22.10.2013 (on the 1st day), reconstruction of the colon was performed. During the operation, a pronounced adhesive process was performed in the abdominal cavity, enterolysis was performed. Rectal stump was isolated with pronounced technical difficulties (adhesive process with the back wall of the bladder, contact bleeding of tissues), then hardware descendorectoanastomosis was applied.

In the postoperative period, he received antibiotic therapy: ceftriaxone 2 g 2 times/day, metronidazole 500 mg 3 times/day for 5 days and antimycotics: caspofungin 70 mg on the 1st day, then 50 mg/day. Routine postoperative anesthesia, thromboprophylaxis with early activation, and early enteral feeding were performed.

On the 10th day, due to the appearance of subfebrile fever, spastic abdominal pain and leukocytosis, the patient was prescribed cefoperazone + sulbactam intravenously 2 g 2 times/day, continued administration of antimycotics (caspofungin). Against this background, there was a positive trend, the above symptoms regressed.

On the 16th day, the patient's general condition worsened, nausea, vomiting, febrile fever, frequent loose stools appeared. On examination, the abdomen was noticeably distended and painful on palpation.

The patient was transferred to the intensive care unit; pseudomembranous colitis was suspected and diagnosed (toxins A and B of *C. difficile* were found in the stool), antibacterial therapy was initiated: *per os* vancomycin 250 mg 4 times/day, metronidazole 500 mg 3 times/day, continued administration of antimycotics (caspofungin). Diagnosis of *C. difficile* was carried out by an immunochromatographic rapid test for qualitative detection of the toxin A and toxin B *C. difficile* antigens in feces (DUO TOXIN A+B-CHECK-1).

Fibrocolonoscopy was not performed in the patient due to the severity of the condition and the high probability of iatrogenic perforation of the dilated intestine during manipulation.

Plain abdominal radiography was performed, which revealed radiographic signs of toxic megacolon (Fig. 3).

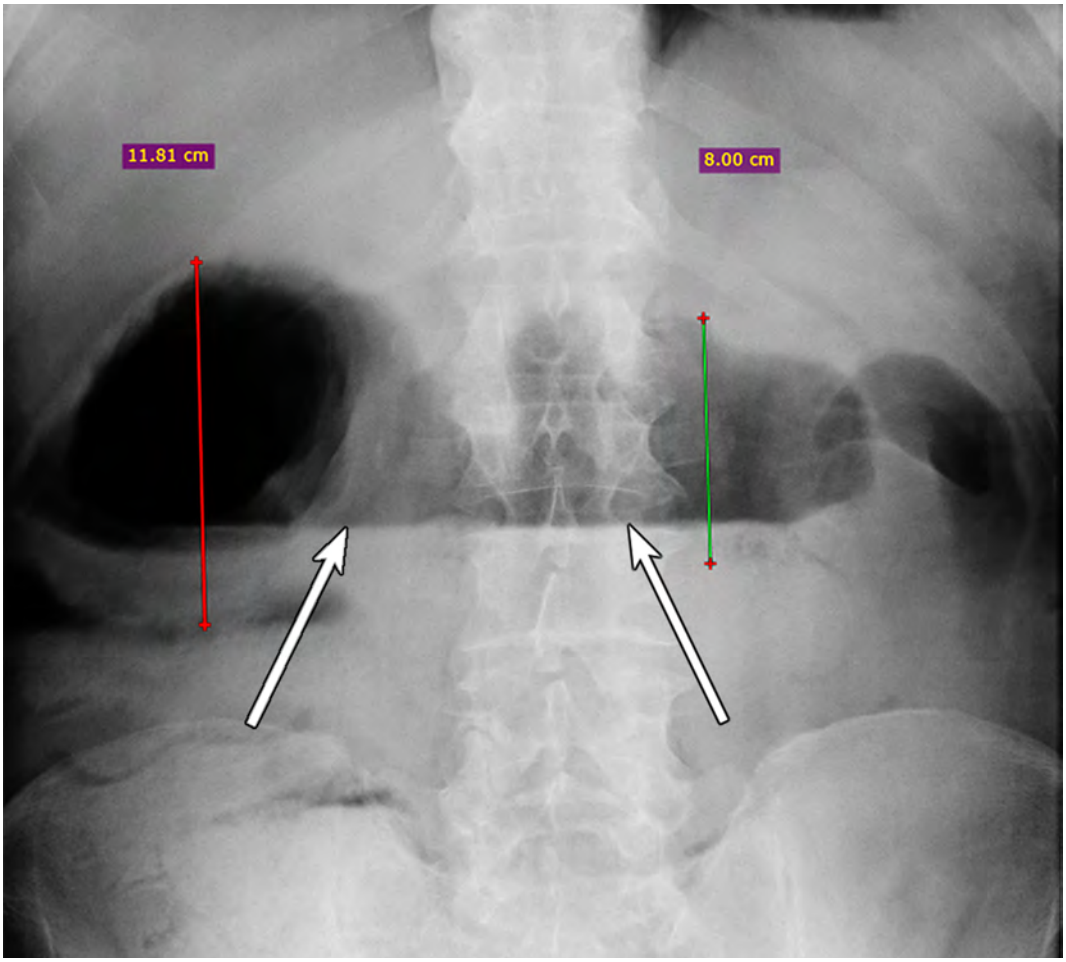

FIG. 3.

Patient L., plain radiograph of the abdominal cavity from 07.11.2013 (photo from the Dicom archive of Irkutsk Regional Cancer Center): pronounced expansion of the colon 11 cm with a wide horizontal level liguid – gas (showed with arrow)

On the 18th day, the patient developed septic shock symptoms, accompanied by a decrease in the level of consciousness to sopor, hypotension, tachycardia, ineffective independent breathing, a decrease in diuresis below 0.5 ml/kg/h, which required prosthesis of vital functions (ALV, infusion of cardiotonics).

It was decided to perform an emergency relaparotomy. During the revision of the abdominal organs, up to 300 ml of odorless light effusion was determined; the colon was swollen to 8–11 cm in diameter throughout; the loops of the small intestine were collapsed to the level of the ileum, increased in diameter at the level of 30 cm from the ileocecal junction due to the accumulation of gases and liquids. A dense infiltrate consisting of a large omentum and retroperitoneal tissue was determined in the left subdiaphragmatic space. Intraoperatively, a toxic megacolon was diagnosed against the background of PMC, a total colectomy and nasointestinal intubation was performed. A colon stump was formed at the level of the upper ampullary rectum with a machine stitch. The preparation is crossed at the level of the terminal part of the ileum. A terminal ileostomy was performed in the right iliac region.

In the postoperative period, the patient's condition remained extremely severe, and prosthesis of vital functions continued: ALV (mandatory ventilation); administration of inotropic drugs (0.5 % dopamine solution 5–10 μg/kg/min).

Due to the development of acute renal damage of prerenal genesis on the 18th day, the patient required renal replacement therapy (RRT), prolonged veno-venous hemofiltration was performed for 96 hours: Aquarius hemoprocessor (Nikkiso Medical, Japan), Aquamax HF-19 hemofilter (Nikkiso Medical, Japan), anticoagulation – unfractionated heparin with the control of blood clotting time and activated partial thromboplastin time. Antibacterial therapy was performed taking into account creatinine clearance and RRT. During the subsequent course of treatment, the patient underwent 4 sessions of prolonged veno-venous hemodiafiltration.

Postoperative anesthesia was sufficient, prolonged epidural anesthesia was performed with a 0.2 % solution of ropivacaine 16–20 mg/hour. Parenteral nutrition was carried out with combined three-component mixtures containing the 3rd generation of fat emulsions with omega-3 fatty acids.

From the 20th day, doripen was prescribed 500 mg 4 times/day by extended infusion, linezolid 600 mg 2 times/day, continued administration of caspofungin 50 mg 1 time/day. Against the background of intensive therapy, the patient's condition stabilized, positive dynamics was noted, tracheal extubation was performed, the nasointestinal tube was removed, enteral nutrition into the nasogastric tube with standard mixtures was started.

The patient underwent daily sanitation tracheobronchoscopy, ultrasound examination of the pleural cavities, if necessary, thoracocentesis (detection of fluid accumulation in the pleural cavities with atelectasis of the lower lobes of the lungs).

On the 22nd day, chest radiography revealed left-sided lower lobe pneumonia. Due to the progression of respiratory failure on the 24th day, the patient repeatedly required prosthesis of lung function.

On the 30th day, caspofungin was discontinued, voriconazole was prescribed (on the 1st day – 600 mg 2 times/day, then 400 mg 2 times/day), clarithromycin 500 mg 2 times/day was added to the prescribing list.

On the 31st day, a tracheostomy was performed due to the predicted prolonged ALV.

From the 37th day, the patient breathed independently through a tracheostomy cannula with insufflation of moistened oxygen.

On the 43rd day, inhalation of sodium colistimethate through a nebulizer 1 million 2 times/day was added to therapy. The radiological dynamics is positive – by 05.12.2013 the infiltration of lung tissue had regressed.

On the 45th day, the patient was transferred from the Department of Anesthesiology and intensive care No. 4 to the specialized department.

On the 55th day, the patient was discharged in satisfactory condition from the coloproctology department of Irkutsk Regional Cancer Center.

The dynamics of the main laboratory parameters, the days of artificial lung ventilation and medications for antibacterial and antifungal therapy are shown in Figure 4.

DISCUSSION OF CLINICAL OBSERVATION

Thus, the clinical example clearly demonstrates that fulminant clostridial colitis is a life-threatening and severe complication. The literature data suggest that performing a total colproctectomy has been a life-saving surgery for many years. The question of the timeliness of its implementation in this pathology is one of the most difficult. A belated decision can lead to further aggravation of the condition, which manifests itself in the form of acute renal damage, respiratory and cardiovascular insufficiency and, in turn, leads to an increase in the percentage of lethal outcomes.

The treatment of complications in the postoperative period also requires a versatile approach in intensive care, highly qualified personnel, and the availability of expensive equipment for prosthesis of vital functions.

Given that total colproctectomy is a complex surgical procedure that not every surgeon is capable of performing, the issue of deciding on timely surgical treatment in small clinics is very difficult. In such cases, we believe that the issue of timely transfer of the patient to a multidisciplinary institution should be addressed. Such a decision can be made after consultations with expert specialists – both face-to-face and using telemedicine.

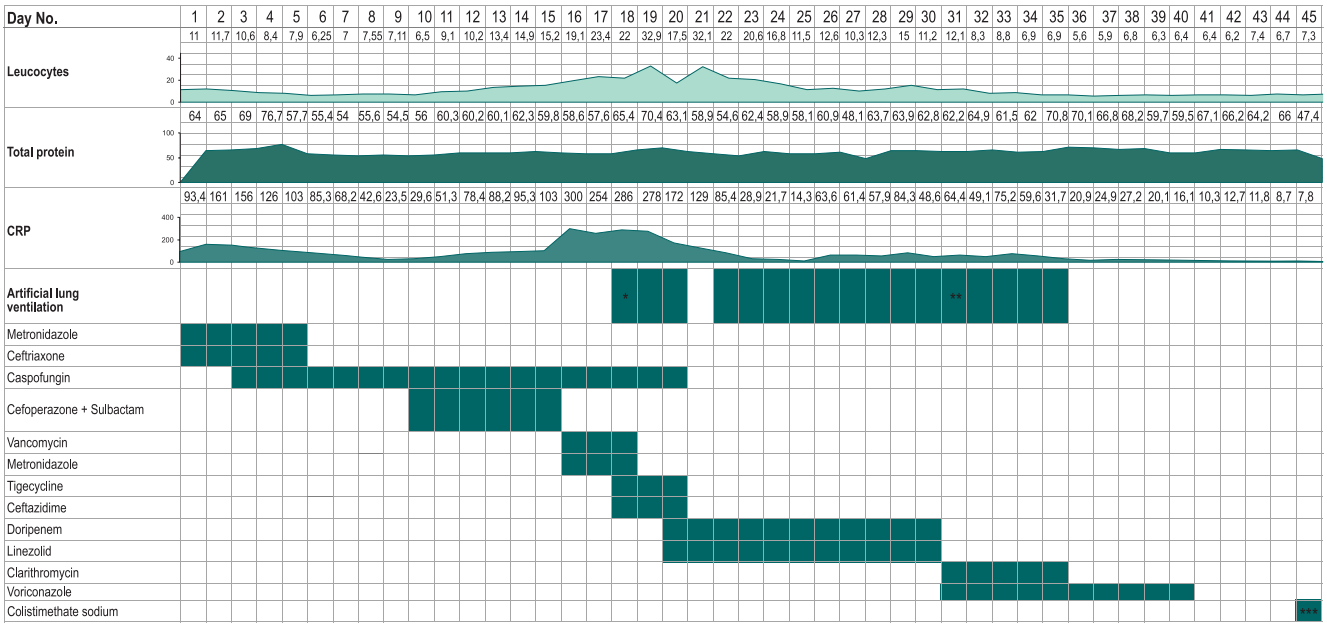

FIG. 4.

*Dynamics of laboratory parameters, days of artificial lung ventilation and medications for antibacterial and antifungal therapy: : * – day of the surgery – colproctectomy; ** – day of the surgery – tracheostomy; *** – administration of sodium colimestate continued until the 55th day*

CONCLUSION

1. In recent years, there has been increased interest in the problem of clostridial infection in clinical practice, in particular, in oncological hospitals. This is due to an exponential increase in morbidity and a high risk of CDI complications.

2. The widespread and uncontrolled use of antibacterial drugs creates prerequisites for the development of resistance of clostridial microorganisms to therapy and the appearance of highly virulent strains of bacteria.

3. The development of pseudomembranous colitis significantly increases the duration of hospitalization, increases treatment costs and can significantly worsen prognosis, increasing the likelihood of death.

4. An important and still unresolved problem is the lack of a unified approach to laboratory microbiological diagnosis of clostridial infection in our country, which leads to a delay in diagnosis, irrational antibiotic therapy and the spread of the pathogen inside the medical institution.

5. To reduce the risk of developing clostridial infection, it is recommended to rationally prescribe antibacterial drugs and reduce, if possible, the duration of hospitalization, especially in people over 65 years of age.

6. When choosing the surgical scope of surgery between total colectomy and hemicolectomy, it is worth remembering that total colectomy is the surgery of choice for patients diagnosed with toxic megacolon. A total colectomy is a potentially life-saving surgery.

7. Postoperative mortality after total colectomy is increased in patients with preoperative acute renal failure, cardiovascular insufficiency requiring vasopressors, and respiratory insufficiency requiring ALV. Therefore, the decision to carry out the surgery should be made before the development of organ failure.

8. A clinical example clearly demonstrates the development of pseudomembranous colitis, complicated by toxic megacolon. The clinical case presented by us represents a late decision on the need for total colproctectomy, which led to the development of multiple organ dysfunction, and only the comprehensive and timely use of respiratory, renal replacement, hepatoprotective, antibiotic and antifungal therapy led to the successful treatment of this patient.

9. In our opinion, the optimal period for surgical intervention was the 16th day. Against the background of deterioration of the general condition, nausea, vomiting, febrile fever, frequent loose stools appeared. During the radiography, a diagnosis of toxic megacolon was made, but at the same time the patient was hemodynamically stable, there were no symptoms of respiratory and renal insufficiency.

Conflict of interest

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

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EXPERIMENTAL RESEARCHES

EFFECT OF A SORBENT COMPOSITION BASED ON ALUMINUM OXIDE AND POLYDIMETHYLSILOXANE ON THE REPRODUCTIVE SYSTEM OF *DB/DB* FEMALE MICE WITH GENETICALLY DETERMINED OBESITY AND TYPE 2 DIABETES MELLITUS

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ABSTRACT

Metabolic syndrome, obesity, type 2 diabetes mellitus are characterized by the accumulation of toxic metabolic products in the internal environment of the organism. The development of innovative medicines based on a sorbent matrix modified with biologically active molecules remains relevant. The sorbent composition from aluminum oxide and polydimethylsiloxane is considered promising.

The aim of the study. *To investigate the effect of the sorbent composition from aluminum oxide and polydimethylsiloxane on the uterus and ovaries of db/db mice with obesity and type 2 diabetes mellitus.*

Materials and methods. *The sorbent composition (0.665 g/kg in 200 µl of distilled water) was administered to 14-week-old animals through an intragastric tube once a day for 7 days. The comparison groups were female rats injected with placebo (daily intragastrical administration of 200 µl of water for 7 days) and intact animals. Digital images of light-optical preparations stained with hematoxylin and eosin were processed using Image-Pro Plus 4.1 software. In the ovaries, the numerical density of primordial, primary, secondary follicles and corpuses luteum was determined. The width of the uterus layers, the diameters of the blood and lymphatic vessels, the width of the interstitial fissures in both organs were measured. The statistical significance of differences was determined using the Mann – Whitney test.*

Results. *In the myometrium and endometrium of the uterus of db/db mice, dilatation of arteries, veins, lymphatic vessels and edema were noted due to the accumulation of tissue fluid in the interstitium layers. There were no tertiary follicles in the ovaries. The introduction of the sorbent composition contributed to a decrease in the diameters of arteries, veins, lymphatic vessels of the uterus, a decrease in edema in both organs due to the narrowing of the prelymphatic slits, and stimulated an increase in the numerical density of secondary follicles.*

Conclusion. *A corrective effect of the sorbent composition of aluminum oxide and polydimethylsiloxane on prelymphatic slits, blood and lymphatic vessels in the uterus and ovaries in db/db mice with obesity and type 2 diabetes mellitus was revealed.*

Key words: *db/db mice, obesity, type 2 diabetes mellitus, uterus, ovaries, blood and lymphatic vessels, prelymphatic slits*

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ВЛИЯНИЕ СОРБЕНТНОЙ КОМПОЗИЦИИ ИЗ ОКСИДА АЛЮМИНИЯ И ПОЛИДИМЕТИЛСИЛОКСАНА НА ПОЛОВУЮ СИСТЕМУ САМОК *DB/DB* МЫШЕЙ С ГЕНЕТИЧЕСКИ ДЕТЕРМИНИРОВАННЫМ РАЗВИТИЕМ ОЖИРЕНИЯ И САХАРНОГО ДИАБЕТА 2-ГО ТИПА

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РЕЗЮМЕ

Метаболический синдром, ожирение, сахарный диабет 2-го типа характеризуются накоплением токсических продуктов метаболизма во внутренней среде организма. Актуальна разработка инновационных лекарственных препаратов на основе сорбентной матрицы, модифицированной биологически активными молекулами. Перспективной является сорбентная композиция из оксида алюминия и полидиметилсилоксана.

Цель исследования. *Изучение влияния данной сорбентной матрицы на матку и яичники db/db мышей с ожирением и сахарным диабетом 2-го типа.*

Материалы и методы. *Сорбентную композицию (0,665 г/кг в 200 мкл дистиллированной воды) вводили 14-недельным животным через внутрижелудочный зонд 1 раз в день в течение 7 суток. Группами сравнения служили самки, которым вводили плацебо (ежедневно внутрижелудочно 200 мкл воды в течение 7 суток), и интактные животные. Цифровые изображения светооптических препаратов, окрашенных гематоксилином и эозином, обрабатывали в программе Image-Pro Plus 4.1. В яичниках определяли численную плотность примордиальных, первичных, вторичных фолликулов и жёлтых тел. Измеряли ширину слоёв матки, диаметры кровеносных и лимфатических сосудов, ширину интерстициальных щелей в обоих органах. Статистическую значимость различий определяли с помощью критерия Манна – Уитни.*

Результаты. *В миометрии и эндометрии матки db/db мышей отмечались дилятация артерий, вен, лимфатических сосудов и отёк за счёт скопления тканевой жидкости в интерстиции слоёв. В яичниках отсутствовали третичные фолликулы. Введение сорбентной композиции способствовало уменьшению диаметров артерий, вен, лимфатических сосудов матки, снижению отёка в обоих органах за счёт сужения прелимфатических щелей, стимулировало увеличение численной плотности вторичных фолликулов. Заключение. Выявлен корригирующий эффект сорбентной композиции из оксида алюминия и полидиметилсилоксана на прелимфатики, кровеносные и лимфатические сосуды в матке и яичниках у db/db мышей с ожирением и сахарным диабетом 2-го типа.*

Ключевые слова: *db/db мыши, ожирение, сахарный диабет 2-го типа, матка, яичники, кровеносные и лимфатические сосуды, прелимфатики*

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INTRODUCTION

Metabolic syndrome, obesity and type 2 diabetes mellitus are an urgent medical and social problem for society associated with a decrease in the quality of life and life expectancy. Metabolic syndrome, the main feature of which is obesity, includes a group of abnormalities (accumulation of abdominal fat, hyperglycemia, hyperinsulinemia, dyslipidemia, hypertension) leading to diabetes and cardiovascular diseases. The prevalence of metabolic syndrome is high and has been found to affect between 17 % and 46 % of the general population in developed countries [1]. In Russia, according to Rosstat estimates, 5.1 million people were diagnosed with diabetes mellitus and 1.9 million were diagnosed with obesity in 2020 [2]. There has been a significant increase in the prevalence of obesity among young people: over the past twenty years, the proportion of 12–19-year-olds suffering from this disease has increased from 5 to 14 % [3].

An increase in the prevalence of obesity among women of reproductive age leads to an increase in infertility. Metabolic syndrome occurs in 30–33 % of women with impaired reproductive function [4]. Among patients with recurrent endometrial hyperplastic processes, the frequency of this pathology reaches 70 % [5]. 30–70 % of women with polycystic ovary syndrome (PCOS) are overweight and obese. At the same time, PCOS is the most common cause (70 %) of anovulatory infertility [6].

The multifactorial pathogenesis of metabolic syndrome, associated with the involvement of many body systems, determines the complexity and effectiveness of therapy, despite clear ideas about the mechanisms of development of reproductive system pathology [4–6]. In metabolic syndrome and type 2 diabetes mellitus, disorders of carbohydrate, fat, protein, mineral and water-salt metabolism are noted. The accumulation of products of impaired metabolism and edema fluid in the interstitium leads to an increase in the load on the regional lymphatic apparatus. Sorption therapy is aimed at removing toxic products from the interstitium by hemo-, plasma-, and lymphosorption. Long-term studies at the Research Institute of Clinical and Experimental Lymphology – Branch of the Federal Research Center Institute of Cytology and Genetics, Siberian Branch of Russian Academy of Sciences (RICEL – Branch of the ICG SB RAS) have shown that correction with sorbents has a positive effect on the lymphatic system, improves its drainage and detoxification functions [7–9]. RICEL – Branch of the ICG SB RAS is developing a direction for the development of innovative medicines based on the embedding of biologically active molecules into the structure of porous carriers in which the pores of sorbents act as containers for active pharmaceutical ingredients. Currently, a sorbent matrix based on aluminum oxide and polydimethylsiloxane is actively used as a carrier of biologically active molecules of melatonin, lithium, etc. However, we have not found studies on the effect of metabolic syndrome, obesity and type 2 diabetes mellitus on the blood and lymphatic vessels of the pelvic organs in the available literature. To study the effect of the «sorbent + medicine» complexes, it is necessary to investigate the effect of the sorbent itself on the reproductive organs in these conditions. The study of the follicular composition of the ovaries, blood and lymphatic vessels, the prelymphatic slits of the ovaries and uterus, as well as the effect of sorption therapy on these structures in women with metabolic syndrome is impossible due to ethical and methodological problems. Therefore, we selected a model of genetically programmed obesity and type 2 diabetes mellitus in homozygous mice of the BKS.Cg-Dock7^{m}+/+Lepr^{db}/J (*db/db* mice) line.

So far, it is known that *db/db* mice have a point mutation in the leptin receptor gene, have pathological obesity and are characterized by chronic hyperglycemia, atrophy of pancreatic beta cells, hypoinsulinemia and dyslipidemia. The hyperlipidemic metabolic microenvironment caused by such a mutation leads to the accumulation of lipids in the theca cells and ovarian interstitium, progressive lipoapoptotic cytoatrophy of the compartments of follicular granulosa cells of the ovaries and epithelial cells of the endometrium of the uterus, which ultimately contributes to the development of infertility and premature organ involution in these mice [10, 11]. Thus, we consider *db/db* mice with obesity and type 2 diabetes mellitus as a suitable model for studying the drainage function of the abovementioned sorbent composition on the reproductive system of females.

THE AIM OF THE STUDY

To identify the effect of a sorbent composition of aluminum oxide and polydimethylsiloxane on the width of the myometrium and endometrium of the uterus, follicular apparatus and corpuses luteum of the ovaries, the state of blood, lymphatic vessels, uterine and ovarian prelymphatic slits of *db/db* mice with genetically determined development of obesity and type 2 diabetes mellitus.

MATERIAL AND METHODS

Experiment design

Female homozygous mice of the BKS.Cg-Dock7^{m}+/+Lepr^{db}/J (*db/db* mice) line at the age of 14 weeks were kept in controlled barrier rooms of the Laboratory Animal Genetic Resource Center of the Institute of Cytology and Genetics, Siberian Branch of Russian Academy of Sciences (RFMEFI62119X0023). The animals had free access to food (SSNIFF (Germany), balanced granulated feed) and water. The experiments were carried out in accordance with 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 the Principles of Good Laboratory Practice (GLP). The study was approved by the local Ethics committee (Protocol No. 128 dated March 15, 2017).

A powdered sorbent complex of aluminum oxide and polydimethylsiloxane with a particle size up to 0.1 mm, a bulk density close to 1, an average pore volume of up to 0.26 cm^3/g, and a specific surface area of up to 160 m^2/g were used in the work. The sorbent matrix produced in Russia, created on the basis of aluminum oxide and polymethylsiloxane, is a hydrophilic-hydrophobic matrix compatible with biological tissues [12].

The following groups were formed:

1. Intact animals ($n = 6$).
2. Placebo ($n = 7$) – mice who received intragastric 200 µl of distilled water daily for 7 days.
3. Sorbent ($n = 5$) – mice that were injected with a sorbent composition (0.665 g per 1 kg of body weight, diluted in 200 µl of distilled water) through an intragastric tube once a day for 7 days.

Methods

After the animals were removed from the experiment by craniocervical dislocation, the uterus and ovaries were taken for histological examination. The organs were fixed in 10 % neutral formalin, dehydrated in a series of alcohols of increasing concentration and placed in a histomix. Slices with a thickness of 5 µm obtained using the Leica RM2155 microtome (Leica Biosystems, USA) were stained with hematoxylin and eosin. The preparations were examined under an Axioplan light microscope (Carl Zeiss, Germany) connected to a digital camera at magnifications of the eyepiece ×10, lens ×5. Digital images of the preparations were processed using the Image-Pro Plus 4.1 program. The width of the myometrium, endometrium, diameter of blood and lymph vessels, width of interstitial slits (prelymphatic slits) of the uterus and ovaries were measured in microns. To assess the follicular composition of the ovaries of mice, the numerical density of the primordial, primary, secondary follicles and corpuses luteum in ovary slices which passed through the ovarian gate was determined. Follicle identification was carried out according to the classification described in the work of A.E. Katelnikova et al. (2020) [13]. Each class of follicles is characterized by a fixed number of epithelial cells: primordial, consisting of an oocyte and surrounded by a single layer of flattened follicular cells, between which there are slits, and as the follicle forms, epithelial cells connect; primary (single-layered, preantral) and secondary (multilayer, antral) follicles formed by an oocyte and cubic granulosa cells; tertiary (preovulatory) follicles, which later become mature and ready for ovulation.

Statistical processing

Statistical processing was performed using the Statistica 12 software package (StatSoft Inc., USA). The values of the median, the first and third quartiles, and the arithmetic average were determined. The statistical significance of the differences in the compared values was established using the non-parametric Mann – Whitney test. The differences were considered statistically significant at $p < 0.05$.

RESULTS

In female *db/db* mice, unlike normal laboratory mice, no clearly defined inner circular and outer longitudinal layers were found in the uterine myometrium. Between the layers of the myometrium, there was also no expression of the vascular layer between the layers of the myometrium, in which the blood and lymph vessels of the uterus normally pass. Only individual vessels, which were located between edematous muscle fibers and single myocytes, were detected in *db/db* mice. The average diameters of the myometrial vessels in *db/db* mice were: arteries – 11.93 (11.43; 17.46) microns, veins – 20.81 (20.07; 28.39) µm, lymphatic vessels – 30.79 (25.54; 36.86) µm. Average diameters of endometrial vessels: arteries – 11.82 (11.79; 14.18) µm, veins – 24.57 (18.74; 26.35) µm, lymphatic vessels – 23.21 (21.67; 31.50) µm. The muscle layer in the females looked edematous due to dilation of the prelymphatic slits and accumulation of tissue fluid in the interstitium. The average width of the prelymphatic slits in the myometrium was 28.86 (26.66; 32.16) µm, and the average thickness of the myometrium reached 138.61 (125.75; 152.27) µm. The same pattern of edema was detected in the endometrium (Fig. 1a). The average width of the prelymphatic slits in the endometrium was 14.13 (13.78; 14.50) µm, and the average thickness of the endometrium itself reached 347.48 (301.82; 358.44) µm. In the placebo group, the edema pattern persisted and the thickness of the layers did not change.

After treatment with the sorbent composition of aluminum oxide and polydimethylsiloxane of *db/db* mice, the thickness of the uterine layers decreased compared to placebo ($p < 0.0122$) (Fig. 2). Sorbent treatment contributed to the narrowing of the diameters of both the bearing vessels – arteries ($p < 0.0216$) and vessels draining fluid from the organ – veins ($p < 0.0122$), lymphatic vessels ($p < 0.0122$) (Fig. 1b; Fig. 3).

The width of the prelymphatic slits decreased ($p < 0.0122$; Fig. 4a, b), interstitial edema decreased, making the endometrium and muscle layer appear more "dense". A decrease in the diameter of all types of vessels and the width of the prelymphatic slits, a decrease in edema in both layers *db/db* mice uterus after administration of aluminum oxide and polydimethylsiloxane may indicate a pronounced draining effect of this sorbent composition on the interstitium of different uterus layers.

Administration of aluminum oxide and polydimethylsiloxane had no effect on the size of the diameters of blood and lymph vessels in the ovaries, but caused a pronounced tendency to decrease the width of the prelymphatic slits in them ($p < 0.0513$) (Fig. 4b). In the follicular apparatus of the ovaries of female *db/db* mice, tertiary follicles were absent, and the numerical densities of other structures were: primordial follicles – 15.0 (11.75; 17.5) units, primary follicles – 5.0 (3.5; 5.0) units, secondary – 3.0 (3.0; 3.75) units, corpuses luteum – 3.0 (2.25; 3.0) units. The quantitative composition of the ovarian follicular apparatus in animals of the placebo group did not differ statistically significantly.

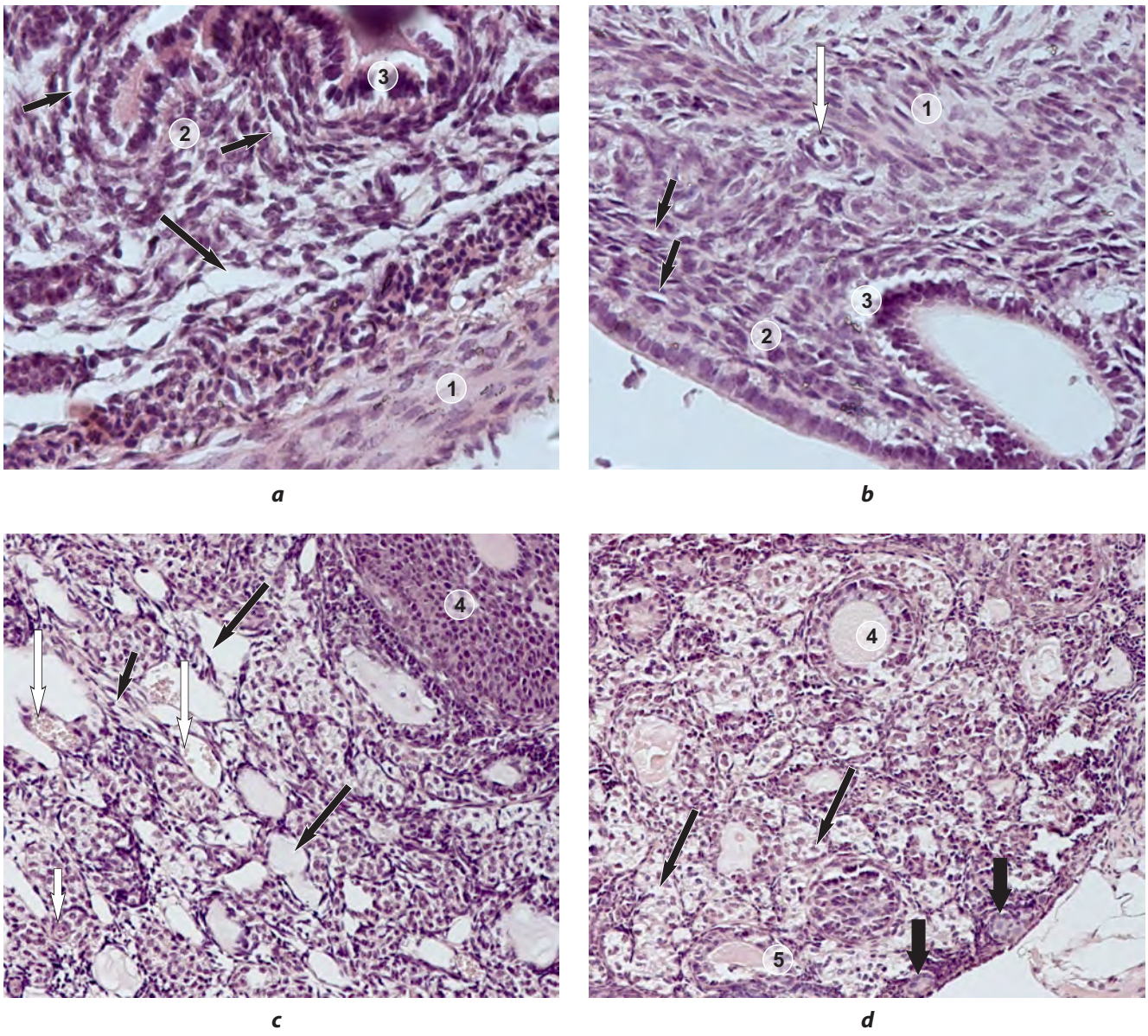

FIG. 1.

*Uterus (**a**, **b**) and ovaries (**c**, **d**) in db/db mice before (**a**, **c**) and after (**b**, **d**) treatment with of the sorbent complex:* 1 *– myometrium;* 2 *– endometrium;* 3 *– uterine glands;* 4 *– secondary follicles; thick black arrows – primordial follicles; long black arrows – lymphatic vessels; short black arrows – prelymphatic slits; long white arrows – veins; short white arrow – artery. Magnification × 400 (**a**, **b**), × 200 (**c**, **d**)*

Administration of the sorbent matrix had no effect on the number of primordial, primary follicles and corpuses luteum, but contributed to an increase in the numerical density of secondary follicles – up to 5.0 (4.0; 6.0) units compared with placebo (2.5 (2.0; 3.0) units; $p < 0.0176$). Perhaps it was the improvement in the drainage of the ovarian interstitium and the removal of toxic metabolic products that contributed to the maturation of secondary follicles.

DISCUSSION

It is known that in obese women, the presence of high levels of lipid droplets in the cumulus and granulosa cells of the ovaries is associated with unfavorable results of gestation and carrying of a pregnancy [14]. Changes in the concentrations of glucose and lipids (free fatty acids/triglycerides) in the intercellular space affect the interstitial and cytoplasmic microchemical environment, which significantly changes the cellular diffusion of nutrients and the rate of active transmembrane flow. For example, it has been shown that a progressive increase in the absorption of interstitial and cytoplasmic lipids is associated with DNA fragmentation of endometrial epithelial cells and follicular granulosa cells of the ovaries in *db/db* mice by lipoinfiltration into the chromatin matrix, that leads to the development of nuclear lipoapoptosis [15]. At the same time, conditions are created in the interstitium to increase the level of free radicals, reactive oxygen/nitrogen species, which in turn contributes to the accumulation of low molecular weight hyaluro-

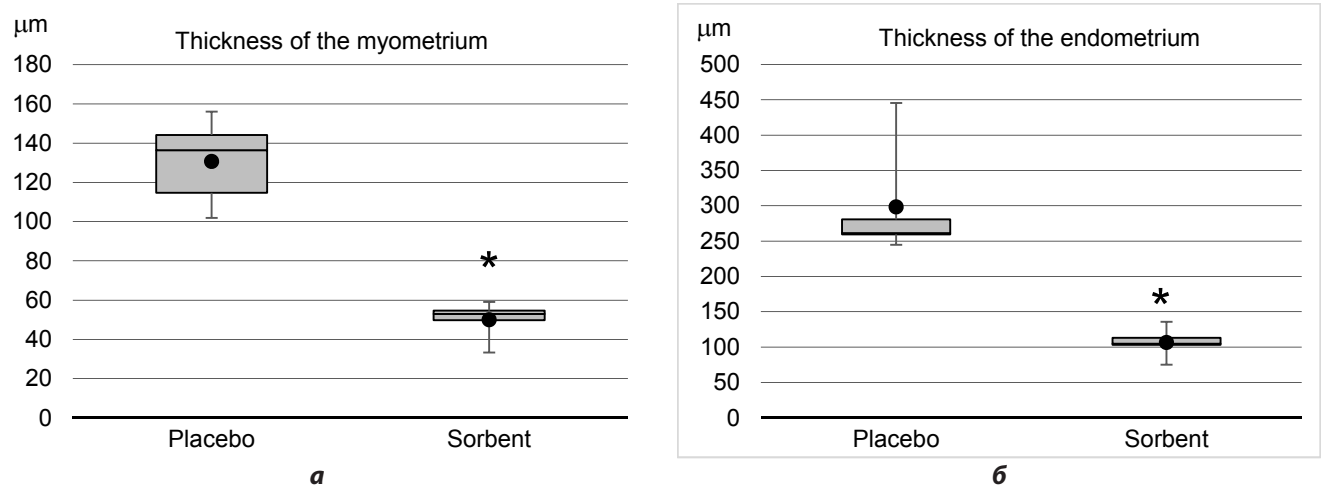

FIG. 2.

*Quantitative assessment of the thickness of the myometrium (**a**) and endometrium (**b**) in the uterus of db/db mice before and after administration of aluminum oxide and polydimethylsiloxane: ▬ – median; ▭ – 25%–75% percentiles; ● – arithmetic average; ✱ – $p < 0.05$*

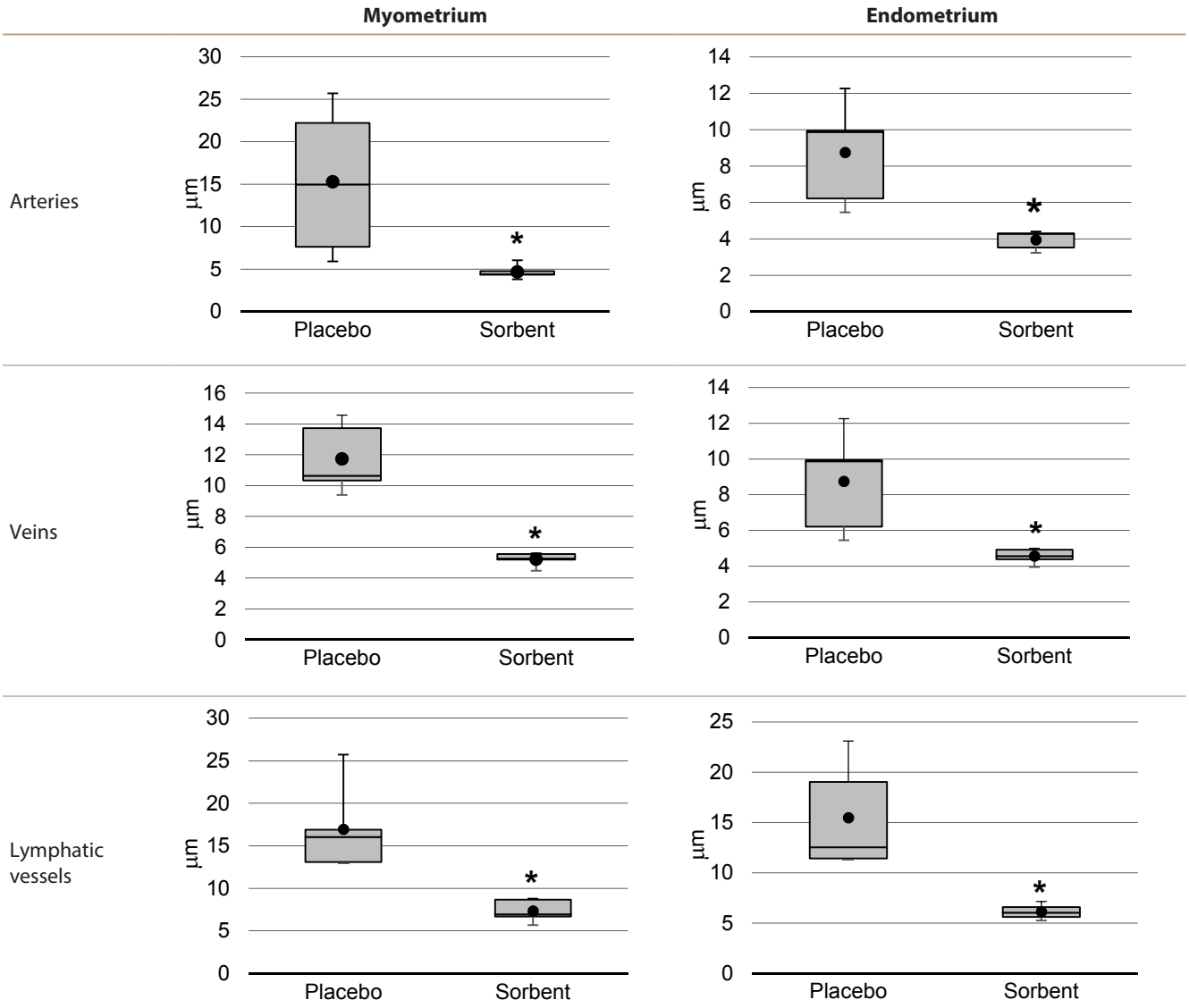

FIG. 3.

Quantitative assessment of the diameters of arteries, veins and lymphatic vessels in the myometrium and endometrium in the uterus of db/db mice before and after administration of aluminum oxide and polydimethylsiloxane: ▬ – median; ▭ – 25%–75% percentiles; ● – arithmetic average; ✱ – $p < 0.05$

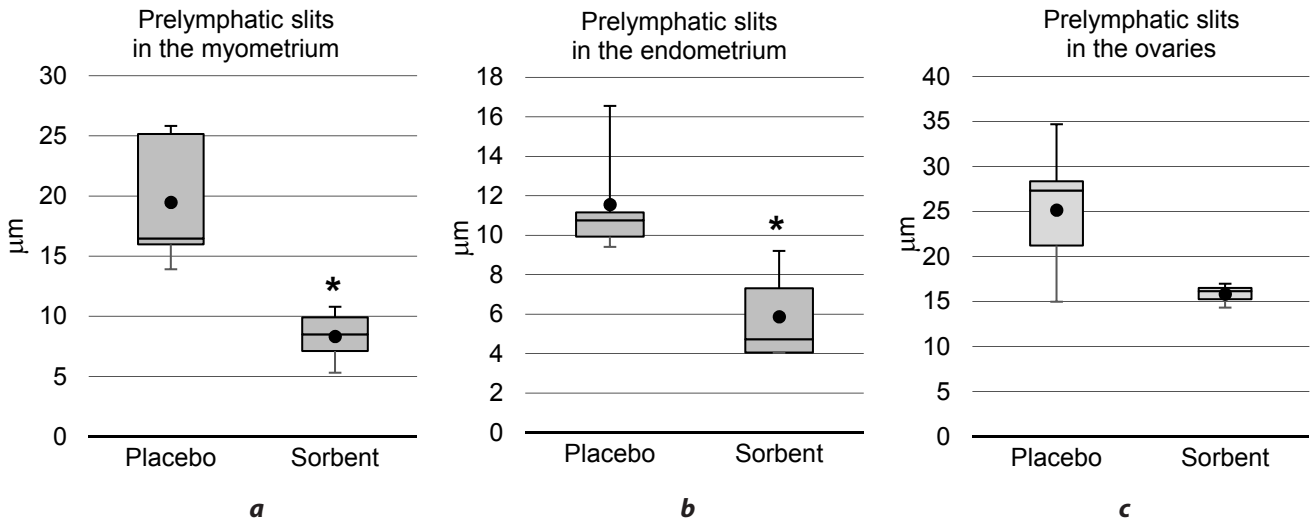

FIG. 4.

*Quantitative assessment of the width of interstitial slits (prelimphatics) in the myometrium (**a**) and endometrium (**b**) of the uterus and in the ovaries (**c**) of db/db mice before and after administration of aluminum oxide and polydimethylsiloxane: ▬ – median; ▭ – 25%–75%th percentiles; ● – arithmetic average; ✱ – $p < 0.05$*

nan of the intercellular matrix. Hyaluronic acid, fragmented to low molecular weight polymers (from 1 to 500 kDa), can not only disrupt the permeability of the blood vessel wall, but also, by binding to the LYVE-1 receptor, destroy the integrity of the endothelial barrier of lymphatic vessels [16, 17]. As a result, we observe dilatation of arteries, veins, lymphatic vessels, interstitial prelymphatic slits of the myometrium and endometrium of the uterus, dilation of the prelymphatic slits in the ovaries and edema due to accumulation of tissue fluid in the interstitial of these organs. Thus, in *db/db* obese mice with type 2 diabetes mellitus, we have already noted an expansion of sinusoidal capillaries and a low level of LYVE-1 expression in the liver [18].

In patients with metabolic syndrome, angiography data are usually provided – when assessing the nature of damage to the coronary arteries, multivessel and polysegmental lesions are more often described [19, 20]. We have not found an analysis of the size of the diameter of veins, lymphatic vessels in the organs of the female reproductive system in the available literature. In our study, it was shown that treatment with the sorbent composition based on aluminum oxide and polydimethylsiloxane has a positive effect on the water homeostasis of reproductive organs, as evidenced by a decrease in the diameters of arteries, veins and lymphatic vessels of the myometrium and endometrium of the uterus, a narrowing of the width of the prelymphatic slits and a decrease of ovarian and uterine tissue edema in *db/db* mice. These results indicate the draining and detoxification effects of aluminum oxide and polydimethylsiloxane.

Obesity affects the hypothalamic-pituitary axis throughout a woman's life. It affects the processes of puberty and is associated with an increased risk of hyperandrogenism and ovarian dysfunction. The association of hyperinsulinemia and hyperandrogenism in disorders of the latters, including PCOS, is well known [5]. The study of the number of follicles in the ovaries of *db/db* mice deserves attention. So, M.V. Denisenko et al. [21] when studying the ovaries of laboratory outbred female mice, counted up to 6 primordial follicles in 1 field of view. It is known that the numerical density of follicles in the ovaries depends on age. A pattern has been traced between the number of primordial follicles in 1 field of vision and the age of a woman: at the age of 18–35, the number of primordial follicles was 7.5 ± 2.5, at the age of 36–55 years – 1 ± 1 (× 240) [21, 22]. The number of all types of follicles in mice decreases significantly every 4 weeks with age [11]. In our study on *db/db* mice, it was revealed that the number of primordial follicles in the ovaries is significantly higher than in ordinary laboratory mice. This is consistent with the results and conclusions of researchers who observed polycystic ovaries in women with metabolic syndrome and type 2 diabetes mellitus [23–25]. Most of the symptoms of polycystic ovaries occur at the beginning of puberty. Persistent hormonal imbalance leads to the formation of multiple small antral follicles and an irregular anovulatory menstrual cycle, that ultimately causes infertility in women. Insulin resistance, cardiovascular diseases, abdominal obesity, psychological disorders, infertility and cancer are also associated with PCOS. Hyperandrogenism causes insulin resistance and hyperglycemia, which leads to oxidative stress and abdominal obesity. As a result, inflammation, production of reactive oxygen species, insulin resistance and hyperandrogenism also increase [26, 27].

Hyperandrogenism affects gene expression in endocrine theca cells, promotes an unbalanced change in the subtypes of granulosa cells [28] and even leads to apoptosis of the latters, whereas the peripheral conversion of androgens to estrogens suppresses the secretion of gon-

adotropins. Q.L. Zhang et al. [29] explain the impaired follicle development (a decrease in the number of preantral and antral follicles) by an increase in insulin-dependent phosphorylation of protein kinase B and levels of caspase-3 in ovaries of mice with hyperinsulinemia. Most of the changes related to ovarian physiology in obesity are the result of transcriptional disorders caused by altered leptin signaling. Increased leptin levels stimulate higher expression of *CART*, which encodes an endogenous neuropeptide that plays a key role in regulating follicle atresia in granulosa cells of overweight and obese animals and humans [30]. Obesity can lead to the activation of inflammatory pathways and the creation of an unbalanced microenvironment around cells, as well as to a higher expression of molecular inflammatory mediators in the follicles themselves, contributing to irreversible damage to the latters [30]. Obese women have elevated levels of C-reactive protein in follicular fluid, indicating that there is a direct effect of the woman's metabolism on the ovarian follicle microenvironment and is a marker of inflammation and oxidative stress. All this contributes to the delay in follicle maturation and the development of anovulation [5, 23, 30]. In our study, it was found that the treatment of *db/db* mice with a sorbent composition based on aluminum oxide and polydimethylsiloxane leads to an increase in the numerical density of secondary follicles, relative to the placebo group. Perhaps it was the improvement in the drainage of the ovarian interstitium and the removal of toxic metabolic products that contributed to the maturation of secondary follicles.

CONCLUSION

Thus, in female *db/db* mice with genetically determined development of obesity and type 2 diabetes mellitus, dilation of the blood and lymph vessels of the myometrium and endometrium in the uterus, expansion of interstitial slits (prelymphatic slits) and edema in the ovaries and both layers of the uterus, a large number of primordial follicles and absence of tertiary follicles in the ovaries are observed. Administration of the sorbent composition based on aluminum oxide and polydimethylsiloxane leads to a decrease in the diameters of the blood and lymph vessels of the uterus, a narrowing of the width of the prelymphatic slits, that contributes to the reduction of tissue edema of reproductive organs, the structuring of the uterine tissue layers and the improvement of the functional activity of the ovaries, as evidenced by an increase in the number of secondary follicles.

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Conflict of interest

The authors of this article declare the absence of a conflict of interest.

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