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Журнал «Acta Biomedica Scientifica» зарегистрирован в Федеральной службе по надзору в сфере связи, информационных технологий и массовых коммуникаций (РОСКОМНАДЗОР). Свидетельство о регистрации СМИ – ПИ № ФС 77–69383 от 06 апреля 2017 г.

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.

Соучредители – Федеральное государственное бюджетное научное учреждение «Научный центр проблем здоровья семьи и репродукции человека» (ФГБНУ НЦ ПЗСРЧ) (664003, Иркутская обл., г. Иркутск, ул. Тимирязева, д. 16), Федеральное государственное бюджетное научное учреждение «Иркутский научный центр хирургии и травматологии» (ИНЦХТ) (664003, Иркутская обл., г. Иркутск, ул. Борцов Революции, д. 1), Федеральное государственное автономное учреждение «Национальный медицинский исследовательский центр «Межотраслевой научно-технический комплекс «Микрохирургия глаза» имени академика С. Н. Фёдорова» Министерства здравоохранения Российской Федерации (ФГАУ «НМИЦ «МНТК «Микрохирургия глаза» им. акад. С. Н. Фёдорова» Минздрава России) (127486, г. Москва, Бескудниковский б-р, д. 59А).

Журнал включён в Реферативный журнал и базу данных ВИНИТИ. Сведения о журнале публикуются в международной справочной системе по периодическим и продолжающимся изданиям «Ulrich's Periodicals Directory». Журнал индексируется в таких базах данных, как РИНЦ, Scopus, DOAJ и др.

Журнал «Acta Biomedica Scientifica» входит в «Перечень ведущих рецензируемых научных журналов и изданий, выпускаемых в Российской Федерации, в которых должны быть опубликованы основные научные результаты диссертаций на соискание учёной степени кандидата и доктора наук».

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EDITOR-IN-CHIEF'S PREFACE

EDITOR-IN-CHIEF'S PREFACE TO ISSUE 2, 2023

Sergey I. Kolesnikov

Member of RAS

Dear readers of the journal and its authors!

We are happy to present you the next issue of our journal. There are quite a lot of interesting articles and reviews in it, but as leading articles I would name three publication articles that carry new information on the still relevant problem of COVID-19 and post-COVID syndrome.

The article of N.V. Semenova et al. (Irkutsk) revealed the persistence of changes in thyroid status in women in the post-reproductive period for 12 months after the moderate course of COVID-19, which requires special attention of clinicians.

In their article M.Yu. Shkurnikov and S.I. Kolesnikov (Moscow) showed for the first time that in patients who recovered from a severe form of COVID-19, the level of hsa-miR-19b-3p miRNA, which is able to bind to regions of SARS-CoV-2 encoding proteins that suppress intracellular mechanisms of immunity (NSP3, NSP9). In addition, this microRNA is able to stimulate the functional activity and proliferation of cytotoxic T-lymphocytes. The results can be used in the development of antiviral drugs based on RNA interference, as well as in the development of predictive test systems.

The work of A.Yu. Karateev (Moscow) is devoted to mathematical modelling of the effectiveness of anti-epidemic measures using original models of cellular automata with intercellular boundaries. This simple model successfully describes the course of the epidemic and allows you to predict the dynamics of those infected and the measures being introduced.

A large block is devoted to various technologies or epidemiological studies of **surgical pathologies**. Very important are works of M.B. Tatarinova et al. from the Irkutsk Branch of S. Fyodorov Eye Microsurgery Federal State Institution on preoperative management of ophthalmosurgical patients taking new anticoagulants, as well as of Yu.V. Malysheva et al., who proved that surgical failure of operations in glaucoma is accompanied by initially high concentrations of IL-6, IL-8, TGF-β in the tear and suppression of MMP-9 and VEGF-A in the postoperative period.

In the work of I.V. Pendyurin et al. from Novosibirsk, the results of surgical treatment of sacral schwannomas were analyzed and their connection with the surgical approach was shown.

The study of A.N. Zharikov et al. from Barnaul proves the effectiveness of the use of VAC-systems in the surgical treatment of unformed small intestinal fistulas in postoperative peritonitis. V.S. Panteleev et al. from Ufa, evaluated the results of surgical treatment of alveococcosis and developed an algorithm for diagnosis and treatment, which reduces the time of surgery, blood loss and the number of complications.

Research by E.B. Topolnitskiy et al. from Tomsk in the experiment showed the feasibility of using a two-layer metal knitwear made of titanium nickelide when replacing a thoracoabdominal defect.

I would especially single out the work of N.V. Protopopova et al. from Irkutsk, dedicated to a new technology – laser hatching in programs with thawed embryo transfer in women with tubal-peritoneal infertility.

Two works are presented by scientists from Vladivostok. So, Atamas O.V. and Antonyuk M.V. once again proved that diabetes mellitus, long smoking history, low HDL cholesterol levels are associated with severe atherosclerosis. A.V. Yurenko et al. revealed the role of disturbances in fatty acid metabolism and plasmalogen synthesis, an increase in inflammatory lipid mediators in the dysfunction of small airways in bronchial asthma aggravated by obesity.

The therapeutic section is also represented by works from the institutions of St. Petersburg. So, E.N. Zabroda et al. showed that individuals with high sleep reactivity to stress are characterized by greater anxiety in combination with sleep disturbance. Scientists-paediatricians A.N. Zavyalova et al. revealed that in advanced

stages of chronic kidney disease in children with overweight, the proportion of fat mass predominates, and the proportion of active cell mass is lower, which indicates the depletion of protein reserves and possible insufficient intake from food.

In the joint work of authors from Omsk and St. Petersburg (Kostyukova I.V. et al.), it has been shown that against the background of a decrease in the incidence and mortality from tuberculosis, there is an alarming change in the structure of bacterial excretion with a predominance of multiple and extensive drug resistance, as well as a 10-fold increase in the incidence of tuberculosis with HIV infection.

Interesting data are presented in two experimental works. One of them, authors from Tomsk and Tyumen, headed by the Member of the Russian Academy of Sciences S.V. Popov, showed the involvement of PKCδ, PI3-kinase and ERK1/2 kinase in the infarct-limiting effect of the opioid peptide deltorphin II. They deny the essential role of superoxide and hydroxyl radicals in reperfusion injury of the heart after coronary occlusion.

The work of A.V. Akhova and A.G. Tkachenko from Perm showed a significant activation of soxRS-regulon system genes after osmotic stress caused by NaCl, which may provide pre-adaptation of bacteria to harmful factors, including antibacterial drugs.

I have no doubt that the reader will be interested in reviews and meta-analyses based on the databases of PubMed, Cochrane, E-Library, Scopus, Springer and foreign recommendations.

First of all, this is a review by S.V. Kolomentsev et al. from St. Petersburg, which outlines the provisions on perioperative ischemic stroke (definition, risk factors, pathogenesis, predictive models), strategies for pre- and intraoperative prevention.

A group of Novosibirsk scientists led by V.G. Selyatitskaya (Sorokin M.Yu. et al.) continues the tradition of research into the role of the circadian rhythm in regulating metabolism. This time, the protective factors in the circadian regulation of energy metabolism that prevent the development of diabetes mellitus and cardiovascular diseases in individuals with the so-called "metabolically healthy" type of obesity are discussed. This is important for determining evidence-based diet and exercise regimens, approaches to therapy.

Close to the topic is the review by I.E. Pleshchev et al. from Yaroslavl, Ivanovo and Moscow on the prevalence of sarcopenia in the elderly age group, the causes of its occurrence and modern methods of prevention and physical rehabilitation of elderly patients.

Quite informative is the review by Z.S. Saakyan et al. from Yakutsk and Irkutsk about mathematical modelling of the degree of risk of rupture of various types of cerebral arteries, on which preoperative planning and the choice of tactics for surgical treatment of patients are based.

For the first time in the 30-year history of our journal, we publish a review in English by V.A. Vorobev et al. from Irkutsk on the effectiveness of the extended program for the recovery of urological patients.

A number of articles (Karapetyan G.S. and Shuyskiy A.A. from Moscow; Zhdanova L.V. and Laperdina M.L. from Ulan-Ude; Styazhkina S.N. et al. from Izhevsk) are devoted to interesting and important isolated cases from clinical practice or the use of new drugs for treatment.

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ПРЕДИСЛОВИЕ ГЛАВНОГО РЕДАКТОРА К № 2 (2023)

Колесников Сергей Иванович

академик РАН

Уважаемые читатели журнала и его авторы!

Представляем Вам очередной номер журнала. В нём достаточно много интересных статей и обзоров, но в качестве передовых статей я назвал бы три статьи, несущие новую информацию по всё ещё актуальной проблеме COVID-19 и постковидного синдрома.

В статье Н.В. Семёновой и соавт. (Иркутск) выявлено сохранение у женщин в пострепродуктивном периоде изменений тиреоидного статуса на протяжении 12 месяцев после среднетяжёлого течения COVID-19, что требует особого внимания клиницистов.

В статье М.Ю. Шкурникова и С.И. Колесникова (Москва) впервые показано, что у пациентов, выздоровевших после тяжёлой формы COVID-19, в плазме крови статистически значимо повышен уровень микроРНК hsa-miR-19b-3p, которая способна связываться с регионами SARS-CoV-2, кодирующими белки, подавляющие внутриклеточные механизмы иммунитета (NSP3, NSP9). Кроме того, данная микроРНК способна стимулировать функциональную активность и пролиферацию цитотоксических Т-лимфоцитов. Результаты могут быть использованы при разработке противовирусных препаратов на основе РНК-интерференции, а также при разработке прогностических тест-систем.

Работа А.Ю. Каратеева (Москва) посвящена математическому моделированию эффективности противоэпидемических мер с помощью оригинальных моделей клеточных автоматов с межклеточными границами. Эта простая модель успешно описывает ход эпидемии и позволяет прогнозировать динамику инфицированных и вводимые меры.

Большой блок посвящён различным технологиям либо эпидемиологическим исследованиям **хирургических патологий**. Очень важные работы Иркутского филиала ФГАУ «НМИЦ «МНТК «Микрохирургия глаза» имени академика С.Н. Фёдорова» Минздрава России М.Б. Татариновой и соавт. по предоперационному ведению офтальмохирургических больных, принимающих новые антикоагулянты, а также Ю.В. Малышевой и соавт., доказавших, что хирургическому неуспеху операций при глаукоме сопутствуют исходно высокие концентрации ИЛ-6, ИЛ-8, TGF-β в слезе и подавление MMP-9 и VEGF-A в послеоперационном периоде.

В работе И.В. Пендюрина и соавт. из Новосибирска проанализированы результаты хирургического лечения шванном крестца и показана их связь с операционным доступом.

В исследовании А.Н. Жарикова и соавт. из Барнаула доказывается эффективность применения VAC-систем в хирургическом лечении несформированных тонкокишечных свищей при послеоперационном перитоните. В.С. Пантелеевым и соавт. из Уфы оценены результаты хирургического лечения альвеококкоза и разработан алгоритм диагностики и лечения, что позволяет сократить время операции, кровопотерю и количество осложнений.

Исследование Е.Б. Топольницкого и соавт. из Томска в эксперименте показало целесообразность использования двухслойного металлотрикотажа из никелид титана при замещении торакоабдоминального дефекта.

Я бы особо выделил важную для практики работу Н.В. Протопоповой и соавт. из Иркутска, посвящённую новой технологии – лазерному хэтчингу в программах с переносом размороженного эмбриона у женщин с трубно-перитонеальным бесплодием.

Две работы представлены учёными из Владивостока. Так, О.В. Атамась и М.В. Антонюк в очередной раз доказали, что с тяжёлым атеросклерозом ассоциированы сахарный диабет, длительный стаж курения, пониженный уровень холестерина ЛПВП. А.В. Юренко и соавт. выявили роль нарушений метаболиз-

ма жирных кислот и синтеза плазмалогенов, увеличение воспалительных липидных медиаторов в дисфункции малых дыхательных путей при бронхиальной астме, отягощённой ожирением.

Терапевтический раздел представлен также работами из учреждений Санкт-Петербурга. Так, Е.Н. Заброда и соавт. показали, что лица с высокой реактивностью сна к стрессу характеризуются большей тревожностью в сочетании с нарушением сна. Учёные-педиатры А.Н. Завьялова и соавт. выявили, что при продвинутых стадиях хронической болезни почек у детей с избыточной массой тела значительно преобладает доля жировой массы, а доля активной клеточной массы ниже, что свидетельствует об истощении белковых запасов и возможного недостаточного поступления с пищей.

В совместной работе авторов из Омска и Санкт-Петербурга (Костюкова И.В. и соавт.) на фоне снижения заболеваемости и смертности от туберкулёза показано тревожное изменение структуры бактериовыделения с преобладанием множественной и широкой лекарственной устойчивости, а также рост в 10 раз заболеваемости туберкулёзом с ВИЧ-инфекцией.

Интересные данные приводятся в двух экспериментальных работах. Одна из них, авторов из Томска и Тюмени во главе с академиком РАН С.В. Поповым, показала вовлечение ПКСδ, PI3-киназы и ERK1/2 киназы в инфаркт-лимитирующий эффект опиоидного пептида дельторфина II. Они отрицают существенную роль супероксидного и гидроксильного радикалов в реперфузионном повреждении сердца после коронарооккклюзии.

Работа А.В. Аховой и А.Г. Ткаченко из Перми показала значимую активацию генов системы soxRS-регулона после осмотического стресса, вызванного NaCl, что может обеспечивать преадаптацию бактерий к вредящим факторам, в том числе к антибактериальным препаратам.

Я не сомневаюсь, что заинтересуют читателя обзоры и метаанализы, основанные на базах данных PubMed, Cochrane, E-Library, Scopus, Springer и зарубежных рекомендациях.

Прежде всего это обзор С.В. Коломенцева и соавт. из Санкт-Петербурга, в котором изложены положения о периоперационном ишемическом инсульте (определение, факторы риска, патогенез, модели прогнозирования), стратегии пред- и интраоперационной профилактики.

Продолжает традицию исследований роли циркадного ритма в регулировании метаболизма группа новосибирских учёных под руководством В.Г. Селятицкой (Сорокин М.Ю. и соавт.). На этот раз обсуждены защитные факторы в циркадной регуляции энергетического метаболизма, препятствующие развитию сахарного диабета и сердечно-сосудистых заболеваний у лиц с так называемым «метаболически здоровым» типом ожирения. Это важно для определения научно обоснованных режимов питания и физических нагрузок, подходов к терапии.

Близок к теме обзор И.Е. Плещёва и соавт. из Ярославля, Иваново и Москвы по распространённости саркопении в пожилой возрастной группе, причинам её возникновения и современным методам профилактики и физической реабилитации пожилых пациентов.

Достаточно информативен и обзор З.С. Саакяна и соавт. из Якутска и Иркутска о математическом моделировании степени риска разрыва различных типов церебральных артерий, на чём основаны предоперационное планирование и выбор тактики хирургического лечения пациентов.

Впервые в 30-летней истории нашего журнала публикуется обзор на английском языке В.А. Воробьева и соавт. из Иркутска об эффективности расширенной программы восстановления урологических больных.

Ряд статей (Карапетян Г.С., Шуйский А.А. из Москвы; Жданова Л.В. и Лапердина М.Л. из Улан-Удэ; Стяжкина С.Н. и соавт. из Ижевска) посвящены интересным и важным единичным случаям из клинической практики или применению новых препаратов для лечения.

Для цитирования: Колесников С.И. Предисловие главного редактора к № 2 (2023). *Acta biomedica scientifica.* 2023; 8(2): 5-8. doi: 10.29413/ABS.2023-8.2.1

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ASSESSMENT OF THE EFFECTIVENESS OF RESTRICTIVE EPIDEMIC CONTROL MEASURES USING ORIGINAL MODELS OF CELLULAR AUTOMATON

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ABSTRACT

Background. *The ongoing COVID-19 pandemic, the human casualties caused by it, and the possibility of new epidemical threats make the search for effective countermeasures actual. One of the most effective tools, as the experience of the COVID-19 pandemic has shown, is restrictive measures of various types, which are especially significant with medical countermeasures being unavailable or insufficient. At the same time, the topic of restrictive measures and their mathematical modeling, especially given its importance, is not sufficiently disclosed in the scientific literature.*

The aim. *To determine the possibility of assessing the effectiveness of restrictive epidemic control measures using original models of cellular automata with intercellular boundaries.*

Methods. *To determine the impact of restrictive measures on the dynamics of the daily increase in infected people, an original cellular automaton with intercellular boundaries was developed, which makes it possible to simulate epidemic control measures of varying stringency. In the simulations carried out using the Monte Carlo method with subsequent statistical processing, we studied the impact of restrictive measures of varying stringency on the number of infected people, the duration of the epidemic, and the quality of forecasting. The final series of experiments simulated the spread of the COVID-19 virus in Germany in the first half of 2020.*

The results *show that even a simple cellular automaton model with boundaries successfully describes the course of the epidemic and allows us to assess the effectiveness of restrictive measures. The dependence of the daily increase in infected people on the stringency of measures is presented; it is shown what characteristics of the population can influence this dependence. It was found that the measures of medium severity (40–50 % according to the Stringency Index) have the least predictable effect; they can cause both rapid localization of the focus and the spread of the epidemic to a large part of the population. Weak and strong measures give a more predictable effect.*

Conclusion. *Cellular automaton models with intercellular boundaries have great potential for modeling the impact of restrictive measures on the course of an epidemic, making it possible to predict the dynamics of infected people based on the population data and the restrictive measures being introduced.*

Key words: *COVID-19, epidemic, restrictive measures, mathematical modeling, agent-based models, cellular automaton*

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ОЦЕНКА ЭФФЕКТИВНОСТИ ПРОТИВОЭПИДЕМИЧЕСКИХ ОГРАНИЧИТЕЛЬНЫХ МЕР С ПОМОЩЬЮ ОРИГИНАЛЬНЫХ МОДЕЛЕЙ КЛЕТОЧНЫХ АВТОМАТОВ

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РЕЗЮМЕ

Обоснование. *Продолжающаяся пандемия COVID-19, связанные с нею человеческие жертвы, возможность возникновения новых эпидемических угроз актуализируют поиск эффективных мер противодействия. Одним из наиболее эффективных инструментов борьбы, как показал опыт пандемии COVID-19, оказались ограничительные меры различного характера, особенно значимые в условиях, когда медицинские меры противодействия отсутствуют или недостаточны. Вместе с тем тема ограничительных мер и их математического моделирования, особенно с учётом её важности, раскрыта в недостаточной степени.*

Цель исследования. *Определение возможности оценки эффективности противоэпидемических ограничительных мер с помощью применения оригинальных моделей клеточных автоматов с межклеточными границами.*

Методы. *Для определения влияния ограничительных мер на динамику ежедневного прироста инфицированных разработан оригинальный клеточный автомат с межклеточными границами, позволяющий моделировать противоэпидемические меры различной строгости. В проведённых численных экспериментах по методу Монте-Карло с последующей статистической обработкой изучалось воздействие ограничительных мер различной строгости на количество инфицированных, продолжительность эпидемии, качество прогнозирования. В заключительной серии экспериментов моделировалось распространение вируса COVID-19 в Германии в первой половине 2020 года.*

Результаты *показывают, что даже простая модель клеточного автомата с границами успешно описывает ход эпидемии и позволяет оценить эффективность ограничительных мер. Представлена зависимость ежедневного прироста инфицированных от строгости мер; показано, какие характеристики популяции могут влиять на эту зависимость. Выявлено, что наименее предсказуемый эффект имеют меры средней строгости (40–50 %, согласно Stringency Index), при которых может наступить как быстрая локализация очага, так и распространение эпидемии на большую часть популяции. Слабые и строгие ограничения дают более предсказуемый эффект.*

Заключение. *Модели клеточных автоматов с межклеточными границами имеют большой потенциал для моделирования влияния ограничительных мер на ход эпидемии, позволяя прогнозировать динамику инфицированных на основе данных о популяции и вводимых ограничительных мерах.*

Ключевые слова: *COVID-19, эпидемия, ограничительные меры, математическое моделирование, агентно-ориентированные модели, клеточный автомат*

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The ongoing COVID-19 pandemic, the associated deaths, economic and non-economic losses, and the possibility of new epidemic threats make the issue of finding effective measures to counter such threats urgent. The experience of the COVID-19 pandemic has shown that restrictive measures of various kinds are one of the most effective tools for control. These measures are particularly important when new viruses emerge that have no medical countermeasures or they are inadequate. It is therefore not surprising that countries that were leading in international rankings of The Most Efficient Health Care (Bloomberg), The Global Health Security Index (Nuclear Threat Initiative, Johns Hopkins University, The Economist Intelligence Unit) and counting on their institutional capacity were not at their best during the pandemic [1]. Restrictive measures have been much more successful (especially in the initial phase before vaccines and treatment protocols were developed).

Restrictive measures have already been the subject of a number of studies aimed both at identifying their main types and assessing the degree of restrictions imposed (see, for example, the Oxford University project [2]) and at examining the correlation between restrictions and the severity of the pandemic [3, 4, etc.]. However, it should be noted that the topic of restrictive measures, especially in view of its importance, has not yet been sufficiently addressed. A number of questions still need to be clarified: what is the optimal degree of constraint? What types of constraints are most effective? Does the order in which different types of restrictions are imposed matter? At the same time, it is obvious that the answers to these questions cannot be automatically transformed into solutions for specific situations. Local specificities must be considered when making decisions. This raises the following question: what country or regional characteristics influence the effectiveness of restrictive measures?

This study explores the possibility of assessing the effectiveness of restrictive measures using original models of cellular automata (CAs). Cellular automata are a type of agent-based models used, in particular, in epidemiological studies for simulating the spread of epidemics in a population consisting of individual agents (cells). The fundamental novelty of the CA discussed in this paper is the intercellular boundaries by which the restrictive measures introduced are modeled.

It is considered that the CA concept was introduced into science in the late 1940s by J. von Neumann for modeling complex, spatially extended systems [5], based on the idea of S. Ulam [6]. It is also fair to say that "cellular automata have been invented many times under different names, and somewhat different concepts have been used under the same name" [7]. A. Burks [8], J. Holland [9], G. Hedlund [10], S. Wolfram [11, etc.] et al. have made significant contributions to the development of the cellular automaton theory and their use. CAs allow modeling mass processes of different nature: dissemination of information, opinions, protest activity (both in real life and in virtual social networks); group formation and the emergence of segregation; urban growth; territorial expansion of states; military conflicts, etc.

Cellular automata are a popular tool for modeling epidemics. N. Bailey [12, etc.] was one of the first to describe the use of CAs for modeling epidemics. He was followed by the papers of D. Mollison [13], S. Jakowitz et al. [14], and a number of other researchers [15, 16, etc.]. Of note are the papers of domestic authors: M.M. Bashabshekh and B.I. Maslennikov [17], D.K. Gorkovenko [18], A.V. Shabunin [19] and others. The COVID-19 pandemic, which triggered a wave of publications on epidemic modeling, contributed to the growing interest in models using CAs [20, 21, etc.] However, for all the abundance of publications, the potential of cellular automata for modeling restrictive measures is not actually used.

The advantages of CAs are visibility, relative ease of creating a model and conducting experiments with it. An important argument in support of the use of CAs is also the fact that simulation modeling with their contribution does not require ultra-high computing power and can be performed on an ordinary personal computer.

The basic element of a cellular automaton is a *cell*, which, most often, has the form of a square and within the framework of epidemic modeling can be interpreted as an individual, a small group (family) or a territorial community (residents of a city block, village, etc.). A set of contiguous cells form a "playing space", or CA *grid*; most often the grid has the form shown in Figure 1. In different models, a cellular grid can be interpreted as both a geographic space and a conventionally depicted population. By using cell geometry and grid shape, it is possible to increase or decrease the number of neighbors a cell has and thus account for the density of connections between individuals (or groups) in the simulated population, as well as the infectivity of the pathogen.

A cell may be in one of several *states*; minimally, there may be only two such states (e. g., a cell may be infected or uninfected). The transition of a cell from one state to another is determined by a set of *rules* that, in the simplest case, take into account 1) the current state of the cell and 2) the current state of its neighboring cells. For example, we can set the following rule: if a cell has two or more infected neighbors, the cell itself also becomes infected (see Fig. 1). This rule can be complicated by indicating that infection occurs in the presence of two or more infected neighbors not always, but with some probability, taking into account, on the one hand, the infectiousness of the pathogen, and on the other hand, the innate or acquired resistance of the individual. In more complex models, an individual's liability to disease may additionally be a function of age, gender, lifestyle, occupation, presence of chronic diseases, etc.

Cellular automata allow us to consider that the degree to which an infected cell is dangerous to its neighbors may change over time. This may take into account not just the average duration of the incubation period and the average duration of infectiousness, but the presence of different forms of the disease course (e. g., mild, moderate, severe), for each of which a different function of the change in infectiousness over time is defined.

Thus, the state of a cell can be influenced by a large number of factors related to both the properties of the cell itself, the properties and state of the cell's distant neighbors, and random factors.

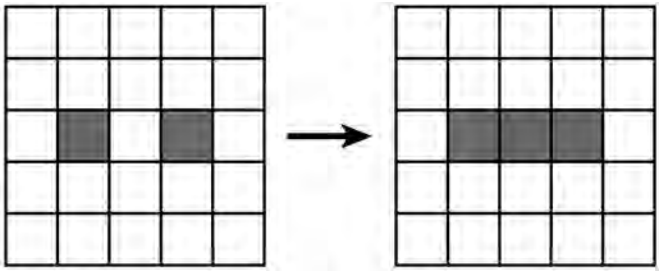

FIG. 1.
Two consecutive steps of the cellular automaton leading to the infection of the central cell

Classical CAs are *discrete* dynamic systems – that is, time in them is measured in steps, and the system life consists as if of separate static frames.

Due to their simplicity and the possibility to almost infinitely increase the set of cell properties, the set of states and the set of rules for changing states, epidemic models constructed using CAs successfully compete with classical models using systems of ordinary differential equations – SIS, SIR, SIRS, SEIRD – and similar models, the development of which began in the 1920s [22]. The use of CAs is also supported by the fact that models using ordinary differential equations are so-called *mean-field models*, i. e., models that assume a random and uniform distribution of healthy and infected individuals in the population. This assumption does not take into account that epidemics are most often focal, i.e. the distribution of infected and healthy people is not even. CA models allow us to account for the uneven distribution in the population of healthy and infected individuals.

THE AIM OF THE STUDY

To determine the possibility of assessing the effectiveness of restrictive epidemic control measures based on original models of cellular automata with intercellular boundaries.

Achieving the objective required the following tasks to be solved:

- searching for how restrictive measures can be implemented within the CA model;
- creation of an original CA model simulating the introduction of restrictive measures, in the R language;
- carrying out numerical experiments with the created CA model;
- analyzing the results of numerical experiments.

The method proposed in this article can be used by both epidemiologists and decision-makers when planning the introduction of restrictive measures to determine their necessary level of severity, as well as when assessing the effectiveness of measures already implemented.

MODELING OF RESTRICTIVE MEASURES

Restrictive measures introduced at the state, regional and local levels (as the experience of countering COVID-19 has clearly shown) are primarily aimed at reducing physical contacts of the population, during which the virus is transmitted. That is, it is the introduction of barriers to the virus by stopping contact or reducing its intensity. These measures include: transfer of employees to remote work; closing catering facilities; transferring students to distance learning; restrictions on visiting public places; restrictions on going outdoors, etc. Moreover, there are *targeted* restrictive measures aimed at reducing (or even eliminating) the contacts of an infected individual who poses a risk to others.

To model the restrictive measures, we used a special type of cellular grid *with boundaries* not previously encountered by the authors in the scientific literature[1]. Thus, while in the typical CA the grid consists only of cells, in the proposed CA additional objects, i. e., boundaries between neighboring cells, are introduced.

The introduction of restrictive measures can be interpreted as closing part of the boundaries between cells. If the boundary is closed, it becomes impenetrable – the pathogen cannot be transmitted through it. Closure of the boundary between two cells in practice can be expressed as the transfer of two employees to remote work, as a result of which their physical contacts are interrupted, as the cessation of face-to-face communication between two classmates when school classes are canceled, etc. It is quite obvious that in practice not all imposed restrictions are "impenetrable", just as the reverse is also true: without the imposition of restrictive measures, the boundaries between cells are not completely "transparent" either – contact between an infected individual and an uninfected one does not necessarily result in the latter becoming infected as well. In this paper, we restrict ourselves to models with fully impermeable boundaries, showing that even such simplified models allow us to describe well the effect of restrictive measures. At the same time, in more complex models it is justified to use partially permeable boundaries, i.e. those that with the introduction of restrictive measures reduce permeability, reducing the probability of virus passage from cell to cell, but not excluding it completely.

In the presented figure (Figure 2), the closed boundaries are shown as black rectangles. Assume that the virus can only transmit to the nearest cells in 4 directions (up, down, left, right), but not diagonally – i. e., we will use the so-called von Neumann's neighborhood of range $r = 1$. The center cell in the figure has two open (top and right) and two closed (left and bottom) boundaries. Direct infec-

[1] The concept of boundary is found in some papers on CAs, but most often it is a matter of distinguishing different zones of cells on the cellular space, so that the boundary in this case is not a significant element of the model (see, for example, [23]). In our case, the boundaries are a crucial element of the model, affecting the states of the cells and thus the dynamics of the whole system.

tion of the left and lower cells from the center cell is not possible in this case. The pathogen can only be transmitted to the top and right cells. However, there is nothing to prevent the use of other types of neighborhoods, such as the Moore's neighborhood (virus transmission also goes diagonally) or the Margolus neighbourhood (the geometry of which is not static, i.e. at one step the neighbors are some cells, and at another step – others; this type of neighborhood successfully describes periodic changes in the circle of contacts, which is typical, for example, for a working individual, when on weekdays they actively interact with colleagues at work, and on weekends – with family and friends).

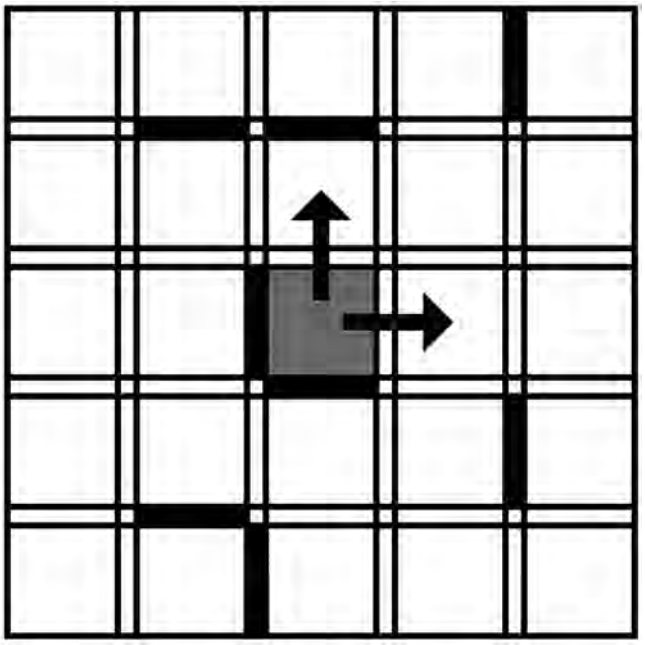

FIG. 2.
Cellular automaton with closed boundaries: the arrows show the directions in which the virus can be transmitted

The severity degree of restrictive measures is described in the model as the proportion of closed boundaries. It is obvious that an increase in the proportion of closed boundaries affects the virus spread rate and the possibility of epidemic outbreaks localization. In applied research, the percentage of closed boundaries can be taken as equal to, for example, the percentage of reduced activity, which is calculated for major world cities [24], or the self-isolation index [25], or taken from sources such as the COVID-19 Government Response Tracker [2].

An important question is which boundaries on the cellular space should be closed. Figure 2 shows a cellular space with 25 cells and 40 boundaries between them, 8 of which (i. e., 20 %) are closed. In the simplest case, the location of closed boundaries can be set randomly, based on the idea that when restrictive measures are imposed, unless they are targeted, it is impossible to predict exactly which individuals will break physical contact.

The random location of closed boundaries (where the only control parameter is the severity of restrictive measures, i. e., the proportion of closed boundaries) creates certain difficulties in modeling the impact of measures on epidemic spread. Closed boundaries can be distributed unevenly over the space, form (or not) extended structures, localize (or not) foci. All this makes it necessary to conduct a series of experiments with the model using the Monte Carlo method with further statistical processing of the findings.

As the experience of COVID-19 and other epidemics shows, restriction policies can be preventive, when restrictions are imposed before the emergence of infected people in a country or region, or reactive (situational, catch-up), when restrictions are imposed after the emergence of infected people. Both of these scenarios can be realized in models using CAs. The first scenario models the spread of an epidemic on a cellular space with already closed boundaries, while the second scenario simulates the epidemic evolving unconstrained until a certain point.

EXPERIMENTAL MODEL DESCRIPTION OF A CELLULAR AUTOMATON

A cellular automaton with the following characteristics was used in the experiments:

- the CA grid is made up of identical square cells;
- a cell can be in two states, uninfected or infected;
- cell neighborhood is Neumann's neighborhood of range $r = 1$ (i. e., the virus from an infected cell can be transmitted only to its four neighbors – top, bottom, left, and right);
- there are 4 boundaries between a cell and the cells that make up its neighborhood, and each boundary can be in two states, open or closed;
- virus transmission from an infected cell to an uninfected cell occurs when there is an open boundary between these cells. To simplify the model and to obtain a clearer dependence of the epidemic on restrictive measures, we consider the option of unconditional infection with an open boundary;
- cell begins to pose a risk to its neighbors in the next step after infection (incubation period is equal to one step), the risk of transmission from an infected cell remains for 5 steps;
- a cellular space has a square shape, its size is 59 × 59 cells (total 3,481 cells and 6,844 cell boundaries);
- the spread of the virus starts from the center cell.

The given space size allows us to trace, first, the nature of the epidemic spread in a closed population, and second, the nature of the epidemic in an unclosed population, since for at least 30 steps (or, interpreting 1 step as 1 day, for 30 days) the spread of the epidemic is affected only by the restrictive measures introduced and is not affected by the edge effect (i. e., there is no edge effect on the cellular space). An example of the described CA functioning is shown in Figure 3.

DESCRIPTION OF EXPERIMENTS

3 series of experiments were conducted aimed at assessing how restrictive measures affect the progression of the epidemic and the severity of its consequences.

The first series of experiments examined what nature, depending on the stringency of restrictive measures (i.e., the proportion of closed cell-to-cell boundaries), the epidemic progression has in a closed population of 59 × 59 cells. First of all, emphasis was placed on studying the rate of virus spread, duration of the epidemic, daily increase in infected persons and peak values.

9 variants of the restrictive measures stringency were considered: the proportion of closed boundaries varied from 0 to 80 % in increments of 10 %. The distribution of closed boundaries on the cellular space was set randomly in each experiment. For each of the 9 variants, 100 experiments were performed, the results of which were then subjected to statistical processing.

The second series of experiments examined the effect of restrictions during the initial phase of the epidemic (lasting for 30 steps) in an unclosed population. Following on from this time, the CA used allows us to model the spread of the virus without the influence of edge effects and to compare the epidemic development under different stringency of restrictions.

In this series, the emphasis was on examining the number of cells affected by the virus over a limited period of time and on the accuracy of estimating the effect of restrictive measures.

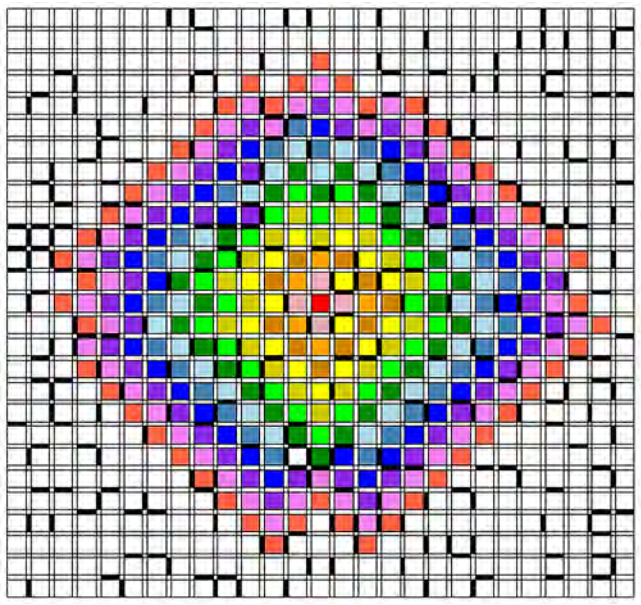

FIG. 3.

An example of the cellular automaton operation indicating the spread of an epidemic with 20 % of closed boundaries at the step 14 (the central part of the cellular space is shown). Cells infected at the same step have the same color. Cells surrounded by closed boundaries from all sides remain uninfected throughout the epidemic

16 variants of the restrictive measures stringency were considered: the proportion of closed boundaries varied from 0 to 80 % in 5 % increments. The distribution of closed boundaries on the cellular space was set randomly in each experiment. For each of the 16 variants, 100 experiments were performed, the results of which were then subjected to statistical processing.

In a third series of 100 experiments, the proposed cellular automaton with boundaries was used to simulate the spread of COVID-19 virus in Germany in the first half of 2020.

The R environment, version 3.4.3, was used to build the model and perform numerical experiments with it. Data statistical processing was performed using R environment and MS Excel 2016 (Microsoft Corp., USA) using standard methods of variation statistics. Hardware: PC with Intel Core i5-1035G1 1.19 GHz processor, 8 GB RAM.

DATA ANALYSIS AND RESULTS

In the absence of restrictions, there is a steady increase in the daily increment of infected from day 1 to day 30. At the same time, visually the zone of infected cells represents a rhombus growing with each step. Starting from day 31, the daily growth rate is reduced due to the edge effect, and the zone of infected cells turns from a rhombus into an octagon and gradually fills the entire cellular space. This results in a population of 3,481 cells being completely infected in 59 days.

The imposition of weak restrictions (10–30 % of closed boundaries) has almost no effect on this pattern, only slightly pushing the peak forward in time. Imposing tighter restrictions (40–50 % of closed boundaries) reduces the peak, lengthens the course of the epidemic, and protects some part of the population (usually not very significant) from infection. Strict restrictions (60 % or more closed boundaries) allow in a large number of cases to localize the epidemic focus, protecting an appreciable part of the population.

Figure 4 shows typical plots (graphs) of the number of daily infections at different levels of restrictive measures stringency.

As can be seen from the graphs, the nature of the epidemic progression does not change much at 10, 20, 30 % restrictions, not very different from the situation of complete absence of restrictions. The fact is that, while making it more difficult for the virus to spread, weak restrictive measures do little to shield the population from the virus, but only slightly reduce the rate of spread. The proportion of cells isolated from the virus is very small, amounting to only 1.5 % at 30 % restriction (see Table 1). In other words, the probability of avoiding infection under such restrictions is only 1.5 % for a cell.

However, even these weak measures have a clear positive effect: they shift the peak of the epidemic to the right, and the end of the epidemic also comes later, reducing the burden on the health system. For instance, a 30 % restriction reduces the burden in the first 30 days of the epidemic by an average of 28 % (see Table 1). Along with this,

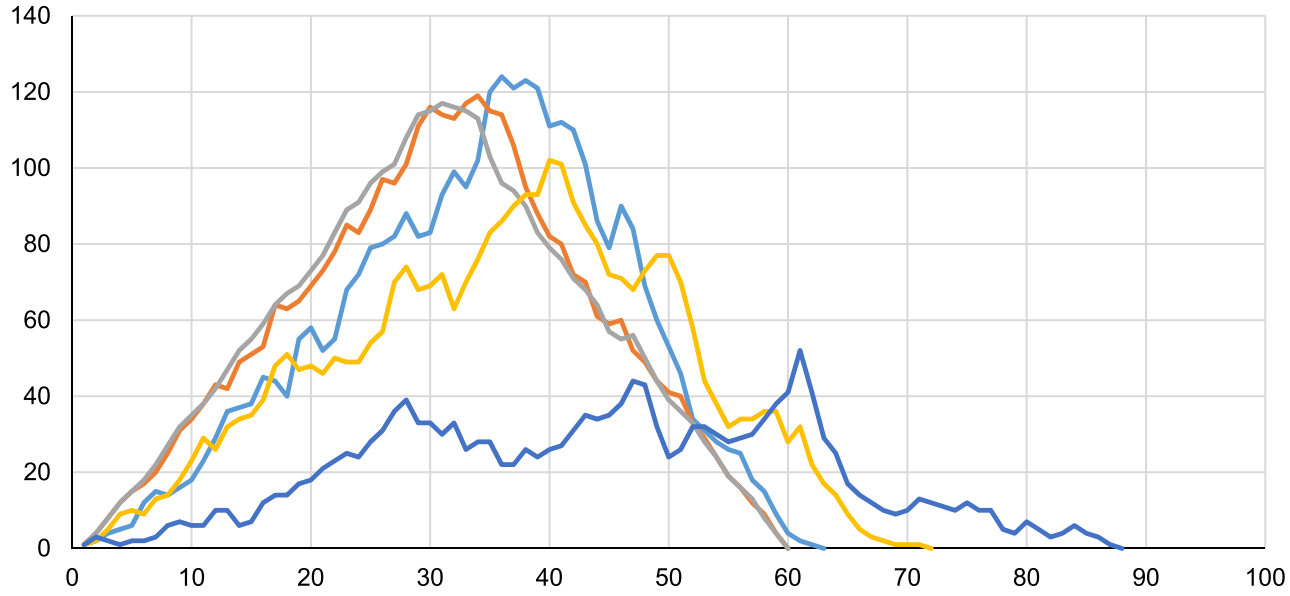

FIG. 4.

Examples of typical dynamics of the incremental growth of infected cells under restrictions of varying stringency. Horizontal axis represents steps; vertical axis – growth of infected cells. Gray – 10 % stringency; orange – 20 % stringency; blue – 30 % stringency; yellow – 40 % stringency; cyan – 50 % stringency

it is also worth noting the possible negative consequences of introducing weak restrictions: in some cases, the peak value may be even higher than in the absence of restrictions (see Fig. 4). Although this excess is usually no more than 10 %, this negative effect should be taken into account when imposing restrictions and forecasting the burden on the health care system.

The imposition of more severe restrictions (40–50 % of closed boundaries) fundamentally changes the picture of the epidemic course: the proportion of isolated population increases significantly (up to 75 %); on average, the public health burden decreases by 83 % in the first 30 days (with 50 % restrictions); the peak value is markedly reduced. The epidemic itself can lengthen considerably. Thus, at 50 % restrictions, the duration of the epidemic doubled or more in 15 % of the experiments.

The most significant effect of more severe restrictions is to increase the likelihood of epidemic localization, so that a significant proportion of the population is protected from infection by the measures. To some extent, as shown above, isolated cells can also occur under weak restrictions. Moreover, with weak restrictions, there is also the possibility of focal suppression, but it is very low, so it is not necessary to rely on such a scenario with weak restrictions. It is at moderate restrictions that a scenario of focal suppression can be considered: at 40 % restrictions, localization occurs in about 10 % of cases, and at 50 % restrictions, in about 30 % of cases[1].

[1] In the context of the experiments, focal localization (suppression) was understood as a situation in which the spread of the virus was stopped by the measures introduced in 25 days or less. This value was chosen based on the fact that if the virus stops spreading during this period, more than 65 % of the population will be protected from it. It is possible to choose a different number of days, which, however, does not change the main conclusion about the marked increase in the cases of focal suppression with increasing stringency of restrictions starting from 40 % restrictions.

An important feature identified in numerical experiments with the model is a wide scatter of the results when introducing moderate severity measures (see Table 1). In particular, while the standard deviation of the isolated cells proportion at weak restrictions did not exceed 0.5 %, at moderate restrictions the standard deviation reaches 25 %, markedly reducing the ability to predict the performance of the measures implemented. Thus, in 100 experiments with 40 % closed boundaries, the minimum proportion of isolated cells was 5.08 % and the maximum was 99.97 %.

Similar scatter is observed for other parameters. At 40 % restrictions, both cases of focus localization within the first week and an increase in the duration of the epidemic up to 100 days are observed. At 50 % restrictions, the difference between the maximum and minimum duration increases even further to almost 200 days.

Thus, one of the main disadvantages of moderate severity measures is the poorly predictable effect of their introduction. Analyzing the behavior of the constructed CA, it can be observed that, as in real life, restrictive measures perform two main functions: isolating a part of the population (1) and reducing the rate of virus spread (2). Both of these functions have positive effects, one by protecting part of the population from infection, the other by reducing the burden on the health system and helping to buy time to mobilize additional resources, develop vaccines, and so on. Under weak restrictions, a scenario is implemented in which the effect of reducing the virus spread rate is more important, since weak restrictions (i. e., a small number of closed boundaries) are unable to protect an appreciable part of the population from infection – single cells are protected. As the number of closed boundaries increases, another scenario is increasingly implemented, in which

the configuration of the closed boundaries is such that a significant part of cells can be shielded from the virus (up to localization of the epidemic focus in the first few steps of CA operation). The higher the number of closed boundaries, the more likely this scenario is to occur.

The wide scatter of indicators and poor predictability of the effect when introducing measures of moderate severity are related to the fact that both the first scenario discussed above and the second one can be implemented with almost equal probability. Such unpredictability is well illustrated by the distribution histogram (Fig. 5). The percentage of isolated cells at 50 % restrictions is shown horizontally, and the occurrence frequency of this result is shown vertically. Focal localization (rightmost column) occurs in one-third of cases, but in a large number of cases localization does not occur and the epidemic affects a significant number of cells. In 21 % of cases, the epidemic covers more than half of the population (leftmost four columns).

Especially important to note is that in such a distribution, focusing on average values can lead to significant errors in predicting the impact of restrictive measures. The average proportion of isolated cells at 50 % restrictions is 74.95 %, but as can be seen from the histogram, the probability of obtaining such a result (let's take the interval from 70 % to 80 %) is only 7 %, which is much lower than the probability of focus localization or spreading the epidemic to a large part of the population.

This finding appears to be one of the most important in our work, allowing us to explain, in particular, the high variability in the results from similar restrictions across countries when dealing with COVID-19.

Moderate restrictive measures make it much more difficult for the virus to spread in a population. Closed boundaries create complex structures on the cellular space, similar to a maze; rather large spaces emerge, partially or completely walled off from the epidemic focus. As a result, the spread of the virus often proceeds in leaps and bounds – sometimes slowly, with small increments of infected people per step, and sometimes rapidly. Wave-like graphs of the infected are typical of moderate restrictions (see Figure 4).

Severe restrictions (60 % or more closed boundaries) turn out to be, as one would expect, the most effective. Al-

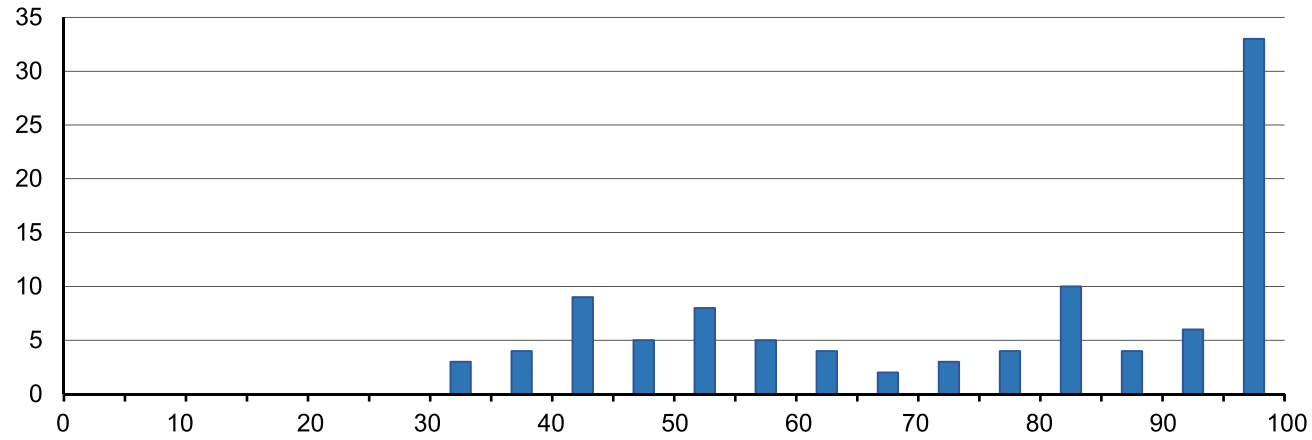

FIG. 5.

Distribution of results of numerical experiments under 50 % restrictions. Horizontal axis represents the proportion of cells (%) that turned out to be isolated by the end of the spread of the epidemic in the population; vertical axis – frequency of occurrence (%)

TABLE 1

STATISTICS OF NUMERICAL EXPERIMENTS

Parameters	Stringency of restrictive measures (%)								
	0	10	20	30	40	50	60	70	80
Average proportion of isolated cells (%)	0.00	0.02	0.27	1.50	14.33	74.95	99.07	99.78	99.92
Standard deviation (%)	0.00	0.03	0.09	0.31	25.38	23.10	1.33	0.26	0.07
Average change in epidemic duration (in days and %)	0.00	+0.2	+0.95	+3.32	+9.19	+7.51	–48.15	–54.28	–56.71
	0.00	+0.34	+1.61	+5.63	+15.58	+12.73	–81.61	–92.00	–96.12
Standard deviation (in days)	0.00	0.53	1.23	2.14	20.53	46.60	12.05	4.45	1.67
Average reduction in health system burden in the first 30 days of the epidemic (%)	0.00	3.60	12.54	28.18	52.59	83.42	96.85	98.89	99.54

ready at 60 % restrictions the proportion of virus-free cells reaches 99 % on average, which makes further increases in the stringency of measures virtually meaningless, especially if we take into account that increasing the stringency of measures in practice is associated with economic, political and other costs. With 60 % restrictions, the burden on the health care system in the first 30 days of the epidemic is reduced by almost 97 %, and the average duration of the epidemic is strongly reduced due to the dominance of cases of epidemic focus localization – such cases were 87 %. While it is still possible that the duration of the epidemic exceeds the duration of the epidemic without restrictions, the range of values for this indicator is substantially reduced compared to the 50 % restrictions. Thus, the results of more severe restrictions are not only more efficient on average, but also more predictable; this is true for both the expected duration of the epidemic and the isolated population proportion.

The second series of experiments examined the effect of restrictions during the initial phase of the epidemic (lasting for 30 steps). Following on from this time, the CA used allows us to model the spread of the virus without the influence of edge effects (i. e., the attenuation of the epidemic in these experiments is not due to the fact that the population is limited, but only to the action of the restrictions) and to compare the epidemic development under different stringency of restrictions.

The results obtained agree with the results of the first series of experiments. Figure 6 shows how the average number of infected individuals on day 30 of the epidemic depends on the stringency of the restrictions (i. e., the proportion of closed cell boundaries). As can be observed, weak restrictions (10–30 % of closed boundaries) lead, on average, to a rather small reduction in the rate of virus spread. 30 % restrictions reduce the number of infections by about 20 %.

The introduction of more severe restrictions (40–50 % of closed boundaries) markedly reduces the rate of virus spread. At day 30, at 50 % restrictions, the number of infections is on average 10 times lower than at no restriction and 8 times lower than at 30 % restrictions.

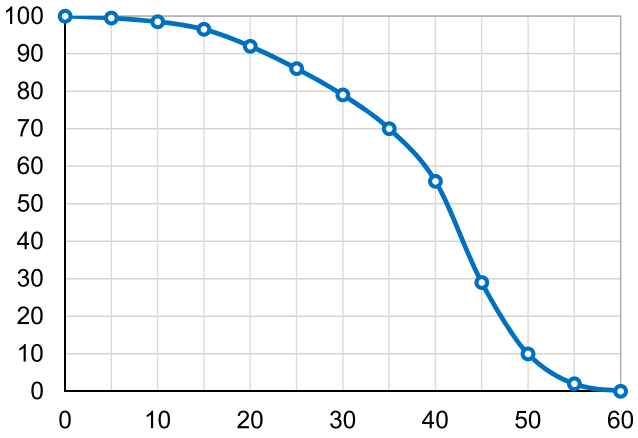

FIG. 6.

Dependence of the proportion of infected cells (%) on the day 30 of the epidemic on the stringency of restrictions (%)

Severe restrictions (60 % or more closed boundaries) almost completely stop the spread of the virus.

The effect of restrictions of varying stringency can be clearly seen in Figure 7, which shows the average number of infections by day as a function of the restrictions severity. Apparently, with 50 % of closed boundaries (and thus weaker restrictions), the number of infections tends to increase gradually. More severe restrictions (55 %) are already able to reverse this trend – the number of new cases is stable and the virus is spreading through the population at the same rate. Even more severe restrictions (60 %) can gradually reduce the rate of spread of the virus and bring the epidemic to a halt.

The high efficiency of severe restrictions demonstrated in the experiments (i. e. when 60 % of the boundaries are closed or more) is in some contradiction with real data, in particular, on the course of the COVID-19 pandemic in various countries and regions, where, according to statistics, the situation improved after the introduction of more severe restrictions (80 % and higher). At a minimum, we can point to three sources of these discrepancies:

1. An important assumption of the conducted experiments with the CA model is that virus spreading occurs in the model already *in the presence* of restrictive measures. The experience of coping with the COVID-19 pandemic and other epidemics shows that, in reality, a different scenario is more often implemented: the main set of restrictive measures is introduced not preventively, but in response to an increasingly complex epidemic situation, i. e. when the virus has already spread over a large area and/or in a significant proportion of the population. In this situation, governments are forced to go for stricter measures.

2. In the model, the closure of the cell-cell boundary completely precludes penetration of the virus. In reality, imposing restrictions does not mean that they are perfectly enforced. Under these circumstances, the introduction of, for example, 60 % restrictions may in practice lead to only 50 % or even 40 % restrictions. Thus, the lower the level of restriction compliance, the more severe the measures must be to achieve the desired effect.

3. Contemporary researchers have pointed out that the CA methodology is somewhat "outdated" for describing epidemics occurring today. Whereas in the past the key factor in the spread of epidemics was precisely "neighborhood" contacts, now, in conditions of high geographical mobility, a significant role is played by "long-distance" contacts, i. e. long-distance migrations of the population associated with recreation, business trips, etc. As a result, the transfer of a virus from one continent to another can occur faster than the transfer from one area of a city to a neighboring one. So, when building a full-fledged model of epidemic spread, it is necessary to consider distant connections, as some modifications of CAs do (see, e. g., [19]). On the other hand, as the practice of confronting epidemics shows, restrictions on movement (especially long-distance travel) are among the first to be introduced, which should significantly reduce the impact of "long-distance travel" on the spread of the virus.

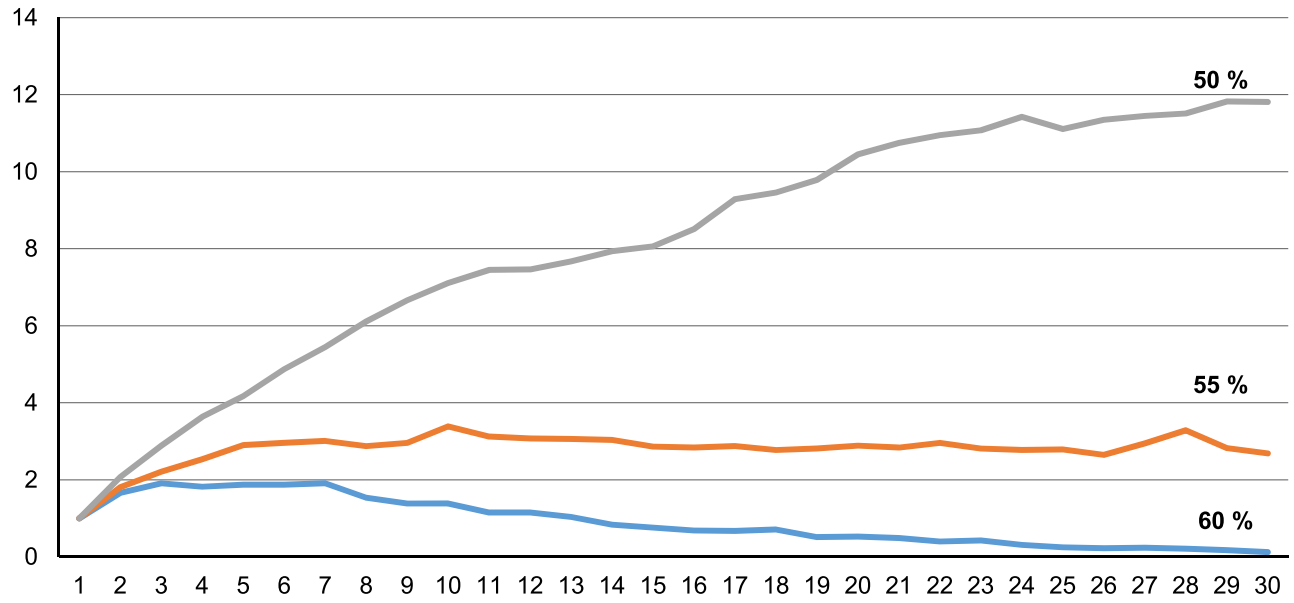

FIG. 7.

Average number of infected cells per step (new cases) depending on the stringency of restrictions

TABLE 2

SIMPLIFIED SEQUENCE OF CHANGES IN THE STRINGENCY OF RESTRICTIONS IN GERMANY DURING THE FIRST WAVE OF THE COVID-19 PANDEMIC

Time period	Time period duration	Stringency Index	Percentage of closed intercellular boundaries	Long-distance connections
1.03 – 9.03	9 days	25	25 %	87.5 %
10.03 – 17.03	8 days	32.87	32.87 %	87.5 %
18.03 – 20.03	3 days	55.09	55.09 %	0 %
21.03 – 04.05	45 days	76.85	76.85 %	0 %
05.05 – 16.05	12 days	64.35	64.35 %	0 %
17.05 – 15.06	30 days	59.72	59.72 %	12.5 %

The aim of the third series of experiments was to test the model on real data: a cellular automaton with boundaries was used to simulate the spread of the COVID-19 virus in Germany during the first wave. The German data were chosen because of their relative reliability and completeness[1].

During the experiments, a larger (101 × 101) CA was used to improve accuracy, which was based on the principles discussed above and included a number of additional rules to better account for the peculiarities of the epidemic in Germany.

1. The number of closed intercellular boundaries changed dynamically, according to Stringency Index (SI) data, an aggregate parameter obtained by researchers at the University of Oxford based on the COVID-19 Government Response Tracker [2]. SI takes into account the stringency of the restrictive measures imposed and is calculated for each day on a scale from 0 to 100. Slightly simplifying the real time variation of SI, the scheme shown in Table 2 was used in the experiments.

2. The closed boundaries in the model were made partially permeable, i.e. the fact that a part of the population could neglect the restrictions and some restrictions could work inefficiently was taken into account. The degree of permeability was found to be 25 %, i. e., with a 25 % probability that the virus was transmitted through a closed boundary (100 % permeability was assumed for open boundaries, as in the previous series of experiments). The degree of permeability of closed boundaries was chosen on the basis of the thesis that permeability is primarily influenced by the behavior of the population and its attitude towards restrictions, which in turn can be operationalized by the level of law-abidingness and the level of trust in the imposed measures. However, as research on the topic of COVID-19 proliferation and confrontation shows, there is currently

[1] Germany is among the leading countries in real-time surveillance and reporting (Global Health Security Index – 2019) and at the same time Germany had one of the highest specific population testing rates during the first wave of the COVID-19 pandemic (see e. g. https://ourworldindata.org/grapher/full-list-total-tests-for-covid-19).

no objective sociological or other data reflecting law-abidingness [26], hence our study used proxy indicators from World Value Survey (WVS) studies to understand the level of non-compliance with restrictive measures [27]. In particular, the responses of German residents to questions about their trust in the World Health Organization (Q88), under whose recommendations the restrictions were imposed, and their attitudes towards tax evasion (Q180) were taken into account. For question Q88, 23.9 % of respondents stated that they do not trust or do not trust WHO at all. For question Q180, 75.7 % said that tax evasion can never be justified. As we can see, both figures allow us to assume a level of non-compliance with restrictive measures of about 25 %.

3. In addition to neighboring cell interactions, the model took into account "long-distance" connections, i. e., interactions between two non-neighboring cells that mimic long-distance travel by residents. The performance of long-distance connections was controlled in the model by two parameters: the average number of such connections per German resident and the restrictions that were imposed on long-distance travel in the relevant time period.

As per statistics, a German resident takes three long-distance trips per year [28]. Thus, it was calculated that per step in the model, $(101 \times 101 \times 3) / 365 = 84$ "long-distance" connections could occur. This quantity was adjusted for the restrictions that were imposed and whose numerical expressions were derived from the data on the SI components that describe restrictions on long-distance movements of residents (C7 and C8 components). As a result, the number of "long-distance" connections ranged from 73 in the initial period of the epidemic to 0 and then rose slightly to 10 (see Table 2). Cells involved in "long-distance" connections were randomly selected.

The plots of infected cell growth obtained from the experiments (Fig. 8) largely coincide with the actual plots of daily infected cell growth (Fig. 9). Similarities can be seen both in the pattern of exponential growth in the number of infected persons during the first weeks of the pandemic and in the shape of the peak apex and the shape of the relatively mild decline in the curve. This indicates that the proposed modeling method can accurately describe the dynamics of disease incidence based on population data and the degree of restrictive measures stringency and, accordingly, can be used to assess the effectiveness of anti-epidemic restrictive measures.

However, discrepancies in the plots related to the comparison of the curve scale are also noticeable. For instance, the termination of exponential growth occurs in the model about 10 days earlier than in reality (if we interpret one step of CA operation as one day). To some extent, such discrepancies can be explained by the fact that the model shows how the number of infected people changes at the time they are infected, while the statistics show the number of infected people at the time they are registered.

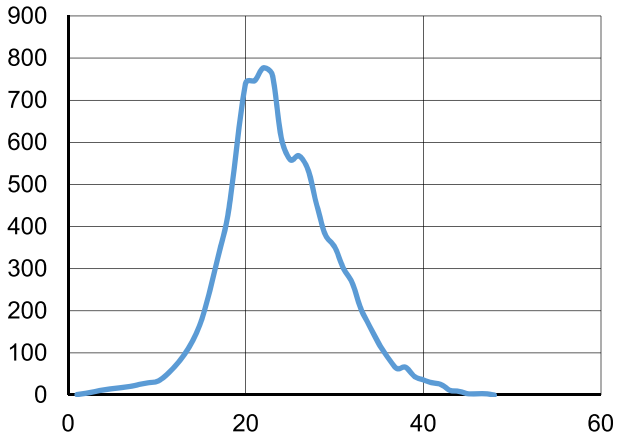

FIG. 8.
An example of a typical dynamics of incremental growth of infected cells in a simulation based on German data

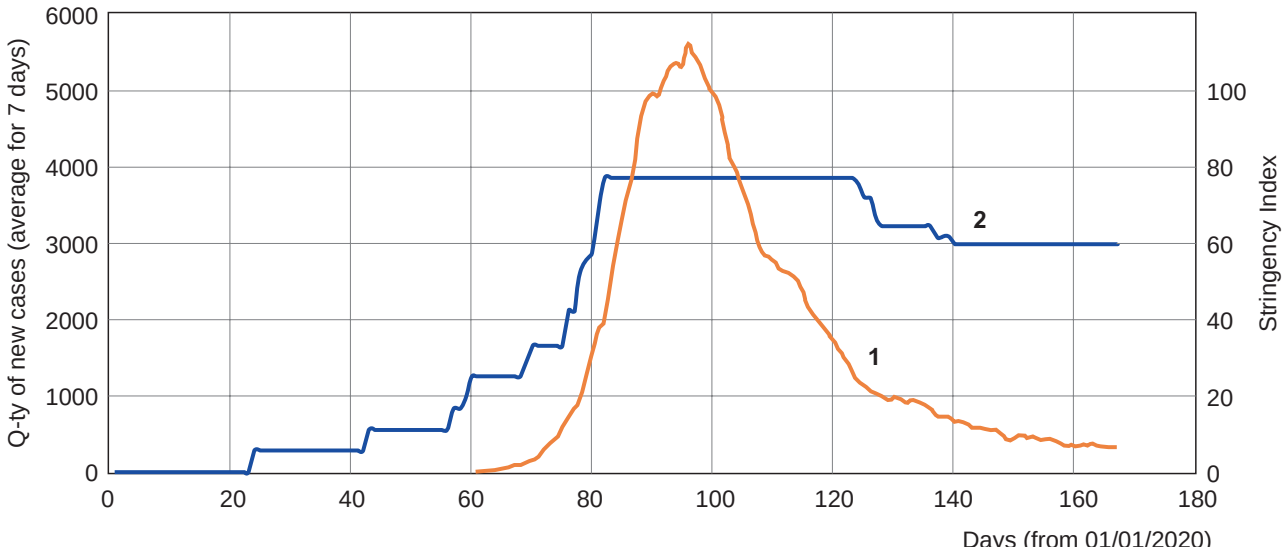

FIG. 9.
The real dynamics of the daily increase in infected people in Germany: **1** *– number of new cases;* **2** *– stringency of the restrictive measures*

Further research in this area could include the development of a model that takes into account more *population characteristics*, as well as *specific types* of restrictive measures, in order to understand the impact of these measures on the epidemic situation and, possibly, to formulate practical recommendations for their effective use, taking into account the specific characteristics of a country or region. Another major issue is the study of the relationship between the length of incubation period, duration of infectiousness and other characteristics of the pathogen (on the one hand) and the effectiveness of restrictive measures and their types (on the other hand). Besides, a number of "technical" issues related to the choice of the optimal grid size of the cellular automaton, to the determination of the CA discrete time and real time ratio, etc., need to be clarified.

CONCLUSION

Study results show that even a rather simple cellular automaton model with boundaries can successfully describe the evolution of an epidemic and evaluate the effectiveness of restrictive measures. The proposed methodology (conducting a series of numerical experiments) makes it possible to estimate the probability of realization of one or another scenario when introducing restrictive measures. An important finding here is that with different levels of restrictions imposed, the quality of prediction is also different. Moderate severity measures (40–50 %) have the least predictable effect, with both rapid localization of the outbreak and spread of the epidemic to a large part of the population. Weak and strong measures give a more predictable effect.

CA models with intercellular boundaries have great potential for modeling epidemics and the impact of restrictive measures. Cellular automata as a kind of agent-based models potentially allow to describe with maximum completeness the population characteristics, specifying age, social, cultural, medical characteristics of each agent, which can influence both the spread of the epidemic and the effectiveness of restrictive measures. In addition, CA models allow to modify and supplement the rules of cell transition from one state to another, increase the number of these states, change the "rules of operation" of intercellular boundaries, taking into account their partial permeability.

All this makes it possible, firstly, to take into account more fully in the model the specifics of the population in a particular region or country and the specifics of its contacts, and secondly, to describe more fully the introduction of restrictive measures and their impact on the behavior of the population and the spread of the epidemic, which, in turn, makes it possible to choose the optimal degree of restrictions stringency, taking into account local characteristics.

Conflict of interest

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

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THE ROLE OF CIRCULATING miR-19b MIRNA IN PREDICTING THE OUTCOME OF COVID-19

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ABSTRACT

Background. *MicroRNAs are short (20–22 nucleotides) non-coding RNAs that can posttranscriptionally regulate gene expression and are considered a regulator of the innate immunity system. Previously, many papers were published on the prediction of the interaction of the single-stranded (+)RNA virus SARS-CoV-2 with human microRNAs, as well as on the profile of circulating microRNAs in patients with COVID-19 of varying severity. However, no works are analyzing the possible contribution of miRNAs circulating in blood plasma to the severity of COVID-19.*
The aim. *To study the features of the blood plasma microRNA profile of patients with different severity of the new coronavirus infection COVID-19 and to evaluate the possibility of microRNA interaction with the SARS-CoV-2 genome.*
Materials and methods. *The results of NGS sequencing of plasma miRNAs of 3 recovered and 8 deceased patients with a highly severe form of COVID-19 were studied. Differentially presented microRNAs were determined using bioinformatics methods, and their binding sites with the SARS-CoV-2 genome were predicted.*
Results. *This study demonstrates that in patients who have recovered from a highly severe form of COVID-19, the level of hsa-miR-19b-3p in the blood plasma is significantly increased. This microRNA makes up about 1.5 % of all circulating microRNAs and can bind to SARS-CoV-2 regions encoding proteins that suppress intracellular immunity mechanisms (NSP3, NSP9). In addition, this miRNA can stimulate the functional activity and proliferation of cytotoxic T-lymphocytes, one of the critical components of acquired cellular immunity against SARS-CoV-2.*
Conclusion. *The results of the study can be used in the development of antiviral drugs based on RNA interference, as well as in the development of predictive test systems to optimize the tactics of treating patients with COVID-19.*

Key words: *miRNA, COVID-19, SARS-CoV-2, miR-19b, disease severity*

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РОЛЬ ЦИРКУЛИРУЮЩЕЙ МИКРОРНК miR-19b В ПРОГНОЗЕ ИСХОДА COVID-19

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РЕЗЮМЕ

Обоснование. *МикроРНК – короткие (20–22 нуклеотида) некодирующие РНК, обладающие способностью постранскрипционно регулировать экспрессию генов, рассматриваются в качестве регулятора системы врождённого иммунитета. Ранее был опубликован ряд работ, посвящённых предсказанию взаимодействия одноцепочечного (+)РНК-вируса SARS-CoV-2 с микроРНК человека, а также особенностям профиля циркулирующих микроРНК у пациентов с COVID-19 различной степени тяжести. Однако практически отсутствуют работы, анализирующие возможный вклад фактически циркулирующих в плазме крови микроРНК в тяжесть течения COVID-19.*

Цель. *Изучить особенности профиля микроРНК плазмы крови пациентов с различной тяжестью течения новой коронавирусной инфекции COVID-19 и оценить возможность взаимодействия микроРНК с геномом SARS-CoV-2.*

Материалы и методы. *Изучены результаты NGS-секвенирования микроРНК плазмы 3 выздоровевших и 8 умерших пациентов с крайне тяжёлой формой COVID-19. С помощью биоинформационных методов определены дифференциально представленные микроРНК, предсказаны места их связывания с геномом SARS-CoV-2.*

Результаты. *В данной работе продемонстрировано, что у пациентов, выздоровевших после крайне тяжёлой формы COVID-19, в плазме крови статистически значимо повышен уровень hsa-miR-19b-3p. Данная микроРНК составляет около 1,5 % от всех циркулирующих микроРНК, способна связываться с регионами SARS-CoV-2, кодирующими белки, подавляющие внутриклеточные механизмы иммунитета (NSP3, NSP9). Кроме того, данная микроРНК способна стимулировать функциональную активность и пролиферацию цитотоксических Т-лимфоцитов – одного из ключевых компонентов приобретённого клеточного иммунитета против SARS-CoV-2.*

Заключение. *Результаты исследования могут быть использованы при разработке противовирусных препаратов на основе РНК-интерференции, а также при разработке прогностических тест-систем для оптимизации тактики лечения пациентов с COVID-19.*

Ключевые слова: *miRNA, COVID-19, SARS-CoV-2, miR-19b, тяжесть течения заболевания*

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OBJECTIVES

In 2019, a new disease called COVID-19, caused by the SARS-CoV-2 coronavirus, was identified. This virus was characterised by rapid, difficult to control spread due to its certain characteristics. The body's first line of defence against viruses, including SARS-CoV-2, is innate immunity, which limits viral entry into cells, translation, replication and virion assembly, and allows detection and destruction of infected cells, coordinates and enhances acquired immunity [1]. Innate antiviral immunity has two components: cellular and humoral immunity, which include macrophages, monocytes, dendritic cells, neutrophils, NK cells, cytokines and interferons, and intracellular immunity. Intracellular immunity includes various families of cytoplasmic receptors and enzymes that can recognise and destroy viruses within a cell [2].

Several studies consider microRNA molecules as a component of the innate immunity system [3–5]. MicroRNAs are short (about 22 nucleotides) non-coding RNAs that regulate gene expression post-transcriptionally. The microRNA sequence includes the so-called seed region, which is located from the 2nd to the 7th nucleotide from the 5'-end of the mature molecule, and which is responsible for the specificity of its binding to target RNAs. It has been shown that host cell microRNAs can act as a component of intracellular immunity, regulating the translation and replication of (+)RNA viruses and altering the pathogenesis of viral infections [6, 7]. Two main effects of the interaction between the viral RNA-genome and microRNA are identified: inhibition of virus translation and slowing down its replication, stabilisation of viral RNA and increasing the rate of virus replication. Moreover, the slowdown of virus replication is primarily associated with the interaction of microRNA with the 3'-untranslated region of the virus [8], and the stabilisation of viral RNA is associated with the interaction with the 5'-untranslated region of the virus [9]. Moreover, microRNAs are able to modulate the activity of both innate [10] and acquired immunity cells [11].

A number of papers have been published on predicting the interaction of the single-stranded (+) RNA virus SARS-CoV-2 with human microRNAs [12–14], as well as the profile of circulating microRNAs among patients with different degrees of COVID-19 severity [15]. However, there are practically no works analysing the possible contribution of microRNAs circulating in plasma to SARS-CoV-2 biogenesis and COVID-19 pathogenesis.

THE AIM OF THE STUDY

To study the features of the blood plasma microRNA profile of patients with different severity of the new coronavirus infection COVID-19 and to evaluate the possibility of microRNA interaction with the SARS-CoV-2 genome.

METHODS

Plasma microRNA profile of patients with COVID-19

Primary microRNA sequencing data (GSE195898) isolated from blood plasma of 3 recovered (two men and one woman) and 8 deceased patients (five men and three women, comparison group) with extremely severe COVID-19, comparable in sex and age, treated at the IRCCS Policlinico San Donato Intensive Care Unit (Milan, Italy) were used for analysis [16]. For each patient, the plasma microRNA profile was assessed at the time of hospital admission (T0) and before discharge or death (T1).

The study was carried out in full compliance with the World Medical Association Declaration of Helsinki. The experimental protocol was approved by the local ethical committee at San Raffaele Hospital (Milan, Italy, Minutes No. 75/INT/2020 dated April 20, 2020).

HTG EdgeSeq miRNA Whole Transcriptome targeted sequencing kit (HTG WTA, HTG Molecular, USA) was used for library preparation. Sequencing was performed on an Illumina NextSeq 500 sequencer (Illumina Inc., USA) using the NextSeq High Output v. 2 75 cycles kit (Illumina Inc., USA) [16].

Sequencing data analysis

The 3'-adapter sequence was removed using Cutadapt v. 2.10. Sequencing read quality control was performed using FastQC v. 0.11.9. After removal of the 3'-adapter sequence, sequencing results were processed using IsoMiRmap [17].

Identification of microRNA binding motifs to the SARS-CoV-2 genome

For each microRNA, SARS-CoV-2 RNA regions reverse-complementary to the 2–7 nucleotide region of the 5'-end of the mature microRNA were identified. Following a common classification [18], such binding regions are labelled "6mer". Interaction of the corresponding microRNA with them leads to suppression of translation and degradation of the target RNA. To determine whether 6mer belongs to regions encoding SARS-CoV-2 proteins, the binding positions were pairwise aligned against the reference sequence of the Wuhan-Hu-1 strain using the stringr and spgs packages.

Statistical analysis

Differences in microRNA representation were analysed using DESeq2 [19]. Comparison of data on the number of microRNA binding motifs to the SARS-CoV-2 genome was performed using the Wilcoxon test. Data processing and statistical analyses were performed in the *R* software environment.

RESULTS

Sequencing results showed that 932 types of microRNAs and their 5'-isoforms were present in the patients' plasma at more than 150 rpm at the time of admission (T0). At T1, 990 types of molecules were identified. Moreover, 54 types of molecules occurred only at T0 and 112 occurred only at T1. The following microRNAs are among the most highly represented at T0 and T1: hsa-miR-22-3p|0, hsa-miR-339-3p|0, hsa-miR-451a|0.

Analysis of differences in microRNA profile between recovered and non-recovered patients showed that the ex-

pression of 46 microRNAs was significantly altered. At T0, subsequently recovered patients had higher expression of hsa-miR-19b-3p|0 (4.5-fold, $p = 0.017$), hsa-miR-25-3p|+1 (4.8-fold; $p = 0.047$). The representation of 291 microRNAs differed significantly at T1. The largest differences in plasma representation between the group of recovered patients and the comparison group were observed for the following microRNAs: hsa-miR-451a|0 (13-fold; $p = 7.65E-07$), hsa-miR-22-3p|0 (4.3-fold; $p = 7.67E-05$), hsa-miR-19b-3p|0 (14-fold; $p = 1.23E-06$).

Several studies have demonstrated the correlation of hsa-miR-451a representation and the level of haemolysis in blood samples [20, 21]. The hypothesis of significance of differences in the representation of microRNAs associated with haemolysis between the comparison groups at T0 and T1 was tested (Table 1). At the time of hospital admission, the levels of microRNAs associated with haemolysis and erythropoiesis did not differ between the comparison groups. Meanwhile, all microRNAs associated with haemolysis and erythropoiesis were significantly elevated at T1 in the group of recovered patients.

Comparison of microRNA sets differing between recovered patients and the comparison group showed that only two microRNAs changed codirectionally at T0 and T1 (Table 2).

The possible binding sites of multiple microRNAs differing between the convalescent patient group and the comparison group at T0 and T1 to the SARS-CoV-2 genome were evaluated (Figure 1). A number of microRNAs had no binding sites with the virus genome: hsa-miR-1225-3p|+3, hsa-miR-4498|+1, hsa-miR-6787-5p|+2, hsa-miR-1538|+1, hsa-miR-1307-5p|+1, hsa-miR-7111-5p|+2. The number of microRNA binding sites of hsa-miR-19b-3p|0 and hsa-miR-25-3p|+1 were 12 and 9, respectively. At the same time, the median of binding sites of the other microRNAs was 3. It can be concluded that recovered patients had a significantly

TABLE 1

DIFFERENCES IN THE REPRESENTATION OF MICRORNAS ASSOCIATED WITH HEMOLYSIS BETWEEN THE COMPARISON GROUPS IN T0 AND T1

MicroRNAs	MicroRNA representation in plasma at T0, log2RPM			MicroRNA representation in plasma at T1, log2RPM		
	recovered	deceased	*p value*	recovered	deceased	*p value*
hsa-miR-451a|0	14.4 ± 2.2	13.2 ± 1.7	0.0959	17.4 ± 1.8	13.9 ± 1.6	1.0E-06
hsa-miR-16-5p|0	11.6 ± 1.5	11.6 ± 1.3	0.9370	13.9 ± 1.3	12.1 ± 0.8	2.0E-04
hsa-miR-486-5p|0	11.2 ± 2.8	10.3 ± 1.5	0.1206	13.7 ± 1.8	10.5 ± 1.3	1.0E-05
hsa-miR-93-5p|0	10.7 ± 1.9	10.1 ± 1.2	0.1771	12.8 ± 1.3	10.1 ± 0.9	4.0E-07
hsa-miR-17-5p|0	9.3 ± 1.4	9 ± 1	0.4637	11 ± 1.1	9.4 ± 0.7	4.0E-04
hsa-miR-20a-5p|0	8.9 ± 1	8.9 ± 1	0.7719	11.1 ± 1.1	9.4 ± 0.8	2.0E-04
hsa-miR-107|0	8.8 ± 1.2	8.8 ± 0.7	0.7764	10.2 ± 1.1	9.1 ± 0.4	1.4E-03
hsa-miR-106a-5p|0	8.5 ± 1.2	7.9 ± 0.7	0.1640	10.2 ± 1.3	8.2 ± 0.7	2.0E-05
hsa-miR-20b-5p|0	7.8 ± 0.4	7.6 ± 0.3	0.6410	9.1 ± 0.7	7.7 ± 0.5	2.0E-04

TABLE 2

REPRESENTATION OF CONCOMITANTLY CHANGED MIRNAS IN COMPARISON GROUPS IN T0 AND T1

MicroRNAs	MicroRNA representation in plasma at T0, log2RPM			MicroRNA representation in plasma at T1, log2RPM			
	recovered	deceased	*p value*	recovered	deceased	p *value*	p *value* adjusted for multiple comparisons
hsa-miR-19b-3p|0	11.7 ± 2.9	10.6 ± 1.5	0.017	14.3 ± 1.8	10.6 ± 1.4	1.23E-06	0.003
hsa-miR-25-3p|+1	3.4 ± 1.2	1.9 ± 0.8	0.047	4.7 ± 1.2	2 ± 0.7	2.25E-05	0.046

increased level of microRNAs with a significant number of binding sites to the SARS-CoV-2 genome ($p = 0.048$).

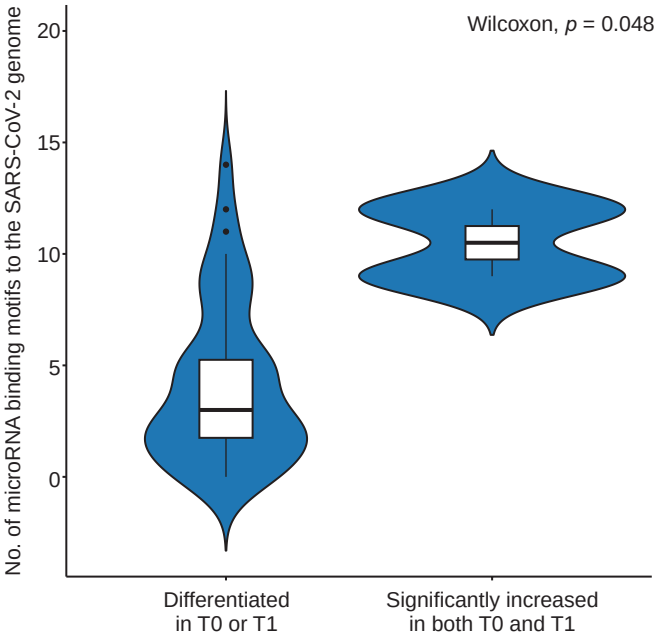

FIG. 1.
The number of possible binding sites of miRNAs in the SARS-CoV-2 genome

The location of possible binding sites of microRNAs to the virus genome was also analysed (Figure 2). The highest number of binding sites was located in the ORF1ab region encoding non-structural proteins. hsa-miR-19b-3p|0 had the highest number of binding sites in the short region (338 nucleotides) encoding the NSP9 protein. Both microRNAs analysed had two possible binding sites each with the extended region (5834 nucleotides) encoding the NSP3 protein. None of the microRNAs had binding sites in the 5`- and 3`-untranslated regions of the SARS-CoV-2 genome.

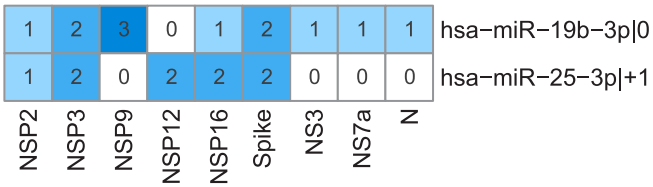

FIG. 2.
Location of possible miRNA binding sites in the SARS-CoV-2 genome

DISCUSSION

Study main result summary

The representation of hsa-miR-19b-3p|0 and hsa-miR-25-3p|+1 microRNAs differed significantly in the plasma of hospitalised patients with extremely severe COVID-19, both at the time of hospital admission and at the time of discharge or death. Both microRNAs have a high number of possible binding sites to the SARS-CoV-2 genome (12 and 9, respectively) compared to other plasma variable microRNAs. The majority of the binding sites are in the parts of the virus genome encoding NSP3 and NSP9.

Study main result discussion

MicroRNAs are short non-coding single-stranded RNA molecules of about 22 nucleotides in length that function as post-transcriptional regulators of gene expression [22]. MicroRNA molecules are involved in many processes including development, proliferation and apoptosis. In addition, microRNAs are associated with many pathological processes [23]. Determination of microRNA expression profiles can act as a method for classifying, diagnosing and predicting disease course [24]. MicroRNAs are detected in various biological fluids and have remarkable stability, emphasising their possible role as promising minimally invasive diagnostic and prognostic markers [25]. Moreover, several studies consider microRNA molecules as a component of the innate immunity system [3–5].

In this study, we studied the profile of circulating microRNAs in the plasma of extremely severe COVID-19 patients. The representation of hsa-miR-19b-3p|0 and hsa-miR-25-3p|+1 differed significantly in the plasma of patients with different COVID-19 outcomes, both at the time of hospital admission and at the time of discharge or death. MicroRNAs can act as a component of intracellular immunity by regulating translation and replication of (+) RNA viruses and altering the pathogenesis of viral infections [6, 7]. Considering that the lung tissue area is 75 to 100 m^2 and that it is abundantly blood supplied [26], it is conceivable that circulating microRNAs could penetrate infected alveocytes and interact with SARS-CoV-2 [27]. hsa-miR-19b-3p|0 significantly elevated in the group of recovered patients is among the most highly represented in plasma. It accounts for more than 1.5 % of all circulating microRNA molecules. This work shows that this microRNA has a significant number of binding sites to the SARS-CoV-2 genome. They are detected in its most stable part which is ORF1ab.

The largest number of hsa-miR-19b-3p|0 binding sites is in the 338-nucleotide long region encoding the NSP9 protein. The NSP9 protein is able to bind to 7SL RNA, component of signal recognition particles, thereby disrupting protein transport into the endoplasmic reticulum and onto the cell membrane [28]. The major histocompatibility complex (MHC) class 1 molecules are produced in the endoplasmic reticulum. Disruption of their synthesis may contribute to impaired antiviral activity of cytotoxic T lymphocytes. In addition, hsa-miR-19b-3p|0 is able to bind to the region encoding the NSP3 protein. NSP3 and NSP4 are responsible for the formation of double-membrane vesicles in the infected cell that protect the virus from the mechanisms of intracellular innate immunity [29]. Thus, hsa-miR-19b-3p|0 highly represented in the plasma of recovered COVID-19 patients is able to bind to virus regions encoding proteins responsible for suppressing intracellular immunity mechanisms.

Moreover, hsa-miR-19b-3p|0 is able to potentiate the activity of cytotoxic T lymphocytes. Levels of hsa-miR-19b-3p|0 were found to be significantly elevated in peripheral blood mononuclear cells of patients in long-term HIV remission. Overexpression of hsa-miR-19b-3p|0 promotes $CD8^+$ T-cell proliferation as well as interferon-γ and granzyme B expression by inhibiting $CD8^+$ T-cell apoptosis induced by anti-CD3/CD28 stimulation. The target of miR-19b was found to be the *PTEN* gene [30].

CONCLUSION

Previously, many papers were published on the prediction of the interaction of the single-stranded (+) RNA virus SARS-CoV-2 with human microRNAs, as well as on the profile of circulating microRNAs in patients with COVID-19 of varying severity. However, there are practically no works analysing the possible contribution of microRNAs circulating in plasma to SARS-CoV-2 biogenesis and COVID-19 pathogenesis. This study demonstrates that in patients who have recovered from a highly severe form of COVID-19, the level of hsa-miR-19b-3p in the blood plasma is statistically significantly increased. This microRNA is present in plasma in significant amounts and is able to bind to SARS-CoV-2 regions encoding proteins that suppress intracellular immune mechanisms. Morevover, it is able to stimulate the functional activity and proliferation of cytotoxic T lymphocytes, a key component of adaptive cellular SARS-CoV-2 immunity. The results of the study can be used in the development of antiviral drugs based on RNA interference, as well as in the development of predictive test systems to optimize the tactics of treating patients with COVID-19.

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Conflict of interest

The authors declare the absence of a conflict of interest.

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THYROID STATUS AND TNF-ALPHA IN POST-REPRODUCTIVE WOMEN WITH COVID-19 AND 12 MONTHS AFTER THE DISEASE

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ABSTRACT

The aim. *To assess the thyroid status and its relationship with tumor necrosis factor alpha (TNF-alpha) in post-reproductive women in the acute phase of the COVID-19 of moderate course and 12 months after the disease.*

Materials and methods. *85 women aged 45 to 69 years were divided into groups: women without COVID-19, not vaccinated, with no antibodies to COVID-19 (IgG) – control group (n = 15); women in the acute phase of COVID-19 of a moderate course, accompanied by pneumonia – main group (n = 57); patients from the main group who agreed to be examined 12 months after having COVID-19 (n = 14); women with IgG in blood who deny any symptoms of COVID-19 in the last 12 months – asymptomatic COVID-19 (n = 13). Using hormone replacement therapy and the presence of thyroid disease in history were the exclusion criteria from the study.*

Results. *75.4 % of patients with COVID-19 had euthyroidism, 12.3 % had subclinical hyperthyroidism. An increase of free thyroxine (free T4) level in women with COVID-19 as compared to the control group (p = 0.004) and the group with asymptomatic COVID-19 (p = 0.054) was found. There was no statistically significant difference in the level of thyroid stimulating hormone between the groups. The level of C-reactive protein in women with COVID-19 was naturally higher as compared to the control group (p = 0.009) and the group of asymptomatic patients (p = 0.001). A lower TNF-alpha level was found in the group of patients without clinical signs of COVID-19 as compared to the control group (p = 0.007) and the group with COVID-19 (p = 0.00007). The analysis of correlation relationships revealed a positive correlation between of free T4 and TNF-alpha levels in women with COVID-19 (r = 0.38; p = 0.004).*

Conclusions. *The moderate course of COVID-19 in the post-reproductive women is associated with an increase of free T4 level, which positively correlates with TNF-alpha level. Twelve months after COVID-19, thyroid status in women remains at the level of the acute phase of the disease.*

Key words: *COVID-19, long-term consequences, thyroid status, tumor necrosis factor alpha, post-reproductive period*

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ТИРЕОИДНЫЙ СТАТУС И ФНО-АЛЬФА У ЖЕНЩИН В ПОСТРЕПРОДУКТИВНОМ ПЕРИОДЕ С COVID-19 И ЧЕРЕЗ 12 МЕСЯЦЕВ ПОСЛЕ ЗАБОЛЕВАНИЯ

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РЕЗЮМЕ

Цель исследования. *Оценка тиреоидного статуса и его взаимосвязь с фактором некроза опухоли альфа (ФНО-альфа) у женщин в пострепродуктивном периоде в острую фазу среднетяжёлого течения COVID-19 и через 12 месяцев после заболевания.*

Методы. *85 женщин в возрасте от 45 до 69 лет были разделены на группы: женщины, не болевшие COVID-19, не привитые, с отсутствием антител к COVID-19 (IgG) – контроль (n = 15); женщины в острой фазе COVID-19 со среднетяжёлым течением, сопровождающимся пневмонией – основная группа (n = 57); пациентки из основной группы, согласившиеся пройти обследование через 12 месяцев после COVID-19 (n = 14); женщины с наличием в крови IgG, отрицающие какие-либо симптомы COVID-19 за последние 12 месяцев – бессимптомное течение COVID-19 (n = 13). Женщины, принимающие заместительную гормональную терапию, имеющие в анамнезе заболевания щитовидной железы, были исключены из исследования.*

Результаты. *У 75,4 % пациенток с COVID-19 отмечен эутиреоз, в 12,3 % случаев – субклинический гипертиреоз. Выявлено повышение уровня свободного тироксина (Т4св.) в группе женщин с COVID-19 по сравнению с контролем (p = 0,004) и группой переболевших COVID-19 бессимптомно (p = 0,054). Не выявлено статистически значимой разницы по уровню тиреотропного гормона между исследуемыми группами. Уровень С-реактивного белка в группе женщин с COVID-19 был закономерно выше по сравнению с контролем (p = 0,009) и с группой переболевших бессимптомно (p = 0,001). Выявлен более низкий уровень ФНО-альфа в группе переболевших без клинических признаков по сравнению с контролем (p = 0,007) и с группой с COVID-19 (p = 0,00007). При анализе корреляционных взаимосвязей выявлена положительная корреляция между уровнем Т4св. и ФНО-альфа у женщин с COVID-19 (r = 0,38; p = 0,004).*

Заключение. *Среднетяжёлое течение COVID-19 у женщин в пострепродуктивном периоде ассоциировано с повышением Т4св., который положительно коррелирует с уровнем ФНО-альфа. Через 12 месяцев после COVID-19 тиреоидный статус у женщин сохраняется на уровне острой фазы заболевания.*

Ключевые слова: *COVID-19, отдалённые последствия, тиреоидный статус, фактор некроза опухоли альфа, пострепродуктивный период*

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OBJECTIVES

The COVID-19 pandemic caused by the SARS-CoV-2 virus has left its mark in history as a public health emergency of international concern. Although it is predominantly a respiratory disease, evidence suggests that it is characterized by multi-organ system damage [1, 2]. With increasing age, the vulnerability to moderate and severe COVID-19 and subsequent complications is known to be higher [3], with men having a more severe course of the disease [4], but in old age, sex differences in the course of COVID-19 are leveled off, which may be associated with a decrease in estrogen levels in women in the post-reproductive period [5].

Age-related estrogen deficiency in women is considered to be one of the principal causes of the various organs disorders development, including Thyroid disease [6–10]. The incidence of most Thyroid disorders is known to be high in postmenopausal and elderly women, which may lead to increased cardiovascular risk, bone fractures, cognitive impairment, depression and mortality [10].

Some studies have shown that the thyroid gland may be a target organ for SARS-CoV-2 with the development of its dysfunction during and after COVID-19 [11–13]. Thyroid status evaluation after prolonged time from recovery after COVID-19 has been performed in few studies [14, 15], and there are no studies in the available literature evaluating thyroid status 12 months after COVID-19. This may be due to the assumption of a duration of up to 6 months for the Post-COVID-19 syndrome, which is why most studies are limited to this time frame [16–18]. In addition, studies do not indicate the effect of age on changes in thyroid status in COVID-19, which may play a role in analyzing the findings [19]. Equally, it is of interest to evaluate the correlations of thyroid hormones with tumor necrosis factor alpha (TNF-alpha) in COVID-19. This cytokine plays a significant role in the pathogenesis of thyroid cancer [20], and in COVID-19 there is a statistically significant increase in its level, and TNF-alpha is considered a risk factor for death in patients with severe or critical COVID-19 [21].

THE AIM OF THE STUDY

Thyroid status evaluation in post-reproductive women with COVID-19 of a moderate course during the acute phase and 12 months after the disease, as well as correlations of thyroid hormones with tumor necrosis factor alpha in the acute phase of COVID-19.

MATERIALS AND METHODS

A short-term longitudinal case-control study was conducted.

The study included 94 women aged 45 to 69 years. In order to be selected into the main cohort, 64 women hospitalized in the Irkutsk Regional Infectious Clinical Hospital in the period from June 2020 to March 2021 with laboratory-confirmed PCR test for the presence of SARS-CoV-2 virus and moderate course of COVID-19 accompanied by pneumonia were examined. Upon admission of the patients to the hospital, questionnaires and analysis of medical records, general clinical examination, and computed tomography were performed. After clinical and anamnestic examination, 7 women with a history of thyroid disorder (manifest forms of the disease: autoimmune thyroiditis – 3; thyrotoxicosis – 1; hypothyroidism – 1; Graves' disease – 1; nontoxic goiter – 1) were excluded. Thus, the main cohort included 57 women (mean age 58 ± 6.33 years). Fourteen women who were called for a clinical and anamnestic examination agreed to be examined after 12 months from those who had survived COVID-19.

Thirty women who denied any symptoms of COVID-19 and had not been vaccinated in the past 12 months were examined to form a control group. The presence of COVID-19 IgG antibodies in blood was determined in all women, after which two groups were formed: without IgG (n = 17) and with IgG (n = 13). Two women with thyroid dysfunction were excluded from the group without IgG; thus, 15 women (mean age, 56 ± 6.52 years) formed the control group for comparison with the main cohort and the group of women who agreed to be examined 12 months after COVID-19. 13 women with IgG in their blood formed a separate group with asymptomatic COVID-19 (mean age, 54 ± 7.59 years).

The use of hormone replacement therapy (HRT) was a criterion for not including women in the study.

All study participants were examined by a general practitioner-cardiologist with calculation of body mass index (BMI), measurement of blood pressure, body temperature, and electrocardiogram. To exclude the presence of COVID-19 at the time of the study, an appropriate rapid test (RAPID BIO, Russia) was performed.

Venous blood was used for laboratory tests, which was collected from 8.00 to 9.00 AM on an empty stomach in accordance with generally accepted requirements. Blood was centrifuged for 10 min at 1,500 rpm, serum was separated and stored at –40 °C until assayed.

Thyroid status indicators were determined: free thyroxine (free T4), thyroid stimulating hormone (TSH)), IgG, C-reactive protein (CRP) and TNF-alpha levels. IgG level was determined on Multiscan Go analyzer (Thermo Scientific, Finland) using Vector-Best commercial kits (Russia). The concentration of free T4 (pmol/l) and TSH (mmol/l) was determined by enzyme immunoassay on Microplate reader ELx808 analyzer (USA) using Alkor Bio commercial kits (Russia); CRP (pg/ml) and TNF-alpha (pg/ml) – using Vector-Best commercial kits (Russia).

Informed consent to participate in the study was signed by each woman. The study protocol was reviewed and approved by the Biomedical Ethics Committee of Scientific Centre for Family Health and Human Reproduction Problems (excerpt from the minutes of the meeting No. 6.1 of June 19, 2020).

Statistical analysis

No pre-calculation of sample size was made. The data obtained were processed in Statistica 10 program (StatSoft Inc., USA). The proximity to the normal law of distribution of quantitative signs was evaluated by visu-

al-graphical method, as well as Kolmogorov – Smirnov (K-S) test (with Lilliefors correction) and Shapiro – Wilk (S-W) test. Data for age and BMI are presented as arithmetic mean ± standard deviation (m ± σ); for laboratory parameters, as median and interquartile range (Me [Q1; Q3]). Intergroup differences for independent samples were analyzed using the Kruskal – Wallis test (ANOVA) and the median test followed by post hoc comparisons using the Mann – Whitney U test. Intragroup differences were analyzed using the Wilcoxon test. Pearson correlation analysis with determination of correlation coefficient (*r*) was used to analyze the relationships between the indicators. The significance level was taken as 5 % (0.05).

RESULTS

In the first step, we analyzed the distribution of COVID-19 patients according to specified free T4 and TSH intervals. In most cases, euthyroidism was noted in the patients, and subclinical hyperthyroidism was registered in 12.3 % of cases (Table 1).

TABLE 1

DISTRIBUTION OF PATIENTS WITH COVID-19 DEPENDING ON THYROID STATUS

Free T4 10.0-23.2 pmol/l	TSH 0.23–3.4 mmol/l	Patients with COVID-19
↔	↔	43 (75.4 %)
↔	↑	3 (5.3 %)
↔	↓	7 (12.3 %)
↑	↔	2 (3.5 %)
↓	↔	1 (1.75 %)
↓	↑	1 (1.75 %)

Note. ↔ – within reference values; ↓ – below reference values; ↑ – above reference values

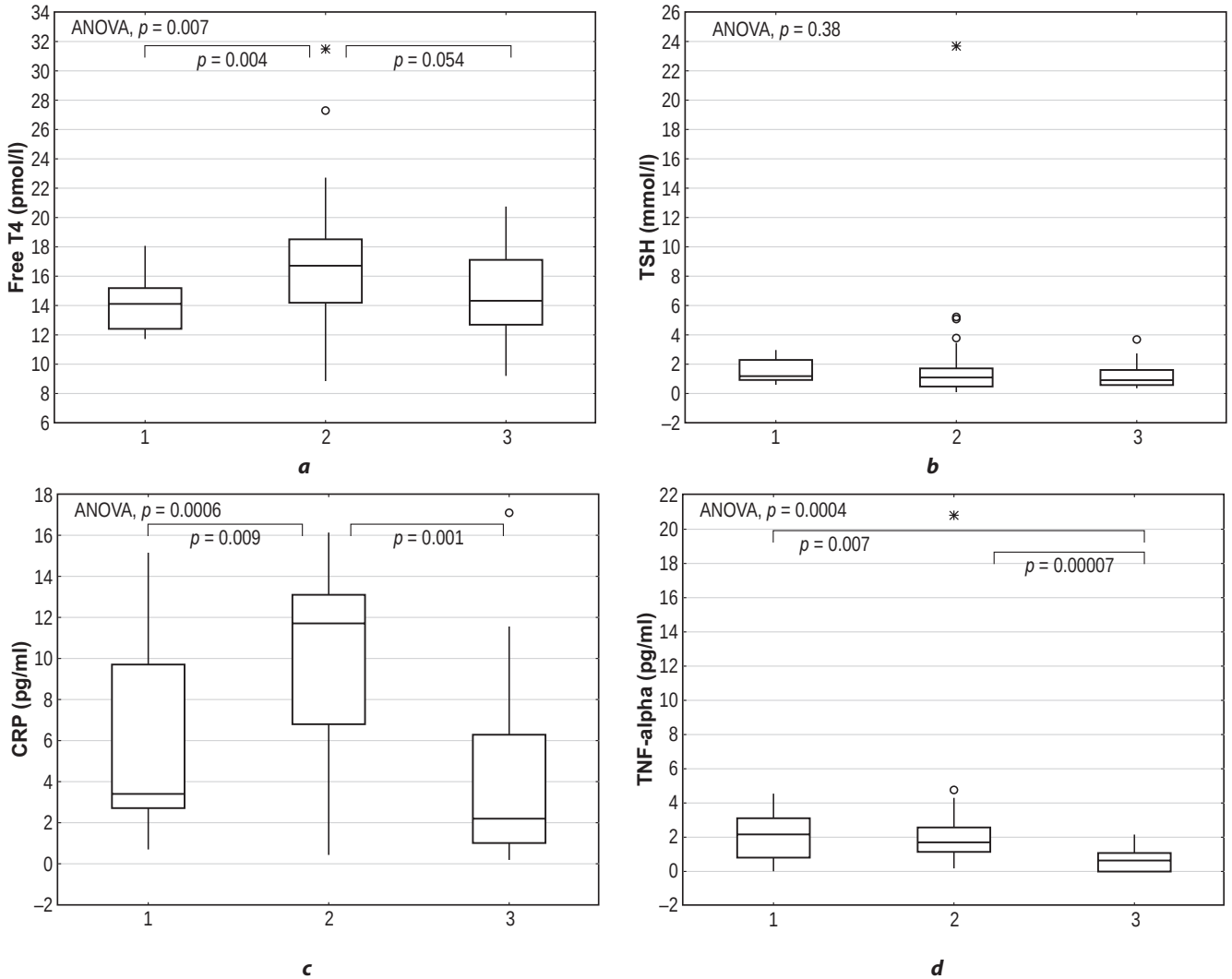

FIG. 1.

*Levels of free thyroxine (**a**), thyroid-stimulating hormone (**b**), C-reactive protein (**s**) and tumor necrosis factor alpha (**d**) in women of post-reproductive age in control group (1; n = 15), group with COVID-19 (2; n = 57) and group with asymptomatic course of the disease (3; n = 13)*

Then we performed an intergroup comparative analysis of the levels of the hormones studied, the results of which indicate an increased free T4 level in the group of women with COVID-19 (16.7 [14.2; 18.5] pmol/l) compared with controls (14.1 [12.4; 15.2] pmol/l) and the group of COVID-19 asymptomatic survivors (14.3 [12.7; 17.1] pmol/l) (Fig. 1a). No statistically significant difference in TSH level was found between the studied groups (Fig. 1b).

Moreover, the level of CRP in the group of women with COVID-19 (11.7 [6.8; 13.1] pg/ml) was consistently higher compared with the control (3.4 [2.7; 9.7] pg/ml) and the group with asymptomatic course of the disease (2.2 [1; 6.3] pg/ml) (Fig. 1c).

We found lower TNF-alpha levels in the group of survivors without clinical signs (0.6 [0.01; 1] pg/ml) compared with controls (2.11 [0.77; 3.08] pg/ml) and the group with COVID-19 (1.7 [1.1; 2.5] pg/ml) (Fig. 1d). When analyzing correlations, a significant positive correlation between free T4 and TNF-alpha levels was revealed (Fig. 2).

In the final phase of the study, we performed a comparative analysis of the investigated parameters in women in the acute phase of COVID-19 and in the same women 12 months after recovery. We did not find any differences in both thyroid hormone levels, CRP and TNF-alpha levels (Fig. 3).

DISCUSSION

This study demonstrates for the first time the results of thyroid status assessment in women of post-reproductive age in the long-term post-COVID-19 period. Previous studies of patients with COVID-19 have found a correlation of low free triiodothyronine (free T3), free T4, and TSH concentrations with COVID-19 severity and mortality [22–24]. However, the results of M. Chen et al. did not demonstrate any changes in free T4 content with decreased levels of free T3 and TSH; at the same time, thyroid hor-

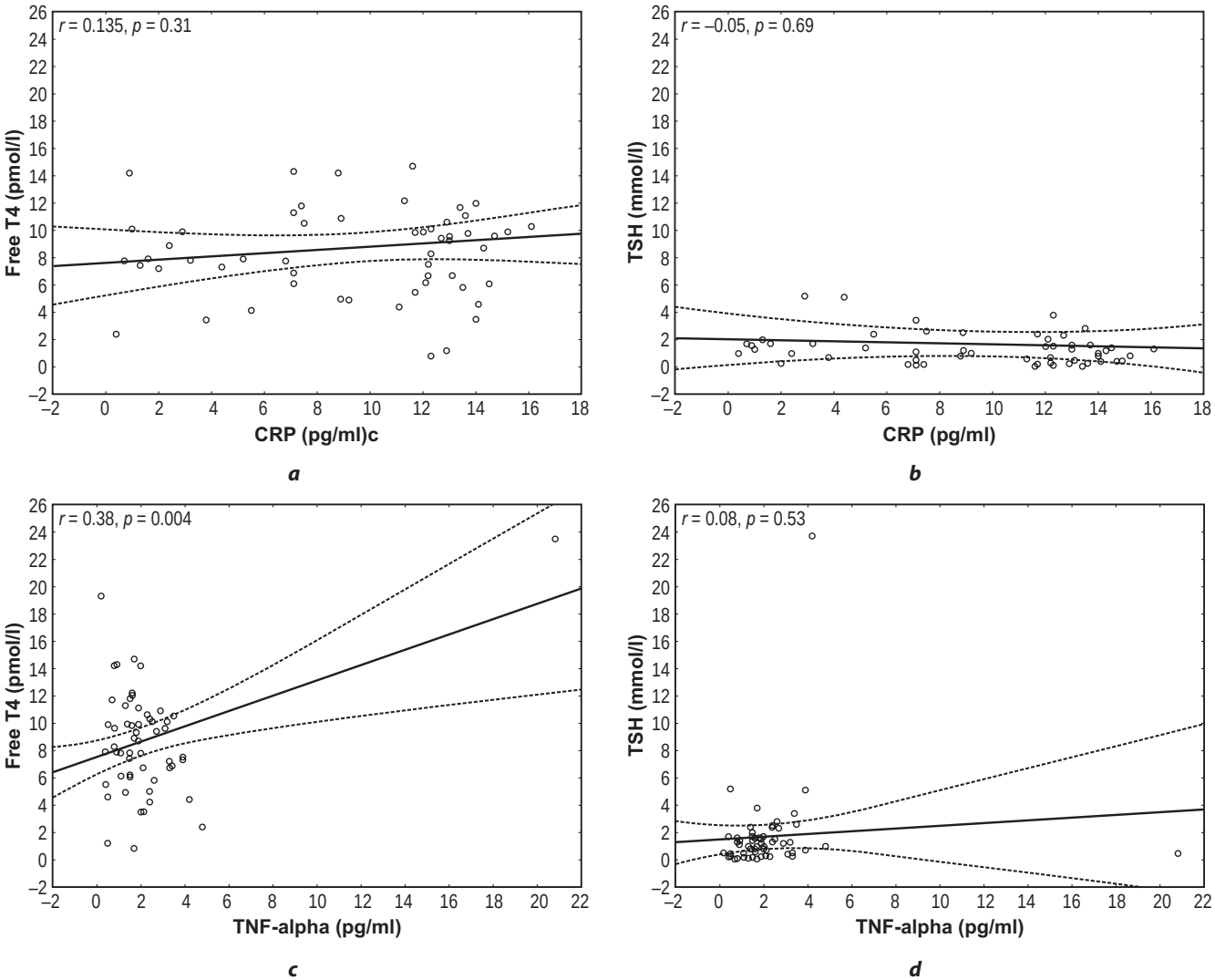

FIG. 2.

*Correlations between the levels of C-reactive protein (**a**, **b**), tumor necrosis factor alpha (**c**, **d**) and thyroid hormones in women of post-reproductive age in the acute phase of COVID-19*

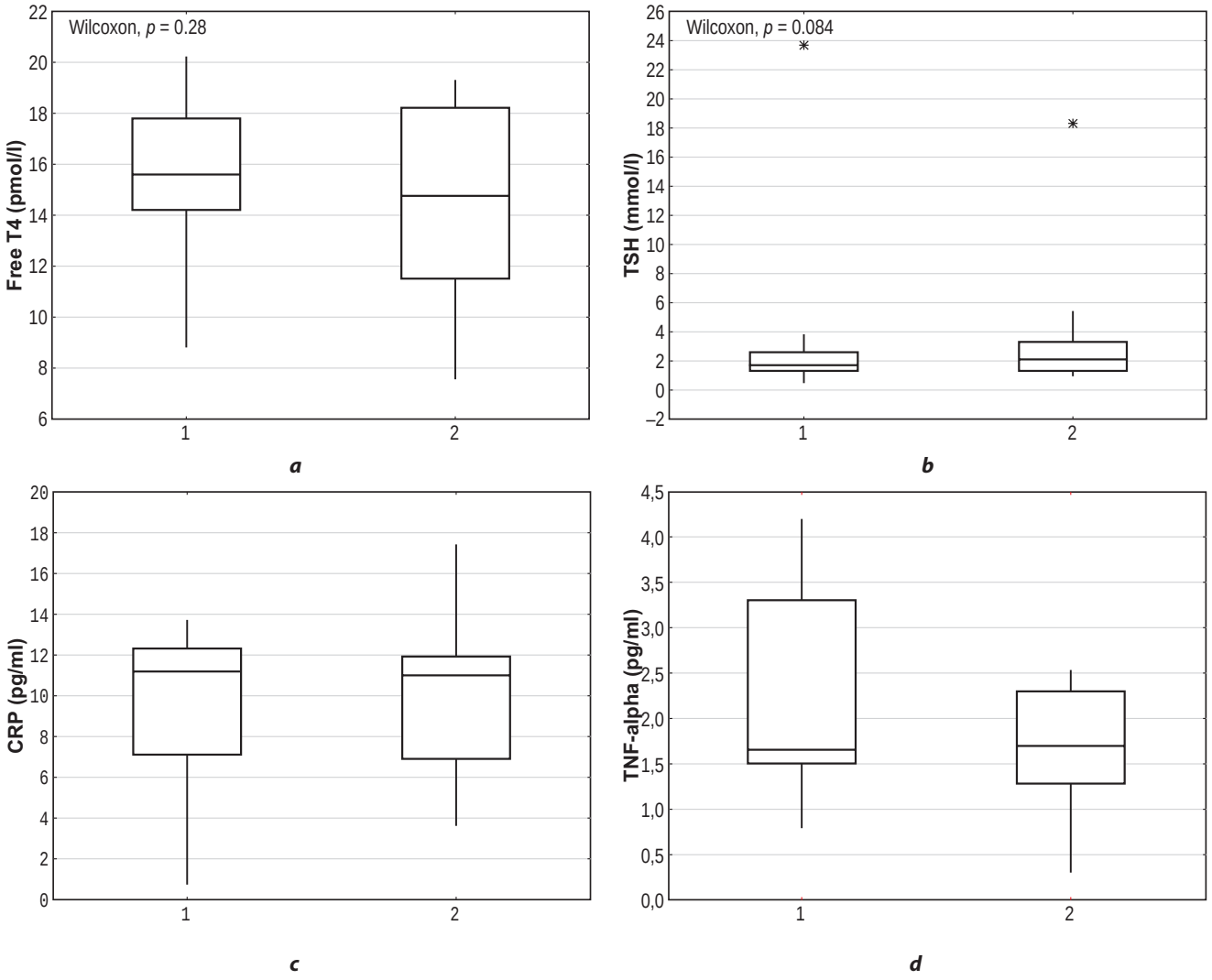

FIG. 3.

*Levels of free thyroxine (**a**), thyroid-stimulating hormone (**b**), C-reactive protein (**c**) and tumor necrosis factor alpha (**d**) in women of post-reproductive age in groups with COVID-19 (1; n = 14) and 12 months after COVID-19 (2; n = 14)*

mone levels in patients immediately after recovery corresponded to control values [25]. The results obtained in our study suggest an increase in free T4 in the acute phase of the disease, which is not consistent with the few studies presented above. As it is known, the main function of free T4 is the activation of metabolic processes, which is accompanied by an increase in cellular oxygen consumption [26]. This may be related to changes in free-radical homeostasis in the acute phase of COVID-19 [27–31], shown in a number of studies to be associated, in particular, with changes in thyroid function. Moreover, our study revealed a positive correlation between free T4 and one of the key mediators of immune response, inflammation and apoptosis – TNF-alpha, the level of which is increased in autoimmune thyreopathies [32]. When assessing TNF-alpha levels in hypo- and hyperthyroidism, an increase in its level was shown, while a decrease in the cytokine level was noted with normalization of thyroid function only in patients with hyperthyroidism after adequate treatment [33].

This study did not reveal a higher level of TNF-alpha in patients in the acute phase of the disease compared with controls, which may be related to the post-reproductive age of women in the compared groups, since it is known about the increased level and important role of this cytokine in aging processes [34]. In addition, the participants of this study were patients with moderately severe COVID-19, and significant elevation of TNF-alpha was observed in severe and critical disease [21]. It should be noted that TNF-alpha levels are lower in asymptomatic women of the same age, even compared to controls, which probably indicates better functioning of their organs and systems and a more adapted immune system. The results of an earlier study demonstrated higher physical and emotional health scores in this group [35].

When thyroid status was assessed 12 months after the disease, the persistence of elevated free T4 levels was found, as well as control TSH levels in women. There are few studies on thyroid status in the post-COVID-19 period. Thus, E. Urban et al. showed that its volume 2–7 months after COVID-19

is smaller than in healthy people, without a significant difference in the content of thyroid hormones [14], and B. Khoo et al. found an increase in the level of TSH in COVID-19 survivors up to control values after 52–108 days from the moment of hospitalization [15].

In our opinion, the wide age range of the patient sample in previous studies may be one of the reasons for their inconsistency with the results of the present study of thyroid hormone levels in COVID-19. Meanwhile, there is increasing evidence that the reference range of TSH increases with age, which may be related to the impaired feedback of the pituitary gland to the target organ in the elderly [19]. The results of the study by N. Milinković et al. in which 22,860 age-differentiated serum samples were analyzed, showed an increase in TSH levels in men over 70 years of age, while in women no differences between age groups were found. In addition, sex differences in free T4 levels have been found in the 31–40 and 41–50 age groups, with lower values in women [36]. Based on the above, it seems essential to take into account age and gender aspects when assessing thyroid status.

Study limitations

Study limitations include the small number of patients who agreed to be evaluated 12 months after COVID-19 and the small number of women who were assigned to the control group due to the high prevalence of SARS-CoV-2.

CONCLUSION

The results of the study indicate long-term changes in thyroid status in women in the post-reproductive period who have survived COVID-19 and the need to monitor it in the long-term post-COVID-19 period in order to timely diagnose the development of pathologic conditions of the thyroid gland.

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Conflict of interest

The authors of this article confirm that there is no conflict of interest.

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OBSTETRICS AND GYNAECOLOGY

ASSISTED HATCHING IN CRYOPRESERVATION PROTOCOLS IN PATIENTS WITH TUBOPERITONEAL INFERTILITY

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ABSTRACT

At present, the problem of increasing the effectiveness of programs of assisted reproductive technologies and successful infertility treatment is still relevant. Assisted hatching used in the devitrificated embryo transfer facilitates the exit of the embryo from the pellucide zone. Yet the clinical efficacy of assisted hatching is relevant and debatable. There are no clear indications for the use of this technology, and no groups of patients have been identified.

The aim of the study. *To assess the effectiveness of laser hatching in the frozen-thawed embryo transfer programs in patients with tuboperitoneal infertility.*

Materials and methods. *We examined 300 women with tuboperitoneal infertility who had their embryos frozen for transfer. Inclusion criteria: age from 18 to 35 years; tuboperitoneal infertility; embryos cryopreserved for transfer. Exclusion criteria: age more than 36 years; other infertility factors. Women were divided into 2 groups: group 1 – women who had a frozen-thawed embryo transfer with preliminary laser hatching (n = 137); group 2 – control group (n = 163).*

Results. *There were no differences between the groups in the mean age, body mass index and the age at menarche. According to the results of the embryological stage, there were also no differences in the number and quality of frozen embryos. The pregnancy rate in the group with preliminary laser hatching was 44.5 %, which is significantly higher than in the control group (42.3 %; $p \leq 0.001$). We also found statistically significant differences in pregnancy outcomes: in the frequency of spontaneous miscarriages – 13.1 % and 20.2 % respectively ($p \leq 0.001$), in the frequency of term deliveries – 30.7 % and 22.1 % respectively ($p \leq 0.001$).*

Conclusion. *In our study, the using laser hatching in women with tuboperitoneal infertility positively affected the embryos implantation in the cryopreservation protocols. Pregnancy and live birth rates are higher after using hatching technology, and the frequency of miscarriages up to 12 weeks is lower. This provide an opportunity to further study the effect of hatching on long-term outcomes, such as gestation course and childbirth.*

Key words: *assisted reproductive technologies, cryopreservation protocol, frozen-thawed embryo, cryopreservation, assisted hatching*

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ПРИМЕНЕНИЕ ВСПОМОГАТЕЛЬНОГО ХЭТЧИНГА В КРИОПРОТОКОЛАХ У ПАЦИЕНТОК С ТРУБНО-ПЕРИТОНЕАЛЬНЫМ БЕСПЛОДИЕМ

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РЕЗЮМЕ

В настоящее время проблема эффективного преодоления бесплодия в программах экстракорпорального оплодотворения остаётся по-прежнему актуальной. Технология вспомогательного хэтчинга, используемая при переносе девитрифицированного эмбриона, направлена на облегчение высвобождения эмбриона из блестящей оболочки. Однако вопрос его клинической эффективности остаётся крайне актуальным и противоречивым.

Цель исследования. *Оценка эффективности применения лазерного хэтчинга в программах с переносом размороженного эмбриона у женщин с трубно-перитонеальным бесплодием.*

Материалы и методы. *Было обследовано 300 женщин, страдающих трубно-перитонеальным бесплодием, которые имели криоконсервированные эмбрионы. Критерии включения: возраст от 18 до 35 лет включительно; трубно-перитонеальное бесплодие; наличие криоконсервированных эмбрионов для переноса. Критерии исключения: возраст 36 лет и старше; наличие других факторов бесплодия. Далее были сформированы две группы: группа 1 – женщины, у которых перенос размороженных эмбрионов выполнялся с проведением предварительного лазерного хэтчинга (n = 137); группа 2 – группа контроля (n = 163).*

Результаты. *Группы сравнения не различались по среднему возрасту, индексу массы тела, возрасту менархе. По результатам эмбриологического этапа также не выявлены различия по количеству и качеству замороженных эмбрионов. Частота наступления беременности в группе исследования с проведением лазерного хэтчинга составила 44,5 %, что статистически значимо выше, чем в группе контроля (42,3 %; $p \leq 0,001$). Также нами были выявлены статистически значимые различия в исходах беременностей: в частоте самопроизвольных выкидышей – 13,1 % и 20,2 % соответственно ($p \leq 0,001$), срочных родов – 30,7 % и 22,1 % соответственно ($p \leq 0,001$).*

Заключение. *В нашем исследовании применение лазерного хэтчинга благоприятно повлияло на имплантацию в криопротоколах. Однако связь между хэтчингом и долгосрочными исходами, такими как течение беременности и роды, требует дальнейшего изучения.*

Ключевые слова: *вспомогательные репродуктивные технологии, криопротокол, размороженный эмбрион, криоконсервация, вспомогательный хэтчинг*

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RELEVANCE

At present, the problem of increasing the efficiency of programs of assisted reproductive technologies and successful infertility treatment is still relevant. Data published in the 26th annual report of the Register of Assisted Reproductive Technologies (ART) of the Russian Association of Human Reproduction (RAHR) show that the pregnancy rate in in vitro fertilisation (IVF) programs in 2020 was: per cycle – 28.9 % (in 2019 –32.3 %), per puncture – 30.0 % (in 2019 – 33.3 %), per embryo transfer – 34.8 % (in 2019 – 38.5 %) [1]. In programs with frozen-thawed embryo transfer, the pregnancy rate per cycle was 41.3 % (41.8 % in 2019) and per embryo transfer was 42.1 % (43.0 % in 2019) [1]. Despite significant progress in the field of assisted reproductive technologies, it is impossible to improve the outcome of ART programs without studying the mechanisms of implantation.

The outcome of ART programs is influenced by many factors. The main ones are endometrial condition, embryo quality and delivery to the implantation site [2, 3]. However, during the preimplantation development stage, the embryo is inside a glycoprotein shell, which is called the zona pellucida (pellucid zone) [4, 5]. Failure of the embryo to emerge from the zona pellucida often results in failure in ART programs and spontaneous pregnancy does not occur.

The human oocyte is surrounded by the zona pellucida shell composed of specific glycoproteins. After fertilization, blocking mechanisms are triggered in the zona pellucida and plasma membrane of the oocyte to prevent the penetration and fusion of additional spermatozoa [6, 7]. After fertilization, the zona pellucida shell persists and surrounds the developing human embryo for a few more days. Then, the embryo emerges from the shell and establishes cell contacts between the trophectoderm and the endometrial epithelium for subsequent implantation [8]. In embryology, the term «hatching» refers to the emergence of an embryo from the zona pellucida shell: spontaneous hatching occurs at the blastocyst stage, when the shell ruptures and the blastocysts emerge through the resulting gap. The zona pellucida rupture is caused by the following factors: release of proteolytic enzyme – cathepsin – by the cells of trophectoderm, which in turn dissolves the area of the shell; mechanical rupture of the zona pellucida by a blastocyst due to an increase in size [9, 10].

At the embryological stage, various factors may affect a blastocyst, such as the composition of the culture medium, intracellular zinc concentration in the oocyte, impaired synthesis of metalloproteinases and the use of cryopreservatives for the embryo freezing, leading to thickening of the zona pellucida, which necessitates preliminary assisted hatching [11–13].

Assisted hatching is an embryological technique that aims to increase the embryo implantation rate by eliminating the cause of incomplete hatching. Several types of assisted hatching are used in embryological practice: mechanical, laser-assisted, chemical or enzymatic. During mechanical hatching, the zona pellucida shell is pierced with a micro-needle, but there is a risk of rupture or loss of blastomeres and there may be an increased incidence of multiple pregnancies [12–14]. During chemical hatching, an acid is applied to the shell, which if the technique of the procedure is not followed can be destructive to the blastomeres adjacent to the hole made. The zona pellucida shell dissolves on contact with acid, so the embryo is immediately removed and washed several times to remove any traces of acid [14].

The laser is ideal for microsurgical procedures because the energy is easily focused on the target area, creating a controlled and precise orifice that is coordinated between operators. Using an optical lens, the laser beam is directed tangentially to the embryo through the zona pellucida in non-contact mode or touches the embryo and performs hatching in contact mode [14]. Femtosecond laser pulse technology is currently used in cell surgery. According to the study of M.M. Rakityansky et al., the use of femtosecond laser pulses allows for precision perforation of the zona pellucida without affecting its cells. A femtosecond laser scalpel-tweezers are used to perform laser-assisted hatching. An optical mammalian embryo biopsy technique has been developed. It allows non-contact isolation of material from the embryo for preimplantation diagnosis of its condition. The results of the study show that about 90 % of embryos subjected to such operations retained the ability to develop to the blastula stage [15].

A. Alteri et al. who compared mechanical, chemical and laser-assisted hatching reported the superiority of the latter [16]. In the study by C. Liu et al. use of laser-assisted hatching during transfer of a single frozen-thawed blastocyst shows higher rates of implantation, pregnancy and live birth [17].

Despite the large number of existing embryological techniques, the issue of clinical efficiency of programs of cryopreserved embryo transfer remains highly relevant and controversial. Considering the above arguments, the aim of the study was to evaluate the efficiency of laser hatching in programs with frozen-thawed embryo transfer among female patients with tuboperitoneal infertility.

MATERIALS AND METHODS

The study was conducted at the ART department of the Regional Perinatal Center of the State Budgetary Healthcare Institution «Irkutsk Regional Clinical Hospital, winner of the "Mark of the Honor" award from 2018 to 2021. A total of 300 women receiving ART treatment for tuboperitoneal infertility (ICD-10: N97.1) were examined. After the IVF program, the patients' embryos were vitrified for further transfer. The inclusion criteria were: age range from 18 to 35 years inclusive; tuboperitoneal infertility; embryos vitrified for transfer. Exclusion criteria: age 36 years and older; other infertility factors; endometriosis; uterine anomalies; absence of vitrified embryos; male

infertility; use of donor material (donor oocytes, sperm and embryos).

Patients with cryopreserved embryos were divided into two groups depending on the use of laser-assisted hatching: group 1 – women who had a frozen-thawed embryo transfer with preliminary laser hatching (n = 137); group 2 – control group which consisted of women who had a frozen-thawed embryo transfer without hatching (n = 163). All patients gave a voluntary informed consent to participate in the study. The work with the patients was carried out in accordance with the ethical standards of the World Medical Association Declaration of Helsinki (ed. 1964, 2012). The conduct of this study was approved by the local ethical committee of the Irkutsk State Medical Academy of Postgraduate Education – Branch Campus of the Russian Medical Academy of Continuing Professional Education of the Ministry of Health of Russia (Minutes No. 12 dated 14.11.2017).

Clinical and medical history data, IVF program and embryological stage, cryopreservation protocol and pregnancy rate of the compared groups were studied after the performed manipulations.

Controlled ovarian stimulation in IVF cycles was performed according to a protocol with gonadotropin-releasing hormone antagonists. The average doses of gonadotropins used were not statistically different between groups. The ovulation trigger is chorionic gonadotropin at a dosage of 6500 IU. The method of fertilization is IVF or intracytoplasmic sperm injection (ICSI).

At the cleavage stage, embryo quality was assessed according to the classification of J. Lens et al., at the blastocyst stage – according to the classification of D. Gardner et al. (1999). Embryos were vitrified (ultra-fast cryopreservation) using Kitazato reagent kit (Japan). Thawing was carried out according to the manufacturers' recommendations. Preparation of the endometrium for thawed embryo transfer was performed with step-by-step administration of estrogen and progesterone preparations according to the days of embryo culturing. In all cases, transfer of one or two embryos of excellent and good quality was performed when endometrial thickness of 8 mm or more was achieved. The efficiency of this method was assessed by pregnancy rate and long-term outcomes (spontaneous abortion and term birth).

Statistical processing was carried out with preliminary assessment for compliance with the normal distribution (the Gaussian distribution), then by nonparametric methods using the Statistica v. 10.0 basic package (StatSoft Inc., USA). The parametric Student's test (t-test) was used to test the statistical hypothesis of equality of two independent samples in the case of normally distributed continuous variables. The nonparametric Mann – Whitney test was used for pairwise comparison of groups in the case of distribution of values different from the normal distribution. The χ^2 test, the χ^2 test with Yates's correction for continuity and the two-tailed Fisher exact test were used in the analysis of 2 × 2 contingency tables. Differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

At the first stage, we analyzed the clinical and medical history data. The comparison groups did not differ in the patients' average age, body mass index and age at menarche. The average duration of infertility in the studied patients also had no statistical differences and was 5.1–5.9 years ($p = 0.6$). A history of pelvic inflammatory diseases was found in more than 95 % of cases (group 1 – 95 % of cases; group 2 – 96.6 % of cases; $p = 0.9$); reconstructive surgeries on fallopian tubes were performed in more than 70 % of cases (group 1 – 77 % of cases; group 2 – 77.5 % of cases; $p = 0.5$), but no statistically significant differences were found.

In the study groups, secondary infertility was more frequent than primary infertility: in group 1, primary infertility was registered in 47.7 % and secondary infertility in 52.3 % of cases; in group 2, in 38 % and 62 % of cases, respectively. One third of all women studied had a history of childbirth (31.1 % and 32.7 %, respectively). Half of the women had medical abortions (50.9 % and 51.7 % respectively). It is important to note that half of the subjects had a history of ectopic pregnancies (55.2 % and 53.2 %, respectively; $p = 0.4$), which led to fallopian tube surgery, but the differences were not statistically significant.

Hormonal panel analysis (Table 1) showed that hormone values were within reference values, but the control group had statistically higher levels of follicle-stimulating hormone (FSH) and progesterone than the assisted hatching group (7.2 ± 1.9 and 6.3 ± 1.9 mIU/ml, respectively ($p = 0.012$); 31.2 ± 22.1 and 24.5 ± 16.3 nmol/L, respectively ($p = 0.012$)). The control group also differed statistically significantly in low Anti-Müllerian hormone (AMH) levels (3.2 ± 1.5 ng/ml) from the assisted hatching group (5.4 ± 2.6 ng/ml; $p < 0.001$) and had a statistically significantly lower number of antral follicles (6.1 ± 2.4 and 8.6 ± 3.9, respectively; $p < 0.001$), but all women studied had adequate ovarian reserve and number of embryos for cryopreservation.

Embryological stage and pregnancy outcomes were further analyzed in our study and the data are presented below (Table 2). Spontaneous abortions were defined as pregnancies that ended before 12 weeks. Term birth was defined as birth from 37 weeks onwards.

According to the results of the embryological stage (Table 2), the comparison groups did not differ in the number of cryopreserved and thawed embryos and embryo quality. Optimal endometrial thickness at the time of embryo transfer was also achieved in the study groups, but the differences were not statistically significant.

The pregnancy rate and outcomes deserve special attention: pregnancy occurred in 44.5 % of cases in the study group with laser-assisted hatching, in the control group – in 42.3 % of cases ($p < 0.001$); spontaneous abortion rates were 13.1 % and 20.2 %, respectively ($p < 0.001$). In group 1, 30.7 % of pregnancies ended in birth, in the control group – 22.1 %; the differences were statistically significant ($p < 0.001$). Therefore, preliminary assisted hatching increased the pregnancy rate in the study group and favorably influenced the pregnancy outcome compared to the control group.

TABLE 1

CLINICAL AND ANAMNESTIC CHARACTERISTICS IN THE STUDIED GROUPS

Indicators	Group 1 (*n* = 137)	Group 2 (*n* = 163)	*p value*
	M ± SD; Me (25th; 75th percentiles)		
Baseline FSH, mIU/ml	6.3 ± 1.9; 6.3 (0.6; 9.1)	7.2 ± 1.9; 7 (2.7; 9.4)	0.012*
Baseline LH, mIU/ml	6.7 ± 4.3; 6.1 (0.9; 15.6)	5.9 ± 2.8; 5.4 (1.3; 11.5)	0.4
Baseline progesterone, nMol/L	24.5 ± 16.3; 16.1 (0.1; 160)	31.2 ± 22.1; 30.6 (0.2; 84)	0.012*
Baseline AMH, ng/ml	5.4 ± 2.6; 4.9 (1.2; 12.9)	3.2 ± 1.5; 3.3 (0.8; 9.6)	< 0.0001*
Left ovary volume, cm^3	8.3 ± 7.3; 7.1 (1.9; 13.4)	7.4 ± 4.5; 6.7 (1.9; 16.6)	0.1
Right ovary volume, cm^3	11.1 ± 7.1; 8.3 (0.5; 14)	8.1 ± 4.1; 7.6 (0.7; 13.8)	0.1
Number of antral follicles	8.6 ± 3.9 9.0 (5.5; 12.0)	6.1 ± 2.4 5.5 (4.5; 7.0)	<0.001

Note. LH – luteinizing hormone; * – $p \leq 0.05$.

TABLE 2

CRYOPRESERVATION PROTOCOL PARAMETERS IN THE STUDIED GROUPS

Indicators	Group 1 (n = 137)	Group 2 (n = 163)	p value
Vitrification on the 3th day of cultivation, *n* (%)	20 (14.6 %)	21 (12.9 %)	0.7
Vitrification on the 4th day of cultivation, *n* (%)	49 (35.8 %)	74 (45.4 %)	0.2
Vitrification on the 5th day of cultivation, *n* (%)	68 (49.6 %)	68 (41.7 %)	0.4
Number of thawed embryos, M ± SD; Me (25th; 75th percentiles)	1.8 ± 0.4; 2 (1; 2)	1.8 ± 0.3; 2 (1; 2)	0.4
Thawing percentage, M ± SD; Me (25th; 75th percentiles)	85.4 ± 20.9; 100 (33.3; 100)	80.3 ± 23.1; 83.4 (33.3; 100)	0.5
«Day-to-day» cryotransfer, *n* (%)	67 (48.9 %)	61 (37.4 %)	0.2
Completing of cryopreserved embryo growing, *n* (%)	70 (51.1 %)	102 (62.6 %)	0.2
Cryotransfer of 4-day old embryos (morula), *n* (%)	8 (5.8 %)	8 (4.9 %)	0.7
Cryotransfer of 5-day old embryos (blastocyst), *n* (%)	129 (94.2 %)	155 (95.1 %)	0.9
Good quality embryos (for transfer), *n* (%)	60 (43.8 %)	56 (34.4 %)	0.2
Fair quality embryos, *n* (%)	75 (54.7 %)	103 (63.2 %)	0.4
Poor quality embryos, *n* (%)	2 (1.5 %)	4 (2.5 %)	0.5
Endometrial thickness at the time of transfer, M ± SD; Me (25th; 75th percentiles)	10.2 ± 1.4; 10 (8; 12)	10.2 ± 1.1; 10 (8.3; 12)	0.3
Pregnancy rate, *n* (%)	61 (44.5 %)	69 (42.3 %)	< 0.0001*
Spontaneous abortions, *n* (%)	18 (13.1 %)	33 (20.2 %)	< 0.0001*
Term births, *n* (%)	42 (30.7 %)	36 (22.1 %)	< 0.0001*

Note. * – $p \leq 0.05$.

CONCLUSION

Despite the successful development of methods of assisted reproductive technologies (ART), the issues of improving the efficiency of infertility treatment remain relevant. The search for new solutions leads to the introduction of new techniques into routine embryological practice. Currently, laser-assisted hatching is widely used in cryopreservation protocols, but the need and indications for it are not fully studied.

In our study, preliminary laser-assisted hatching increased implantation in cryopreservation protocols and favorably influenced the course of pregnancy and birth among patients with tuboperitoneal infertility. However, the correlation between hatching and long-term outcomes such as pregnancy course and birth requires further study.

Conflict of interest

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

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BIOCHEMISTRY

THE ROLE OF FATTY ACIDS AND LIPID INFLAMMATORY MEDIATORS IN THE DEVELOPMENT OF SMALL AIRWAY DYSFUNCTION IN ASTHMA COMPLICATED WITH OBESITY

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ABSTRACT

Background. *Small airway involvement is important in determining the phenotypes of bronchial asthma. Establishing the mechanisms of dysfunction of small airways will make it possible to predict the course and control bronchial asthma.*

The aim. *To study the association between the modification of the composition of fatty acids, lipid inflammatory mediators (eicosanoids, plasmalogens) and the functional state of small airways and to identify lipid biomarkers for the development of small airway dysfunction in bronchial asthma associated with obesity.*

Materials and methods. *The study included 85 patients with mild, partially controlled asthma. Of these, 39 patients with normal body weight (Group 1) and 46 patients with grade I–II obesity (Group 2). The control group consisted of 30 healthy volunteers. The function of the small airways was assessed according to spirometry and body plethysmography. The composition of fatty acids and plasmalogens in blood plasma was assessed by gas chromatography-mass spectrometry. In the blood serum, the content of thromboxane B2 and leukotriene B4was determined. Statistical processing was performed using the Statistica 6.1 program (StatSoft Inc., USA). Interrelations between pairs of traits were examined using the Spearman correlation test (r). Differences were considered statistically significant at $p < 0.05$.*

Results. *In the combined course of asthma and obesity, dysfunction of the small airways develops against the background of generalized bronchial obstruction. A modification of lipid metabolism was revealed, manifested by an increase in the levels of saturated, monoenoic, n-6 polyunsaturated fatty acids against the background of a deficiency of n-3 polyunsaturated fatty acids and phospholipids with an alkenyl bond – plasmalogens. It has been shown that bronchial asthma, aggravated by obesity, occurs against the background of increased synthesis of inflammatory lipid mediators – eicosanoids (thromboxane B_2 and leukotriene B_4). Evaluation of the correlations between the studied lipids and the function of small airways revealed a high degree of relationship between their elements.*

Conclusion. *An important pathogenetic link in the formation of small airway dysfunction in bronchial asthma aggravated by obesity is a violation of fatty acid metabolism and plasmalogen synthesis, an increase in the formation of inflammatory lipid mediators.*

Key words: *bronchial asthma, small airways, fatty acids, plasmalogens*

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РОЛЬ ЖИРНЫХ КИСЛОТ И ЛИПИДНЫХ ВОСПАЛИТЕЛЬНЫХ МЕДИАТОРОВ В РАЗВИТИИ ДИСФУНКЦИИ МАЛЫХ ДЫХАТЕЛЬНЫХ ПУТЕЙ ПРИ БРОНХИАЛЬНОЙ АСТМЕ, АССОЦИИРОВАННОЙ С ОЖИРЕНИЕМ

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РЕЗЮМЕ

Обоснование. *Поражение малых дыхательных путей (МДП) имеет большое значение в определении фенотипов бронхиальной астмы (БА). Установление механизмов дисфункции МДП позволит прогнозировать течение и контролировать БА.*

Цель исследования. *Изучить взаимосвязь модификации состава жирных кислот, липидных воспалительных медиаторов (эйкозаноиды, плазмалогены) с функциональным состоянием малых дыхательных путей и выделить липидные биомаркеры развития дисфункции МДП при БА, ассоциированной с ожирением.*

Материалы и методы. *В исследование включено 85 пациентов с лёгкой частично контролируемой БА. Из них 39 пациентов с нормальной массой тела (1-я группа) и 46 пациентов с ожирением 1–2-й степени (2-я группа). Группу контроля составили 30 здоровых добровольцев. Функцию МДП оценивали по данным спирометрии, бодиплетизмографии. Состав жирных кислот (ЖК) и плазмалогенов в плазме крови оценивали методами газовой хромато-масс-спектрометрии. В сыворотке крови определяли содержание тромбоксана B_2 ($ТХВ_2$) и лейкотриена B_4 ($ЛТВ_4$). Статистическую обработку осуществляли с использованием программы Statistica 6.1 (StatSoft Inc., США). Взаимосвязи между парами признаков исследовали с использованием критерия корреляции Спирмена (r). Различия считали статистически значимыми при $p < 0,05$.*

Результаты. *При сочетанном течении БА и ожирения развивается дисфункция МДП на фоне генерализованной бронхиальной обструкции. Выявлено нарушение метаболизма липидов, проявляющееся повышением уровней насыщенных, моноеновых, n-6 полиненасыщенных ЖК на фоне дефицита n-3 полиненасыщенных ЖК и фосфолипидов с алкенильной связью – плазмалогенов. Показано, что БА, отягощённая ожирением, протекает на фоне повышенного синтеза воспалительных липидных медиаторов – эйкозаноидов ($ТХВ_2$, $ЛТВ_4$). Оценка корреляционных взаимосвязей изучаемых липидов и функции МДП выявила высокую степень взаимоотношений между их участниками.*

Заключение*. Важным патогенетическим звеном формирования дисфункции МДП при БА, отягощённой ожирением, является нарушение метаболизма жирных кислот и синтеза плазмалогенов, увеличение образования воспалительных липидных медиаторов.*

Ключевые слова: *бронхиальная астма, малые дыхательные пути, жирные кислоты, плазмалогены*

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OBJECTIVES

Bronchial asthma (BA) is still one of the topical problems in medicine. Chronic inflammation of the airways leads to the development of reversible bronchial obstruction. Mechanisms study leading to functional disorders of the respiratory system and the development of various phenotypes of bronchial asthma, including BA complicated with obesity abdominal obesity (AO), remains relevant. A significant role in the development of this phenotype of bronchial asthma is attributed not only to immune, but also to hormonal disorders specific to obesity [1, 2]. In recent years, the role of small airways in the pathogenesis of BA has paid much attention [3, 4]. Small airway dysfunction has an unfavorable effect on the clinical manifestations of the disease, predetermining uncontrolled course, and statistically significantly increases the risks of BA acute conditions [5]. In the vast majority of cases, small airway dysfunction is present in any degree of asthma severity; at the same time, the changes are diagnosed not only against the background of mild generalized bronchial obstruction, but also with normal pulmonary function [6]. The pathophysiologic mechanisms of small airway dysfunction development in BA are under active research, the results of which are contradictory due to the heterogeneity of pathogenesis and clinical manifestations of asthma [7].

Fatty acids (FAs) with their important structural, energetic and signaling functions play a key role in the pathogenesis of bronchopulmonary diseases, as well as in the development of abdominal obesity. Lipids are extremely diverse in terms of their chemical structure and the functions they perform. For example, phospholipids (PLs) and their constituent polyunsaturated fatty acids (PUFAs) are the main components of pulmonary surfactant. Modification of PUFA composition and alteration of surfactant PL molecular species may influence the development of bronchial asthma [8]. Lipid metabolism disorder in AO plays no less significant role [9].

PLs are subdivided into several subclasses of ether lipids – diacyl, alkyl (alkenyl)-acyl, or plasmalogens. The link of disorders in plasmalogens biosynthesis with the development of respiratory diseases and abdominal obesity has been established. This has prompted interest in them as promising therapeutic targets. The presence of plasmalogens in cell membranes affects the properties and functions of membranes and their receptors. Plasmalogens of surfactant play a protective role due to antioxidant activity [10]. The peculiarity of phospholipids and, above all, plasmalogens of cell membranes is that they are carriers of precursors of the most important secondary messengers, such as leukotrienes, prostaglandins, platelet-activating factor (PAF) and some others [11]. Since plasmalogens constitute a large fraction of total lipids in humans, changes in their levels have been shown to affect membrane properties and therefore signaling pathways involved in the inflammatory cascade [12]. The available sporadic data on impaired synthesis of plasmalogens in obstructive lung diseases give grounds to consider these compounds as essential participants in the pathogenesis of bronchopulmonary diseases [13].

Lipid mediators, including derivatives of ω-3 and ω-6 PUFAs – eicosanoids (leukotrienes, thromboxanes), docosanoids, and pro-resolving lipid mediators – play an active role in the development of chronic airway inflammation [14]. Initiation of inflammation in BA is mainly due to the participation of oxidized derivatives of arachidonic acid (ARA); at the same time, oxidized derivatives of docosahexaenoic acid (DHA), dominant in the 2nd position of plasmalogous forms of phospholipids, are assigned the role of a proinflammatory mediator [15]. Fatty acid metabolism and plasmalogens synthesis disorder, increased formation of proinflammatory derivatives is one of the reasons for the aggravation of the course of BA, an important factor in the formation of chronic inflammation in BA and AO [16].

Thus, polyunsaturated fatty acid, plasmalogens are important structural and signaling molecules involved in both the regulation of chronic inflammation and bronchoconstriction. Lipids polyfunctionality, the presence of common etiologic and pathogenetic mechanisms of bronchial asthma and obesity formation determine the special significance of studying the participation of individual lipid classes in the development of systemic inflammation and small airway dysfunction in BA associated with obesity. Determining the role of lipid mediators of inflammation in the formation of disorders of external respiratory function in patients with bronchial asthma will allow to identify therapeutic targets for improving disease control.

THE AIM OF THE STUDY

To study the interrelation of modification of fatty acid composition, lipid inflammatory mediators (eicosanoids, plasmalogens) with the functional state of small airways and to identify lipid biomarkers of the development of small airway dysfunction in bronchial asthma associated with obesity.

MATERIALS AND METHODS

The study was conducted in accordance with the requirements of the WMA Declaration of Helsinki (revision 2013), with the approval of the local ethical committee of the Vladivostok branch of the Far Eastern Scientific Center of Physiology and Pathology of Respiration – Research Institute of Medical Climatology and Rehabilitation Treatment (minutes No. 8 of 28.06.2022) and under the conditions of voluntary informed consent of all included patients and volunteers.

Study design. The study was performed as a prospective single-center randomized study.

It included patients who were on examination and treatment in the clinical department of the Vladivostok

branch of the Far Eastern Scientific Center of Physiology and Pathology of Respiration – Research Institute of Medical Climatology and Rehabilitation Treatment from 2019 to 2021.

Inclusion criteria: patients with mild, partially controlled bronchial asthma, with normal body weight (NBW) and abdominal obesity of grade I–II, aged from 20 to 65 years.

Exclusion criteria: patients with moderate and severe bronchial asthma of uncontrolled course, with chronic obstructive pulmonary disease, occupational diseases of bronchopulmonary system, abdominal obesity of grade III and IV, endocrine diseases and other diseases of internal organs in decompensation stage.

Eighty-five patients with mild, partially controlled bronchial asthma, including 31 men and 64 women, aged 20–65 years (mean age 50.72 ± 15.24 years) were investigated. Patients were divided into two groups based on the Body Mass Index (BMI, Quetelet's index). Group 1 included 39 patients with BA and normal body weight (BMI = 23.32 ± 2.47 kg/m^2), and Group 2 included 46 patients with BA and AO of grade I and II (BMI = 34.06 ± 3.48 kg/m^2). The control group consisted of 30 conditionally healthy volunteers. The groups were comparable in terms of sex and age.

Clinical, laboratory and functional examination of patients was carried out in accordance with the standards of examination of patients with BA and obesity. BA diagnosis was made according to the international consensus criteria for the diagnosis and treatment of bronchial asthma (GINA, 2021). BA history duration was up to 5 years in 49 % of cases and more than 5 years in 51 % of cases. All patients with bronchial asthma received baseline therapy with a fixed combination of a low-dose inhaled glucocorticosteroid (budesonide – 200–400 μg/day) and a long-acting β2-agonist (formoterol) (GINA, 2021; national asthma guidelines). The ACQ-5 (Asthma Control Questionnaire) test was used to assess the level of disease control. ACQ-5 test scores between 0.75 and 1.5 indicated partial control of the disease [17]. To diagnose alimentary-constitutional obesity, the World Health Organization recommendations were followed [18].

Pulmonary function tests (PFTs) were performed using Master Screen Body apparatus (Germany). According to spirometry data, the vital capacity (VC%), expiratory reserve volume (ERV), forced vital capacity (FVC%), forced expiratory volume in 1 second (FEV_1%), the ratio of FEV_1 to VC (FEV_1/VC%), the ratio of FEV_1 to FVC (FEV_1/FVC%), peak expiratory flow rate after exhalation of 25 % FVC ($PEFR_{75}$ %), maximal expiratory flow at 25 % of FVC (MEF_{75}), maximal expiratory flow at 50 % of FVC (MEF_{50}), maximal expiratory flow at 75 % of FVC (MEF_{25}), and maximal mid-expiratory flow ($MMEF_{25–75}$) were estimated in % of the target parameters. To investigate bronchodilator reversibility, a sample with salbutamol (400 μg) was used [19].

Static lung volumes and capacities were assessed by body plethysmography in % of target values: functional residual capacity (FRCplet); residual volume (RV); total lung capacity (TLC); percentage ratio of RV/TLC; airway resistance on inhalation (inhalation resistance) and exhalation (exhalation resistance); total airway (bronchial) resistance (Rtot).

Blood plasma lipids were extracted according to the method of Bligh and Dyer (Bligh and Dyer, 1959). The levels of fatty acids (FAs) and plasmalogens were estimated by the content of FA methyl esters (FAME) and plasmalogen derivatives, dimethyl acetals (DMA), which were determined by gas chromatography-mass spectrometry. The ratio of the plasmalogen level to the corresponding fatty acid methyl ester in terms of the number of carbon atoms was calculated. Fatty acid methyl esters and dimethylacetals were prepared according to the method of Carreau and Duback (1978). FAME peaks identification was done by retention times of individual fatty acid esters and by equivalent chain length values (Christie, 1988). DMAs were identified by comparing their retention times with those of the 16:0DMA and 18:0DMA standards.

The content of eicosanoids was determined by the amount of their stable metabolites in serum – thromboxane B_2 and leukotriene B_4. Minicolumns (Minicolumns for Sample Preparation, USA) were used for sample preparation. Quantitative level of thromboxane B_2 and leukotriene B_4 was determined by immunoenzyme technique using ELISA kits of Enzo Life Sciences (USA). Measurement was performed in flat-bottom 96-well plates on a spectrophotometer (Biotek Power Wave, USA).

Statistical processing of data was performed using the standard statistical software package Statistica 6.1 for Windows (StatSoft Inc., USA). Hypothesis tests for the normal distribution of quantitative signs in groups were performed using Kolmogorov – Smirnov, Shapiro – Wilk and Pearson's chi-squared tests (χ^2). Descriptive statistics are presented in the text as M ± SD (in case of normal distribution of the trait), where M is the mean, SD is the standard deviation, and Me (L_q; U_q) (in case of non-normal distribution of the trait), where Me is the median, L_q is the lower quartile, and U_q is the upper quartile. Statistically significant difference between alternative quantitative parameters with a distribution corresponding to the normal distribution law was assessed using Student's t-test, otherwise using two-samples Wilcoxon test, Mann – Whitney U test, and Kolmogorov – Smirnov test. The interrelation between pairs of traits was investigated using the Spearman's rank correlation coefficient (r). Differences were considered statistically significant at $p < 0.05$.

STUDY RESULTS

Clinical and functional characteristics of the examined patients are presented in Table 1. Pulmonary function indicators according to spirometry in patients with mild BA and NBW (Group 1) were reduced than in control group: expiratory reserve volume % – by 30 % ($p = 0.035$); FEV_1/VC% – by 7 % ($p = 0.008$); FEV_1/FVC% – by 7% ($p = 0.015$); MEF_{75}% – by 26 % ($p = 0.003$); MEF_{50}% – by 29 % ($p = 0.003$); MEF_{25}% – by 27 % ($p = 0.003$); $MMEF_{25–75}$% – by 28 % ($p = 0.009$).

TABLE 1

CLINICAL AND FUNCTIONAL CHARACTERISTICS OF THE EXAMINED PATIENTS

Indicators	Control group (n = 30)	Group 1: BA + IBW (n = 39)	Group 2: BA + AO (n = 46)	Statistical significance level (p)
Age	35.88 ± 8.23	45.86 ± 16.95	59.83 ± 11.6	–
Body mass index, kg/m^2	23.51 ± 2.91	24.55 ± 3.27	34.06 ± 3.48	$p_{c-2} < 0.001$; $p_{1-2} < 0.001$
ACQ test, scores	–	0.9 (0.6; 1.2)	1 (0.5; 1.6)	–
VC, %	105.65 (98.28; 121.7)	107.15 (103.18; 119.1)	107.4 (96; 126.45)	–
IC, %	108.75 (86.18; 115.48)	112.4 (103.7; 127.2)	127.05 (101.8; 146.98)	–
ERV, %	124.64 (108.38; 139.35)	87.21 (76.91; 101.22)	58.71 (50.23; 93.23)	$p_{c-1} = 0.035$ $p_{c-2} < 0.001$ $p_{1-2} = 0.013$
FVC, %	105.51 (99.73; 123)	107.25 (103.91; 114.38)	107 (85.00; 114.51)	–
FEV_1, % of target	104.42 (95.68; 112.01)	94.85 (90.88; 105.55)	85.11 (76.65; 105.81)	$p_{c-2} = 0.004$;
FEV_1/VC, %	74.82 (71.25; 82.85)	69.78 (64.79; 73.83)	64.91 (58.44; 70.99)	$p_{c-2} < 0.001$ $p_{c-1} = 0.008$
FEV_1/FVC, %	78.72 (73.89; 84.38)	73.45 (70.26; 77.06)	70.27 (62.21; 76.25)	$p_{c-1} = 0.015$ $p_{c-2} = 0.002$;
MEF_{75}, %	100.45 (84.08; 117.71)	74.51 (60.03; 84.89)	63.41 (37.55; 87.91)	$p_{c-1} < 0.001$ $p_{c-2} = 0.003$
MEF_{50}, %	82.85 (64.83; 110.43)	59.20 (3.31; 70.05)	42.6 (8.55; 58.05)	$p_{c-1} = 0.003$ $p_{c-2} < 0.001$ $p_{1-2} = 0.039$
MEF_{25}, %	49.15 (41.18; 90.13)	35.65 (26.28; 48.45)	26.5 (23.9; 36.1)	$p_{c-1} = 0.009$ $p_{c-2} < 0.001$
$MMEFR_{25-75}$, %	73.75 (57.55; 97.05)	53.35 (37.18; 59.45)	33 (21.75; 50.4)	$p_{c-1} = 0.001$ $p_{1-3} < 0.001$ $p_{2-3} = 0.042$
Rin, kPa × s/l	0.17 (0.14; 0.2)	0.26 (0.2; 0.33)	0.32 (0.26; 0.38) 23%?	$p_{c-1} = 0.041$ $p_{c-2} = 0.007$
Rex, kPa × s/l	0.22 (0.15; 0.3)	0.38 (0.28; 0.41)	0.4 (0.34; 0.66)	$p_{1-2} = 0.041$ $p_{c-2} = 0.003$ $p_{c-1} = 0.017$
Rtot, kPa × s/l	0.19 (0.14 0.25)	0.31 (0.2; 0.35)	0.36 (0.28; 0.51)	$p_{c-1} = 0.017$ $p_{c-2} < 0.001$ $p_{1-2} = 0.025$
FRC_{plet}, %	100.4 (92.23; 115.2)	106 (96.15; 122.03)	113.5 (103.03; 125.4)	–
RV, %	107.85 (92.68; 118.53)	109.9 (93.15; 143.25)	114.05 (105.1; 139.3)	$p_{c-2} = 0.037$
TLC, %	96.5 (92.6; 115.73)	106,45 (104,63; 111,65)	107.6 (96.75; 115.55)	–
RV/TLC, %	98.15 (91.13; 100.5)	105.4 (94.75; 122.05)	102.6 (93.42; 127.32)	–

Note. Descriptive statistics are presented as M ± SD, where M – mean, SD – standard deviation (in case of normal distribution of the trait); in case of distribution not corresponding to normal distribution – as Me (L_q; U_q), where Me – median, L_q – lower quartile, U_q – upper quartile; IC – inspiratory capacity; ERV – expiratory reserve volume; p_{c-1} – statistical significance of differences between the control group and group 1; p_{c-2} – statistical significance of differences between the control group and group 2; p_{1-2} – statistical significance of differences between Groups 1 and 2. Values are given for $p < 0.05$ only.

The body plethysmography data revealed an increase in inhalation resistance by 53 % ($p = 0.041$) and exhalation resistance by 71 % ($p = 0.017$), total inhalation and exhalation resistance by 63 % ($p = 0.017$), which is specific for bronchial obstruction.

Patients with bronchial asthma and abdominal obesity (group 2) had more pronounced changes in PFT parameters than in the control group. A 53 % decrease in expiratory reserve volume % ($p < 0.001$), 18 % decrease in FEV_1 % ($p = 0.004$), 13 % decrease in FEV_1/VC% % ($p < 0.001$), 11 % decrease in FEV_1/FVC% ($p = 0.002$), 37 % decrease in MEF_{75}% ($p < 0.001$), 48 % decrease in MEF_{50} % ($p < 0.001$), 46 % decrease in MEF_{25}% ($p < 0.001$), 55 % decrease in $MMEF_{25–75}$% ($p < 0.001$) were noted. Body plethysmography revealed an increase in inhalation resistance by 88 % ($p = 0.007$) and exhalation resistance by 82 % ($p = 0.003$), total inhalation and exhalation resistance by 89 % ($p < 0.001$), and RV by 6 % ($p = 0.037$).

Comparative analysis of PFT parameters in patients of Groups 1 and 2 revealed statistically significant differences. For example, patients with bronchial asthma combined with obesity compared to patients with IBW showed a 32 % decrease in expiratory reserve volume ($p = 0.013$), a 28 % decrease in MEF_{50} ($p = 0.039$), a 38 % decrease in $MMEF_{25–75}$ ($p = 0.041$), and a 16 % increase in total inhalation and exhalation resistance ($p = 0.025$).

The data obtained indicate the progression of generalized bronchial obstruction, including at the level of small bronchi in obesity-associated BA.

The result of composition of individual fatty acids and plasmalogens are summarized in Table 2. Comparative analysis of FA plasma composition between patients with BA and NBW (Group 1) and the control group showed a statistically significant increase in the proportion of myristic acid (14:0) by 33 % ($p = 0.044$) and a decrease in digomo-γ-linolenic acid (20:3ω-6) by 27 % ($p = 0.036$).

A statistically significant increase in the proportion of myristic acid (14:0) more than 2-fold ($p < 0.001$) was observed in patient Group 2 with BA in combination with AO compared to controls. Along with the increase in saturated fatty acids (SFAs), the content of some monoenoic acids (MFAs) increased, in particular palmitoleic acid (16:1ω-7) by 28 % ($p < 0.001$), hexadecenoic acid (16:1ω-9) by 21 % ($p < 0.001$) and heptadecenoic acid (17:1) by 40 % ($p < 0.001$).

Modification of PUFA of blood plasma in patient Group 2 was characterized by statistically significant decrease of: eicosapentaenoic acid (20:5ω-3) – by 38 % ($p = 0.012$), and docosahexaenoic acid (22:6ω-3) – by 34 % ($p = 0.012$) in comparison with the control group. There was a trend of 16 % decrease in docosapentaenoic (22:5ω-3) acid. Against this background, an increase in the proportion of α-linolenic (18:3ω-3) acid by 37 % ($p < 0.001$) versus to the control was observed.

Modification of ω-6 PUFA composition was observed: an increase in the levels of γ-linolenic (18:3ω-6) acid by 33 % ($p = 0.015$) and digomo-γ-linolenic (20:3ω-6) by 7 %, and a decrease in adrenic acid (22:4ω-6) by 29 % ($p < 0.001$).

When comparing the parameters of FA composition between the Groups 1 and 2 in BA patients with obesity, an increase in the proportion of saturated fatty acids was found: myristic acid (14:0) – by 62 % ($p = 0.035$), stearic acid (18:0) – by 10 %. The content of MFAs, palmitoleic acid (16:1ω-7) and hexadecenoic acid (16:1ω-9), also increased by 23 % ($p = 0.004$) and 34 % ($p = 0.005$), respectively.

In the comparative analysis between Groups 1 and 2, statistically significant differences in the composition of PUFAs of the ω-3 family were determined. So, in patients of Group 2 the level of eicosapentaenoic FA (20:5ω-3) was lower by 34 % ($p = 0.017$), docosahexaenoic FA (22:6ω-3) – by 35 % ($p = 0.035$) relative to the indicators in Group 1. In addition, the level of α-linolenic (18:3ω-3) acid in Group 2 patients was 36 % higher ($p = 0.038$) than in Group 1.

A modification in the composition of PUFAs of the ω-6 family was identified, which was manifested by an increase in the proportion of γ-linolenic acid (18:3n6) and digomo-γ-linolenic acid (20:3ω-6) by 43 % ($p = 0.015$) and 46 % ($p = 0.042$), respectively, and a decrease the level of adrenic acid (22:4ω-6) by 33 % ($p < 0.001$). The content of arachidonic acid (20:4ω-6) in patients in both groups has no statistically significantly differences. However, the decrease in the proportion of 20:5ω-3 PUFA in Group 2 resulted in an increase in the 20:4ω-6/20:5ω-3 ratio, the value of which was 30 % higher compared to Group 1 ($p = 0.017$) and 15 % higher versus to controls ($p = 0.042$). A disbalance of ω-3 and ω-6 PUFAs with accumulation of ω-6 PUFAs leads to preferential synthesis of their pro-inflammatory metabolites.

In Group 1 (BA and NBW) no statistically significant changes in plasma plasmalogens content were found (Table 2). Group 2 (BA with obesity) showed a decrease in plasmalogens DMA16:0 and DMA18:0. Indeed, DMA16:0 and DMA18:0 levels decreased by 31 % ($p = 0.002$) and 32 % ($p = 0.028$) than in controls and by 41 % ($p < 0.001$) and 34 % ($p = 0.036$) than in Group 1, respectively. The ratio of all DMA16:0 to total palmitic acid in blood (DMA16:0/FAME16:0) decreased by 30.9 % ($p = 0.002$) versus to control and by 85 % ($p < 0.001$) compared to Group 1. The index level of the relative content of all DMA18:0 to total blood stearic acid (DMA18:0/FAME18:0) decreased by 31.8 % ($p = 0.002$) and 25 % ($p < 0.001$) versus to the control group and Group 1 respectively. There were no statistically significant differences in DMA18:1 and DMA20:0 levels between the healthy and BA patient groups.

Thus, the results of this study showed decreased levels of alkenyl-linked phospholipids during the development of AO-associated BA.

The content of pro-inflammatory eicosanoids – thromboxane B2 and leukotriene B4 – in BA patients was investigated (Table 3). Regardless of body weight, a statistically significant increase in blood levels of leukotriene B4 and thromboxane B2 was observed in BA patients compared to the control group. In addition, comparison of indicators between groups of BA patients showed a 43 % ($p = 0.001$) increase in leukotriene B4 and a 15 % ($p = 0.001$) increase

TABLE 2

THE COMPOSITION OF FATTY ACIDS AND PLASMALOGENS IN BLOOD PLASMA OF THE EXAMINED PATIENTS

Indicators	Control group ($n = 30$)	Group 1: BA + IBW ($n = 39$)	Group 2: BA + AO ($n = 46$)	Statistical significance level (p)
14:0	0.48 (0.45; 0.57)	0.64 (0.5; 0.93)	1.04 (0.97; 1.23)	$p_{c-2} < 0.001$ $p_{1-2} = 0.035$ $p_{c-1} = 0.044$
15:0	0.17 (0.15; 0.2)	0.18 (0.16; 0.2)	0.18 (0.17; 0.22)	–
16:0	20.3 (19.38; 21.52)	20.16 (19.41; 21.84)	21.21 (19.77; 22.49)	–
17:0	0.23 (0.2; 0.24)	0.25 (0.22 ; 0.26)	0.22 (0.21; 0.24)	–
16:1ω-9	0.39 (0.37; 0.42)	0.35 (0.34; 0.35)	0.47 (0.44; 0.51)	$p_{c-2} < 0.001$ $p_{1-2} = 0.005$
16:1ω-7	1.52 (1.21; 1.75)	1.58 (1.49; 2.36)	1.95 (1.7; 2.43)	$p_{c-2} < 0.001$ $p_{1-2} = 0.004$
17:1	0.15 (0.12; 0.23)	0.17 (0.15; 0.21)	0.21 (0.16; 0.27)	$p_{1-2}<0.001$ $p_{c-2}<0.001$
18:0	6.39 (5.93; 6.8)	6.59 (6.38; 7.13)	7.03 (6.63; 7.45)	–
18:1ω-9	16.89 (15.02; 17.79)	15.76 (15.01; 16.42)	16.57 (15.79; 17.1)	–
18:1ω-7	1.53 (1.36; 1.61)	1.56 (1.39; 1.67)	1.58 (1.48; 1.62)	–
18:2ω-6	36.44 (35.23; 37.64)	38.09 (37.5; 38.95)	35.58 (34.57; 38.21)	–
18:3ω-6	0.3 (0.23; 0.38)	0.28 (0.17; 0.31)	0.4 (0.32; 0.43)	$p_{c-2} = 0.015$ $p_{1-2} = 0.015$
18:3ω-3	0.37 (0.32; 0.41)	0.36 (0.32; 0.47)	0.49 (0.4; 0.54)	$p_{c-2} < 0.001$ $p_{1-2} = 0.038$
20:1ω-7	0.13 (0.11 ; 0.14)	0.22 (0.19; 0.24)	0.21 (0.15; 0.21)	–
20:1ω-9	0.18 (0.15; 0.21)	0.14 (0.12; 0.18)	0.19 (0.15; 0.21)	–
20:2ω-6	0.23 (0.2; 0.29)	0.22 (0.1; 0.2)	0.22 (0.19; 0.25)	–
20:3ω-6	1.12 (0.98; 1.37)	0.81 (0.74; 1.12)	1.19 (1.12; 1.29)	$p_{c-1} = 0.036$ $p_{1-2} = 0.015$
20:4ω-6	5.93 (5.08; 6.54)	5.44 (4.84; 6.81)	5.35 (4.94; 5.96)	–
20:5ω-3	0.88 (0.63; 1.07)	0.83 (0.36; 2.65)	0.54 (0.4; 0.71)	$p_{1-2} = 0.017$ $p_{c-2} = 0.012$
20:4ω-6/20:5ω-3	6.88 (5.35; 9.2)	6.05 (2.57; 9.42)	7.88 (3.91; 13.36)	$p_{1-2} = 0.037$ $p_{c-2} = 0.042$
22:4ω-6	0.17 (0.15; 0.2)	0.18 (0.15; 0.22)	0.12 (0.1; 0.14)	$p_{c-2} < 0.001$ $p_{1-2} < 0.001$

TABLE 2 (*continued*)

Indicators	Control group (*n* = 30)	Group 1: BA + IBW (*n* = 39)	Group 2: BA + AO (*n* = 46)	Statistical significance level (*p*)
22:5ω-3	0.49 (0.4; 0.54)	0.48 (0.3; 0.6)	0.41 (0.29; 0.51)	–
22:6ω-3	2.3 (1.96; 2.54)	2.35 (1.77; 3.06)	1.52 (1.32; 1.65)	$p_{c-2} = 0.012$ $p_{1-2} = 0.035$
16:0DMA	0.39 (0.36; 0.5)	0.46 (0.41; 0.49)	0.27 (0.24; 0.32)	$p_{c-2} = 0.002$ $p_{1-2} < 0.001$
18:0DMA	0.28 (0.23; 0.32)	0.29 (0.22; 0.3)	0.19 (0.17; 0.23)	$p_{1-2} = 0.036$ $p_{c-2} = 0.028$
18:1DMA	0.14 (0.13; 0.15)	0.12 (0.1; 0.16)	0.11 (0.1; 0.12)	–
20:0DMA	0.18 (0.15; 0.18)	0.13 (0.08; 0.19)	0.18 (0.17; 0.2)	–
DMA16:0/FAME16:0	1.91 (1.64; 2.37)	2.21 (1.93; 2.4)	1.32 (1.09; 1.54)	$p_{c-2} = 0.002$ $p_{1-2} < 0.001$
DMA18:0/FAME18:0	4.03 (3.44; 4.82)	3.68 (3.15; 5.06)	2.76 (2.45; 3.38)	$p_{c-2} < 0.001$ $p_{1-2} = 0.013$

Note. Descriptive statistics are presented as Me (L_q; U_q), where Me is median, L_q is lower quartile, U_q is upper quartile; p_{c-1} is statistical significance of differences between control group and Group 1; p_{c-2} is statistical significance of differences between control group and group 2; p_{1-2} is statistical significance of differences between Groups 1 and 2. Values are given for $p < 0.05$ only.

TABLE 3
THE LEVEL OF EICOSANOIDS IN BLOOD PLASMA OF THE EXAMINED PATIENTS

Indicators	Control group (*n* = 30)	Group 1: BA + IBW (*n* = 39)	Group 2: BA + AO (*n* = 46)	Statistical significance level (*p*)
Lukotriene B4, pg/ml	11.28 (10.43; 12.43)	17.87 (16.4; 18.65)	25.61 (23.03; 29.9)	$p_{c-1} < 0.001$ $p_{c-2} < 0.001$ $p_{1-2} = 0.001$;
Thromboxane B_2, pg/ml	62.15 (56.3; 70.85)	79.9 (78.12; 90.32)	90.24 (87.47; 98.54)	$p_{c-1} = 0.003$ $p_{c-2} < 0.001$ $p_{1-2} = 0.001$

Note. Descriptive statistics are presented as Me (L_q; U_q), where Me is median, L_q is lower quartile, U_q is upper quartile; p_{c-1} is statistical significance of differences between control group and Group 1; p_{c-2} is statistical significance of differences between control group and Group 2; p_{1-2} is statistical significance of differences between Groups 1 and 2. Values are given for $p < 0.05$ only.

in thromboxane B2 if obesity was present. The findings suggest a pronounced inflammatory response in BA associated with obesity.

The presence of the disbalance in the FA composition, formation disorder of their oxidized metabolites and plasmalogen synthesis disorder can provoke the development of chronic inflammation, oxidative stress, which can lead to increased respiratory dysfunction.

In order to establish the role of individual lipids in the development of small airway dysfunction in mild BA with NBW and associated with alimentary-constitutional obesity, a correlation analysis was performed (Tables 4, 5). Correlations were evaluated with regard to PFT parameters reflecting the functional state of small airways.

According to body plethysmography, the criteria for small airway dysfunction are considered to be an increase in RV more than 140 % and RV/TLC more than 125 % of the target values as signs of "air traps", and an increase in FRC more than 130 % of the target value – as an indicator of hyperinflation [19]. Indirect signs of small airway dysfunction in BA also include changes in spirometry. Decreased FVC level is suggested by some authors to be considered as an indicator of «air traps» presence, and $MMEF_{25-75}$ is suggested to be used as a marker of early small airway dysfunction [20].

In the 1st group of patients with BA and NBW, associations were established between the levels of SFA and FWD indicators reflecting the state of small airways (Table 4).

TABLE 4

CORRELATIONS BETWEEN INDICATORS OF DYSFUNCTION OF SMALL AIRWAYS AND FATTY ACIDS, PLASMALOGENS IN PATIENTS OF GROUP 1(SPEARMAN CORRELATION, *r*)

Indicators	FVC%	FEV_1%	$MMEF_{25-75}$%	FRC_{plet}%	RV%	TLC%	RV/TLC%
14:0	–	–	–	0.76 $p = 0.011$	0.59 $p = 0.023$	–	–
15:0	–	–	–	–	0.56 $p = 0.035$	–	0.58 $p = 0.027$
16:0	–	–	–	–	0.57 $p = 0.047$	–	0.6 $p = 0.021$
18:0	–	–	–	0.53 $p = 0.046$	–	–	–
17:1	–	–	–	–	–	0.64 $p = 0.013$	–
16:1ω-9	–	–	–	–	0.57 $p = 0.019$	0.53 $p = 0.046$	–
18:1ω-9	–	–	–	–0.55 $p = 0.036$	–	–	–
18:1ω-7	–	–	–	0.83 $p = 0.011$	–	–	–
18:3ω-3	0.73 $p = 0.003$	0.81 $p < 0.001$	–	–	–	–	–
18:3ω-6	0.57 $p = 0.031$	–	–	–	–	–	–
20:1ω-9	–	–0.69 $p = 0.006$	–0.71 $p = 0.011$	–	–	–	–
20:2ω-6	–	–	–	–0.65 $p = 0.011$	–	–	–
20:3ω-6	0.59 $p = 0.025$	–	–		–	–	–
20:4ω-6		–	–	–0.6 $p = 0.013$	–	–	–
22:4ω-6	–0.81 $p = 0.042$	–	–	–0.59 $p = 0.025$	–	–	–
22:5ω-3	–	–	–	–	–0.62 $p = 0.022$	–	–0.58 $p = 0.028$
22:6ω-3	–	–	–	–	–0.65 $p = 0.011$	–	–0.66 $p = 0.009$
16:0 DMA	–0.55 $p = 0.038$	–0.79 $p = 0.001$	–	–	0.57 $p = 0.029$	–	–
DMA16:0/FAME16:0		–0.88 $p < 0.001$	–	–	–	–	–
18:0 DMA	–0.66 $p = 0.009$	–0.61 $p = 0.019$	–	–	–	–	–

TABLE 4 (*continued*)

Indicators	FVC%	FEV_1%	$MMEF_{25-75}$%	FRC_{plet}%	RV%	TLC%	RV/TLC%
18:1 DMA	–0.53 $p = 0.038$	–	–	–	–	–	–
DMA18:0/FAME18:0	–0.59 $p = 0.023$	–0.59 $p = 0.023$	–	–	–	–	–
20:0 DMA	0.57 $p = 0.029$	–	–	–	–	–	–

Note. Only statistically significant correlations between indicators at $p < 0.05$ are shown.

TABLE 5

CORRELATIONS BETWEEN INDICATORS OF DYSFUNCTION OF SMALL AIRWAYS AND FATTY ACIDS, PLASMALOGENS IN PATIENTS OF GROUP 2 (SPEARMAN CORRELATION, *r*)

Indicators	FVC%	FEV_1%	$MMEF_{25-75}$%	FRC_{plet}%	RV%	TLC%	RV/TLC%
14:0	–	–	–	–	–	–	–
15:0	0.49 $p = 0.019$	0.48 $p = 0.022$	–	–	–	–	–
16:0	–0.66 $p < 0.001$	–	–	–	–	–	–
17:1	–	–	–	–0.59 $p = 0.019$	–	–0.54 $p = 0.036$	–
18:0	–0.47 $p = 0.024$	–	–	0.32 $p = 0.041$	–	–	–
20:1ω-7	–0.51 $p = 0.005$	–0.43 $p = 0.022$	–0.45 $p = 0.005$	–	–	–0.70 $p < 0.001$	0.52 $p = 0.009$
20:4ω-6	–	–	–	–	–	–	0.73 $p = 0.017$
20:5ω-3	–	–	–	–	0,39 $p = 0.034$	0.47 $p = 0.009$	–
20:4ω-6/20:5ω-3	–	–	–	–	–	–	0.78 $p = 0.016$
22:4ω-6	–	–	0.29 $p = 0.044$	–	0.29 $p = 0.046$	–	–
22:5ω-3	–	–	–	–	–	–	–0.58 $p = 0.028$
22:6ω-3 a	–	–	–	–	–	–	–0.66 $p = 0.009$
DMA18:0/FAME18:0	–	–	–	–	–	–	–0.41 $p = 0.029$
20:0 DMA	–	–	–	–0.47 $p = 0.024$	–0.42 $p = 0.048$	–	–
Thromboxane B_2, pg/ml	–0.89 $p < 0.001$	–	–	–0.65 $p = 0.011$	–	–0.86 $p < 0.001$	–

Note. Only statistically significant correlations between indicators at $p < 0.05$ are shown.

Positive correlations of myristic (14:0) and stearic (18:0) acids with FRC_{plet}; myristic, pentadecylic (15:0), and palmitic (16:0) acids with RV; and pentadecylic and palmitic acids with RV/TLC were found.

Positive correlations of MFAs were determined, specifically heptadecenoic (17:1) with TLC; hexadecenoic (16:1ω-9) with RV and TLC; and octadecaenoic (18:1ω-7) with FRC_{plet}. Negative correlations were determined between oleic acid (18:1ω-9) and FRC, eicosenoic acid (20:1ω-9) and FEV_1, $MMEF_{25-75}$.

Assotiations with PFT indicators were also revealed for PUFAs. For ω-3 PUFAs, positive correlations were observed between α-linolenic acid (18:3ω-3), FVC and FEV_1 and negative correlations between docosapentaenoic (22:5ω-3) and docosahexaenoic (22:6ω-3) acids and RV, RV/TLC. Among ω-6 PUFAs, positive correlations of γ-linolenic (18:3ω-6) and digomo-γ-linolenic (20:3ω-6) PUFAs with FVC were observed; negative correlations of eicosadiene (20:2ω-6) and arachidonic (20:4ω-6) PUFAs with FRC_{plet}, and adrenoic (22:4ω-6) PUFA with FVC and FRC_{plet}.

Plasmalogens levels were also correlated with the examined PFT indicators. So, DMA16:0, DMA18:0, and DMA18:0/FAME18:0 had negative correlations with FVC and FEV_1; DMA18:1 with FVC; and DMA16:0/FAME16:0 with FEV_1. Direct correlations were established between DMA16:0 and RV, DMA20:0 and FVC.

In Group 2 (Table 5), assotiation between SFA and external respiratory function were determined. In particular, pentadecylic acid (15:0) had a positive correlation with FVC and FEV_1, while palmitic (16:0) and stearic (18:0) acids had a negative correlation with FVC and a positive correlation with FRC_{plet}. Of the MFAs, heptadecenoic acid (17:1) had negative correlations with FRC_{plet} and TLC, and eicosenoic acid (20:1ω-7) had negative correlations with FVC, FEV1, $MMEF_{25–75}$, and TLC. A positive correlation was observed between 20:1ω-7 and RV/TLC. Of the ω-3 family PUFAs, docosapentaenoic (22:5ω-3) and docosahexaenoic (22:6ω-3) acids had negative correlations with RV/TLC.

Positive correlations were established between arachidonic acid (20:4ω-6) and RV/TLC, adrenoic acid (22:4ω-6) and $MMEF_{25–75}$, and RV. Negative correlations were found between DMA20:0 and FRC_{plet}, RV levels; DMA18:0/FAME18:0 and RV/TLC. We should note the negative correlations of thromboxane B_2 level with such an indicator of PFT as FVC, and indicators reflecting the state of small airways, – FRC_{plet} and TLC.

The established features of the composition of fatty acids, eicosanoids, plasmalogens, their link with PFT indicators demonstrate their participation in the progression of bronchobstruction, formation of air traps and hyperinflation in BA.

RESULTS DISCUSSION

The changes in PFT indicators according to spirometry and body plethysmography revealed in the study show the presence of generalized bronchial obstruction in patients with different body weight and with mild partially controlled asthma. Furthermore, in patients with obesity-associated bronchial asthma, a decrease in the levels of FEV_1, $MMEF_{25–75}$ and an increase in RV were observed, which indicate the development of small airway dysfunction in patients of this group.

When studying the lipid composition of blood plasma by the level of fatty acids, eicosanoids and plasmalogens, the peculiarities of the lipidome in groups of patients with BA were identified. Moreover, the dynamics of blood plasma lipid changes indicates the presence of a systemic chronic inflammatory process in patients with asthma, aggravated by obesity. It has been previously shown that inflammation in BA associated with AO is mediated by hyperproduction of leptin, pro-inflammatory cytokines that lead to systemic chronic low intensity inflammation [21].

In patients with bronchial asthma, only an increase in the proportion of saturated myristic acid was noted. At the same time, correlations with PFT indicators have been established for the majority of SFAs, indicating their involvement in the development of bronchial obstruction and small airway dysfunction. Saturated fatty acids (myristic, stearic, palmitic), on the one hand, play a structural role affecting the packing density of the cell membrane; on the other hand, an increase in their level in biological substrates is always an unfavorable sign from the position of cell signaling functions, since in this case the membrane becomes less susceptible to receptor expression and synthesis of immune mediators. In lung surfactant, saturated fatty acids esterified into complex lipids form a more structured packing of phospholipids, which increases the density of surfactant [22]. Therefore, the chemical composition of surfactant, its enrichment of SFAs can influence the properties and structure of small airways.

Increased levels of monoenoic palmitoleic acid and hexadecenoic acid were observed in patients with bronchial asthma and obesity. MFAs are part of the structure of every cell and play an essential role in the regulation of lipid metabolism [23]. Palmitoleic acid is known to function as a lipokine lipid with hormone-like biological activity. Palmitoleic acid can directly participate in the regulation of insulin resistance and metabolic disorders [24]. In a study by D. Mozaffarian et al. it was shown that increased levels of palmitoleic acid are associated with a better metabolic profile and low development of diabetes [24]. The increased level of palmitoleic acid in patients with bronchial asthma and obesity detected in our study probably indicates a compensatory response of the organism aimed at maintaining lipid homeostasis in order to minimize metabolic disorders.

In the 1st group of patients against the background of normal level of MFA, as well as in the 2nd group MFA associative relations of ω-9 and ω-7 families with PFT indicators characterizing the state of small airways were established, which allows to conclude about the MFA influence of ω-9 and ω-7 families on the formation of small airway dysfunction.

Differences in PUFA composition in groups of BA patients with NBW (Group 1) and BA associated with obesity (Group 2) were identified. In group 2, a change in the PUFA levels of the ω-3 and ω-6 families was observed. Against

the background of decreased levels of eicosapentaenoic (20:5ω-3), docosahexaenoic (22:6ω-3) acids, there was an increase in γ-linolenic (18:3ω-6) acid, digomo-γ-linolenic (20:3ω-6) LC compared to control and Group 1.

Polyunsaturated fatty acids are responsible for increasing the fluidity and viscosity of the cell membrane. PUFAs of the ω-6 family are the main substrates for the synthesis of pro-inflammatory, bronchoconstrictor, vasoconstrictor mediators and precursors of endocannabinoids, lipoxins, while PUFAs of the ω-3 family are considered as precursors of anti-inflammatory, pro-resolving, vasodilatory mediators. ω-3 PUFAs are known to influence inflammatory markers, reducing their levels (C-reactive protein, IL-6, tumor necrosis factor α). Eicosapentaenoic acid (20:5ω-3) is a substrate for oxylipins synthesis (maresins, resolvins, protectins), which are anti-inflammatory, pro-resolving and anti-proliferative mediators providing the most important competing cascade in inflammation towards its resolution [25]. Balance shift between arachidonic (20:4ω-6) and eicosopentaenoic (20:5ω-3) FAs shows disorders in the eicosanoid cycle and, consequently, a high risk of inflammation, which is a prognostically unfavorable sign of increased pro-inflammatory reactions that may lead to the development of small airway dysfunction in the combined course of BA [26].

Interesting correlations were established between the PFT indicators reflecting the state of airways and the composition of PUFAs of the ω-3 and ω-6 families in the groups. For example, for patients with BA and NBW, the greatest number of relations with FVC and FEV_1 were noted. These indicators are known to reflect the presence or absence of bronchial obstruction at different levels and can act as indicators of airway remodeling [27].

In Group 2, in patients with BA and obesity, the highest number of direct relations with PUFAs of the ω-6 family and negative relations with PUFAs of the ω-3 family were determined for indicators reflecting the state of small airways (FRC_{plet}, RV, TLC, RV/TLC). In contrast to Group 1, in the combined course of BA, correlations of eicosopentaenoic acid levels, 20:4ω-6/20:5ω-3 ratio with RV, RV/TLC indicators were established.

The results demonstrate the involvement of PUFAs in the development and progression of small airway inflammation and in the formation of air traps and hyperinflation.

Notably, thromboxane B_2 and leukotriene B_4 were increased in the blood of BA patients regardless of body weight compared to the control group. Eicosanoids as derivatives of arachidonic acid contribute to the development of a marked inflammatory response [26]. In patients with BA and AO, the level of leukotriene B_4 was higher relative to the patients of Group 1. The established inverse correlation between thromboxane B_2 levels and FVC, FRC_{plet}, and TLC in obesity-associated BA indicates the involvement of thromboxane in the development of small airway dysfunction in this group of patients.

The level of plasmalogens in Group 1 did not differ from that of controls. Despite this, negative correlations were found between plasmalogens 16:0DMA, 18:0DMA, 18:1DMA, and 20:0DMA with PFT indicators (FVC, FEV_1). Meanwhile, in Group 2 (bronchial asthma with obesity), the decrease in the levels of plasmalogens containing 16:0 and 18:0 aldehydes and 18:0DMA/18:0FAME ratio was combined with the presence of negative correlations between the indicators reflecting the state of small airways (FRC_{plet}, RV, RV/TLC) and the levels of 20:0DMA and 18:0DMA/18:0FAME. The established correlations indicate the involvement of plasmalogens in the development and progression of small airway inflammation and in the formation of air traps and hyperinflation. Plasmalogens are known to increase the viscosity and tension of lung surfactant. Since the lungs are a direct target of reactive oxygen species, plasmalogens, which are part of surfactant, protect the lungs from their aggressive effects and other environmental factors [28–30]. In the studies of J.E. Sordillo et al. plasmalogens are identified as possible mediators of changes in lung function in people with asthma, including age-related, and can also serve as a potential pharmacological target to improve PFT in people with asthma [13].

Thus, the presence of a large number of positive and negative correlations between lipid mediators and PFT parameters in patients with BA and NBW can be regarded as a more active systemic response to the development of the disease, aimed at preserving internal homeostasis. The association of BA with obesity causes a decrease in the body defenses and disease progression, which is reflected in clinical, laboratory and functional parameters. The assotiations between the indicators of small airway dysfunction and blood plasma lipidome indicate the importance of fatty acids, plasmalogens and eicosanoids in the regulation of the functional state of small airway in BA.

CONCLUSION

Small airway dysfunction develops in patients with mild bronchial asthma associated with abdominal obesity against the background of generalized bronchial obstruction. An important pathogenetic link in the formation of small airway dysfunction is impaired fatty acid metabolism and plasmalogens synthesis, increased formation of inflammatory lipid mediators such as thromboxane and leukotriene. The combined course of BA and alimentary-constitutional obesity is characterized by increased levels of SFAs, MFAs, PUFAs of the ω-6 family and decreased levels of PUFAs of the ω-3 family in plasma. Disorder in the synthesis of lipid mediators (plasmalogens and eicosanoids) in obesity-associated BA determines changes in the molecular mechanisms of immune signaling and antioxidant processes, which underlies the development of chronic systemic and local inflammation in obesity-associated BA. The presence of high correlation of blood lipidome components with functional indices of respiratory organs indicates the pathogenetic role of fatty acids and inflammatory lipid mediators in the formation and progression of small airway dysfunction in BA in combination with obesity, and, consequently, in the aggravation of BA course. Eicosopentaenoic acid, the ratio of PUFAs of ω-6 and ω-3 families (20:4ω-6/20:5ω-3) and thromboxane B_2, which have correlations with markers of small airway

dysfunction characteristic for BA in combination with obesity, may be biomarkers of the formation and progression of airway dysfunction and, consequently, of the aggravation of the course of bronchial asthma. Further studies of lipid triggers of small airway dysfunction will allow the development of technologies to predict the course and control of bronchial asthma.

Conflict of interest

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

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INTERNAL DISEASES

THE ENHANCED RECOVERY PROGRAM IN UROLOGY. SYSTEMATIC REVIEW AND META-ANALYSIS

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ABSTRACT

A systematic review and meta-analysis of data on the problem of enhanced recovery in urology was performed. Inclusion criteria – clinical trials of the enhanced recovery program in urology.

***The aim of the study** was to assess the significance of the enhanced recovery program (ERP) in the provision of surgical care in the "urology" profile.*

***Materials and methods.** The systematic review was performed according to the guidelines for the presentation of systematic reviews and meta-analyses by PRISMA. The registration number in the international system Prospero was received (CRD42022358982). The review included 364 studies. Studies in urologic oncology were excluded from the meta-analysis. The meta-analysis included 15 studies involving 2293 subjects. A comparison was made between the application of ERP and the standard treatment protocol.*

***Results.** The use of ERP leads to an expected two-fold reduction in the duration of postoperative length of hospitalization (OR = –1.96; 95% CI: –2.56÷–1.36; p < 0.00001). The reduction in the duration of hospitalization with the use of ERP in urology does not lead to the increased risk of readmission or re-operation (p = 0.35). The risks of developing postoperative complications ≥ Class 2 by Clavien – Dindo classification were comparable in both groups (p = 0.13). The use of ERP increases the expected success of the treatment by 1.74 times (OR = 1.74; 95% CI: 1.08–2.79; p = 0.02). With the use of ERP in reconstructive urology, a significantly lower risk of complications was established (p = 0.02).*

***Conclusion.** The ERP program allows you to reduce the time and cost of treatment, reduce the likelihood of re-hospitalization and achieve better treatment results. The use of ERP is not accompanied by increased risk of complications > Class 2.*

***Key words:** enhanced recovery after surgery, rapid recovery after surgery programs, fast track surgery, FTS, ERAS, enhanced recovery, ERP*

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ПРИМЕНЕНИЕ ПРОГРАММЫ УСКОРЕННОГО ВЫЗДОРОВЛЕНИЯ В УРОЛОГИИ. СИСТЕМАТИЧЕСКИЙ ОБЗОР И МЕТААНАЛИЗ

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РЕЗЮМЕ

Выполнен систематический обзор и метаанализ данных по проблеме ускоренного выздоровления в урологии. Критерии включения – клинические исследования применения программы ускоренного выздоровления в урологии.
***Цель исследования** – оценка значимости программы ускоренного выздоровления при оказании хирургической помощи по профилю «урология».*
***Материалы и методы.** Систематический обзор выполнен согласно методическим рекомендациям по представлению систематических обзоров и метаанализов PRISMA. Получен регистрационный номер в международной системе Prospero (CRD42022358982). В систематический обзор включены 364 исследования. Онкоурологические исследования из метаанализа были исключены. Проанализировано 15 исследований с участием 2293 субъектов. Выполнено сравнение применения программы ускоренного выздоровления (ПУВ) и стандартного протокола лечения.*
***Результаты.** Применение ПУВ приводит к ожидаемому двухкратному сокращению срока послеоперационного пребывания (ОШ = –1,96; 95% ДИ: –2,56÷–1,36; p < 0,00001). Сокращение сроков госпитализации при применении ПУВ в урологии не приводит к увеличению риска повторного обращения или реоперации (p = 0,35). Риски развития послеоперационных осложнений ≥ 2-го класса по универсальной классификации Clavien – Dindo оказались сопоставимы в обеих группах (p = 0,13). Применение ПУВ повышает предполагаемую успешность проводимого лечения в 1,74 раза (ОШ = 1,74; 95% ДИ: 1,08–2,79; p = 0,02). При применении ПУВ в реконструктивной урологии установлен достоверно меньший риск развития осложнений (p = 0,02).*
***Заключение.** Программа ускоренного выздоровления позволяет сократить сроки и стоимость лечения, уменьшить вероятность повторной госпитализации и добиться лучших результатов лечения. Применение ПУВ не сопровождается увеличением риска развития осложнений > 2-го класса по Clavien – Dindo.*

***Ключевые слова:** протокол ускоренного выздоровления, программа ускоренного выздоровления, ПУВ, ускоренное выздоровление*

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The Enhanced Recovery Program (ERP) is aimed at reducing the duration of treatment from the moment of diagnosis to recovery of working capacity [1].

The aim of the study was to assess the significance of the enhanced recovery program in the provision of surgical care in the "urology" profile.

A systematic review and meta-analysis of data on the problem of ERP in urology were performed. Search for sources was performed in the following databases: PubMed, Google Scholar, Cochrane Library, RSCI, Scopus, Web of Science. Scientific publications in Russian and English were selected, available for obtaining directly or through third-party services, as well as the library and subscription of the Irkutsk State Medical University.

When performing the study, the following questions were formulated: How does the use of enhanced recovery protocols affect the outcomes of surgical treatment of urological diseases? Is there any convincing evidence of the superiority of enhanced recovery protocols over the standard approach? Is there an increased risk of complications, re-operation, readmission or death when using enhanced recovery protocols?

OBTAINING EVIDENCE

A systematic search was conducted in accordance with Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement [2, 3].

A systematic review was registered in PROSPERO (identification number CRD42022358982).

In the databases PubMed, Google Scholar, Cochrane Library, RSCI, Scopus, Web of Science, a search was made for studies on the use of enhanced recovery protocols in urology and reviews on the problem in the period from January 1, 1995 to April 1, 2023 (Fig. 1).

The following keywords were used in the search (by continuous OR search): "fast track surgery" , "FTS", "ERAS", "enhanced recovery", "enhanced recovery urology", "enhanced recovery" (in Russian), "ERP" (in Russian).

The results of interest were: 1) reviews of the use of enhanced recovery protocols in urology; 2) cases of application and development of protocols in urology; 3) clinical studies of the results of the use of protocols in urology; 4) systematic reviews and meta-analyses of data on the use of ERP in urology.

This systematic review was aimed at evaluating the developed protocols of enhanced recovery used in urology, as well as at performing a meta-analysis of the results of clinical studies on this problem.

Thus, this review included the following:

1. Publications devoted to the development of protocols for enhanced recovery in urology;
2. Literature reviews, systematic reviews and meta-analyses of enhanced recovery in urology;
3. Publications devoted to the results of clinical trials (randomized and non-randomized) of the effectiveness of enhanced recovery protocols in urology;
4. Publications in English and Russian.

The following was excluded from the review:

1. Publications devoted to application of enhanced recovery protocols in oncology, including urologic oncology;
2. Publications devoted to case-control studies;
3. Publication with no access to the full texts of the article.

Observational studies (cross-sectional and case-control) were excluded from the study due to their low significance when performing meta-analysis. The search revealed two available case-control studies. Duplicate studies, reprints, studies in languages other than the declared ones (English and Russian), animal studies, letters and short messages were excluded.

A tool developed by the National Institutes of Health (NIH) was used to assess the risk of systematic error and the methodological quality of research (to assess the feasibility of integrating the results into the study). The results of the review and analysis were independently verified before the work was completed by the Vice-Rector for Science and the Vice-Rector for Medical Work of Irkutsk State Medical University. The revealed discrepancies in the evaluation of the results were eliminated after discussion by the team of authors.

The database on clinical trials was formed according to the developed form: date of publication; number

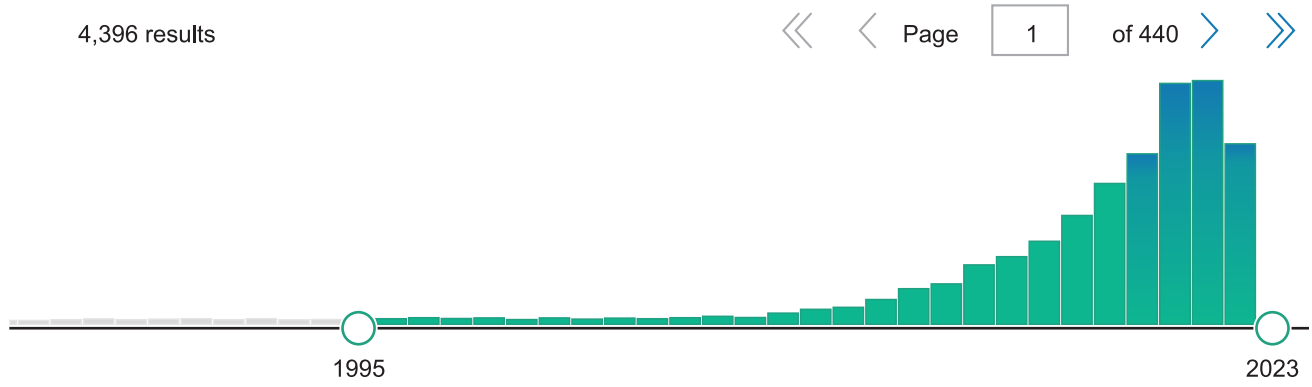

FIG. 1.

Chronometric diagram of search results for "enhanced recovery", "human", "01.01.1995 – 01.04.2023" in the English-language text database of medical and biological publications in PubMed database

of participants; study design; comparison groups; scope of the protocol, the results obtained (nominal, ordinal and predictive).

Meta-analyses that meet the criteria of the study were not revealed during a systematic search. All identified systematic studies of ERP in urology directly relate to the oncourological direction.

The evaluation of the results during the meta-analysis was carried out according to the following parameters: 1) the comparative effectiveness of the treatment; 2) the comparative probability of the occurrence of adverse events (complications or readmissions); 3) comparative duration of hospitalization; 4) comparison of predictors of the success of the treatment; 5) differences in mean values, odds ratio (OR), relative risk (RR), chi-squared (χ^2) occurrence of the event or outcome.

EVIDENCE AND STATISTICAL TECHNIQUES

The data obtained were compared in the Microsoft 365 software package (Microsoft Corporation, USA), and the analysis was performed using Stata v. 16 (College Station, TX, USA). Meta-analyses were performed to analyze the combined data on the impact of the use of enhanced recovery protocols on various treatment outcomes, predicting the occurrence of various events.

The systematic review was carried out according to the methodological recommendations for the submission of systematic reviews and meta-analyses of PRISMA [3]. The survey was registered, and a registration number was obtained in the Prospero international system for registration of systematic reviews (CRD42022358982).

Meta-analysis of proportions was performed using the metaprop command in Stata v. 16 and verified in the RevMan ver. 5.4.1 application. The random effects model was applied using the DerSimonian and Laird method. The proportions were transformed using the double inverse Freeman – Tukey arcsine transform, and confidence intervals (CI) were calculated using the Score method. The use of this method makes it possible to include studies with zero or single parameter values in the meta-analysis [4]. Heterogeneity within and between subgroups was estimated by I^2 or χ^2 statistics [5]. The significance was set at 0.05. If neither the χ^2 analysis nor the I^2 test indicated significant heterogeneity between studies, a fixed-effects model was used. In cases of high statistical heterogeneity, a random effects model was used.

A graphic portrait of the results of the meta-analysis is presented in the form of diagrams consisting of a series of horizontal segments showing the RR and 95% CI of individual studies at the point being compared. I2 levels equal to 25 %, 50 % and 75 % are defined as weak, medium strength and pronounced heterogeneity, respectively. The data pool was analyzed by the method of inverse fixed-effect model in cases of low-moderate heterogeneity ($I^2 < 50$ %), and random-effect model in cases of moderate-high heterogeneity ($I^2 > 50$ %).

The results were measured using a risk ratio (RR) representing a confidence interval (95% CI) and a *p*-value [6]. For studies without a control group, a comparison modeling method was used [7].

The publication bias was assessed using the Begg and Mazumdar test, the Egger regression asymmetry test, as well as funnel graphs with an improved contour. Sensitivity analysis was performed to determine the effect of uncertainty on the effect of exposure; the analysis was repeated when performing direct and indirect comparisons. Sensitivity analysis was carried out by excluding studies on one of the analyses. This allowed us to assess whether one study had a significant impact on the results.

The assessment of the risks of blindness for non-randomized clinical trials (NCI) was carried out according to the RoBANS [8] and MINORS [9] criteria.

The evaluation of the quality of research was determined according to the Oxford Recommendations of 2011 by levels of evidence from 1 to 5. Level 1 – data obtained from systematic reviews and/or meta-analyses; Level 2 – randomized clinical trials; Level 3 – non–randomized controlled cohort studies with sufficient follow-up period; Level 4 – series of clinical observations; Level 5 – expert opinion.

The subgroup analysis was performed by testing interactions between subgroups (presented as an unadapted *p*-level). A subgroup analysis was performed to identify possible causes of heterogeneity when comparing the results of direct and indirect comparisons.

SYSTEMATIC REVIEW

The search algorithm on PubMed. Filters: 01.01.95 to 01.04.23; "Human"; "Russian" or "English". Search: "enhanced recovery" or "ERAS" or "fast track" and "urology". Found: 353 publications. A similar algorithm is used for other scientific databases.

A total of 364 studies are included in this systematic review: 45 are devoted to the review of the problem, 4 – to the development of protocols for enhanced recovery, 21 – to clinical studies, 2 – to observational and case-control studies.

Seventeen studies met the criteria for inclusion in this systematic review. The meta-analysis included 15 studies involving 2,293 subjects (Table 1). The research Design flowchart (PRISMA) is shown in Figure 2.

The methodology was evaluated for each study included in the meta-analysis (Table 1). The bias characteristic of the included studies is shown in Figure 3. Most of the included studies had a good or satisfactory level.

A survey among practicing urologists regarding the introduction of ERP elements into their practical activities, was carried out in 2021 [25]. Of the 714 completed questionnaires, 113 (16 %) were found to be reliable. 58 % of the respondents were employees of the university clinic. 61 % of urologists were unfamiliar with or knew little about the enhanced recovery program. Only 20 % of respondents used ERP systematically, guided by the developed proto-

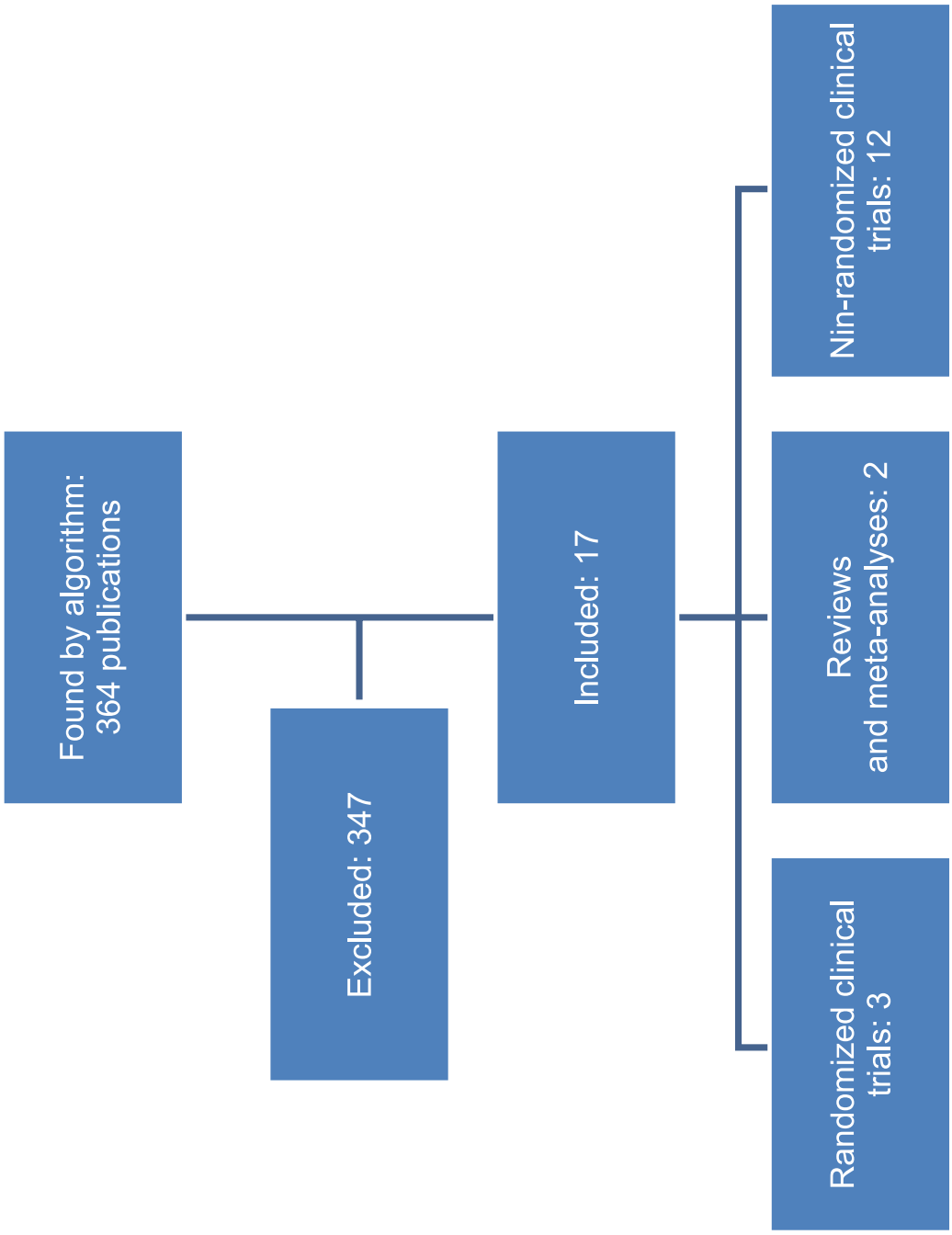

FIG. 2.
Block diagram of the study design

TABLE 1

CHARACTERISTICS OF THE INCLUDED STUDIES

Author, date	Design/ Quality	Number of participants	Comparison group, ERP/Standard	Application	Results: ERP/Standard	Comments
Firoozfard, 2003 [10]	NCT/3	75	25/50	Open nephrectomy	Blood loss: 700/1050 ml; Mortality: 1/1 cases; Complications: CD2 – 4/2 cases, CD3a – 5/5 cases, CD3b – 2/2 cases; Readmission: 1/1 cases. Hospitalization: 4/8 days. VAS POD1: 1/5 points.	Signs of a defect in methodology and statistics
Mansour, 2017 [11]	RCC/2	224	113/111	Laparoscopic and open nephrectomy	Operation time: 156.2 ± 33.2/172 ± 45.5 min. Incision: 6.7 ± 2.4/15.2 ± 3.2 cm/. Blood loss: 165.6 ± 45.2/185 ± 78.5 ml. Hospitalization: 2.8 ± 1/3.9 ± 1.7 days. VAS POD1: 4 ± 1.8/7.2 ± 1.0 points. Disability: 25.3 ± 12.5/41.7 ± 16.5 days. CD2: 15/40 cases. Death: 0/0 cases. Readmission: 0/0 cases.	Poorly described complications
Мазуренко, 2017 [12]	NCT/3	67	29/38	PNL	Stone size: 25.8/27.1 mm. Operation time: 27.98 ± 11.13/26.34 ± 12.21 min. Hospitalization: 27.2/54.7 hours. Complications: CD2 – 5/8 cases, CD3a – 0/1 cases. SFR 4 mm: 91 %/90 % of cases. Death: 0/0 cases. Readmission: 0/0 cases.	Not all sections describe the statistics correctly.
Wong, 2018 [13]	NCT/3	302	126/176	Reconstructive urology, urethroplasty for hypospadias	Duration of catheterization: 10.7 ± 2.8/10.2 ± 2.9 days. Hospitalization: 2 ± 0.5/10 ± 2 days. Complications: CD2 – 7/28 cases, CD3 – 22/61 cases. Success rate: 87/71 cases. Predictor of success: ERP (OD = 0.35, 95% CI: 0.15–0.85; $p = 0.02$).	
Rove, 2018 [14]	NCT/3	39	13/26	Reconstructive urology	Operation time: 277 (189–314)/270 (203–342) min. Hospitalization 5 ± 1/6 ± 1 days. Readmission: 1/7 cases. Complications: CD2 – 9/22 cases, CD3 – 1/10 cases, CD4 – 0/4 cases. Predictor of complications: traditional approach (HR = 0.71, 95% CI: 0.51–0.97).	

TABLE 1 (*continued*)

Author, date	Design/ Quality	Number of participants	Comparison group, ERP/Standard	Application	Results: ERP/Standard	Comments
Haid, 2018 [15]	NCT/3	30	15/15	Reconstructive urology	Operation time: 336.5 ± 21.42/387.9 ± 32.34 min. Hospitalization: 11.93 ± 0.64/19.87 ± 2.04 days. Complications: CD2 – 0/3 cases, CD3 – 0/0 cases.	
Gridley, 2020 [16]	RCC/2	80	52/30	RIRS	Prestenting: 17/7 cases. Access sheath: 43/20 cases. Stones size: 15.3 ± 12.7/15.5 ± 9.4 mm. Operation time: 116 ± 31/122 ± 34 min. Stenting: 8.1 ± 7/6.0 ± 3 days. Opiates: 26/41 cases. VAS > 5 points: 10/7 cases. Death: 0/0 cases. Readmission: 0/0 cases.	There is no description of the developed protocol, the duration of hospitalization is not indicated.
Li, 2020 [17]	RCC/2	235	117/118	PNL	Stone size: 21.03 ± 9.43/19.19 ± 8.16 mm. SFR: 93.2 %/89.8 % of cases. Operation time: 54 ± 12/58 ± 11 min. VAS POD0: 0.79 ± 0.76/2.79 ± 0.98 points. Hematuria: 2/10 cases. Sepsis: 5/6 cases. Complications: CD2 – 14/21 cases, CD3b – 1/1 cases. Hospitalization: 4.6 ± 1/6.2 ± 1.1 days. Nephrostoma: 3.6 ± 1/5.2 ± 1.1 days. Catheter: 2.6 ± 1.0/4.2 ± 1.1 days. Death: 0/0 cases. Readmission: 0/0 cases.	No size specified for SFR.
Han, 2021 [18]	NCT/3	39	13/26	Reconstructive urology	Hospitalization: 5 ± 1/6 ± 1 days.	The study focused primarily on the aspect of anesthesia, complications and details of surgical aspects are not presented.
Chan, 2021 [19]	NCT/3	40	20/20	Reconstructive urology	Hospitalization: 4 (3–29)/9 (2–31) days. Readmission: 6/4 cases. Re-operation: of 3/6 cases. Complications: CD2 – 6/9 cases, CD3 – 3/6 cases.	

TABLE 1 (*continued*)

Author, date	Design/ Quality	Number of participants	Comparison group, ERP/Standard	Application	Results: ERP/Standard	Comments
Shu, 2022 [20]	NCT/3	435	216/219	RIRS	Stone size: 20 (5)/20 (5) mm. Operation time: 75 (50)/90 (50) min. Hospitalization: 2 (1)/3 (1) days. Complications: CD2 – 6/9 cases. Hematuria: 18/35 cases. SFR: 78.3 %/75.8 % of cases.	
Gao, 2022 [21]	NCT/3	341	104/237	PNL	Stone size: 44 ± 21.5/41.7 ± 23.1 mm. Density: 1088 ± 681/1011 ± 591 HU. Fever: 10/12 cases. Operation time: 88.2 ± 46.5/93.6 ± 27.7 min. Sepsis: 4/5 cases. SFR: 91.3/87.5 % of cases. Hospitalization: 4.49 ± 2.4/6.64 ± 3.1 days. Complications: CD2 – 16/20 cases.	
Satvaldieva, 2022 [22]	NCT/3	92	42/50	Reconstructive urology	Operation time: 84 ± 9.7/min. Hospitalization: 38 ± 1.9/hour.	There is no data on complications, there is no data on the control group. Only the analgesic status is presented.
Dutta, 2022 [23]	NCT/3	173	91/82	Reconstructive urology, urogynecology	Operation time: 97.8 (10–268)/82.4 (6–223) min. Complications: CD2 – 4/10 cases, CD3 – 0/1 case. Readmission: 7/12 cases.	A number of important data, such as the duration of hospitalization, are not specified.
Girgiss, 2022 [24]	NCT/3	121	55/66	PNL	Stone size: 38.3 ± 36/30.2 ± 20.6 mm. Operation time: 98.1 ± 35.4/133.3 ± 39.4 min. Hospitalization: 1.22 ± 1.47/1.31 ± 1.95 days. Complications: CD2–3 10/9 cases. Readmission: 7/8 cases.	

Note. CD – Clavien – Dindo classification of complications; VAS – visual-analog pain scale; POD – postoperative day; PNL – percutaneous nephrolapaxy; RIRS – retrograde intrarenal surgery; URS – ureteroscopy; HU – Hounsfield units for measuring X-ray density; SFR – freedom from residual concretions; OR – odds ratio; RR – risk ratio.

col. Of the 24 elements recommended by ERAS, 15 were implemented on average. About half of them face administrative or collective problems with the implementation of ERP.

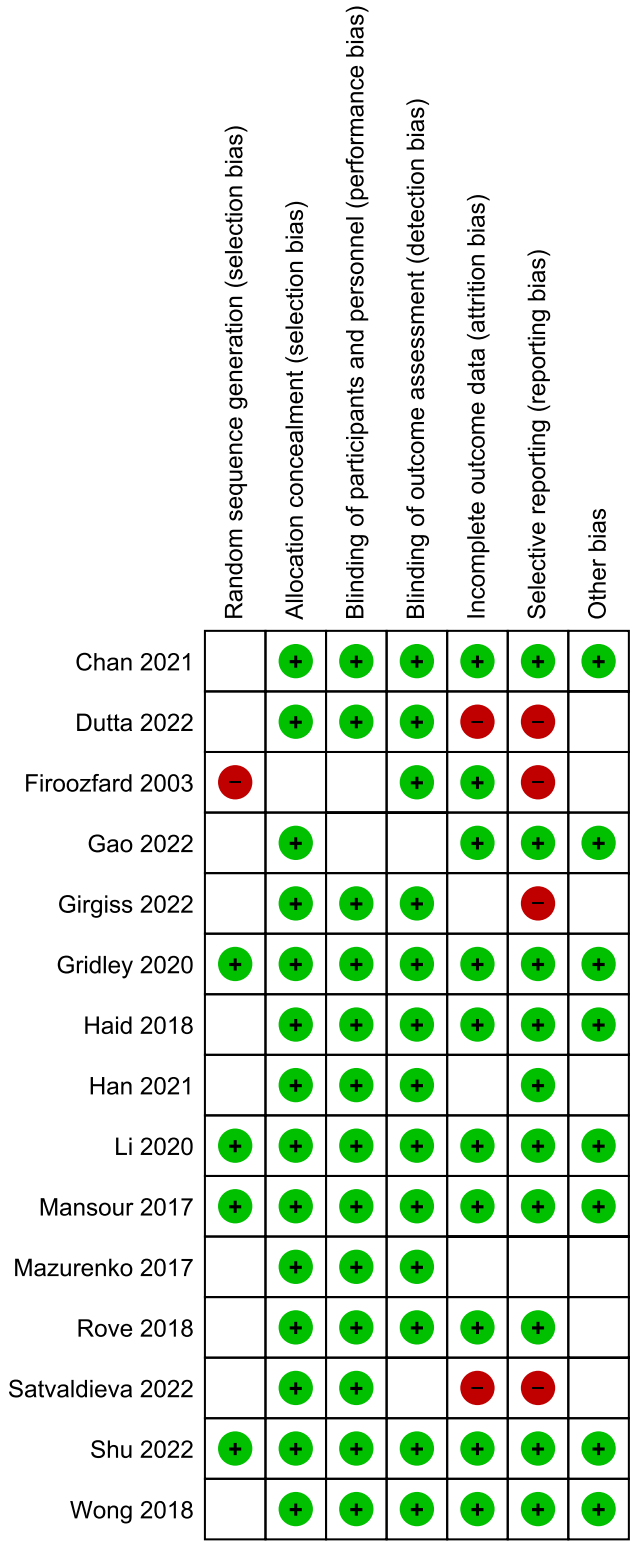

FIG. 3.
Characteristics of the bias of the included studies

Also of scientific interest is the description of the methodology and the process of developing a protocol for enhanced recovery in pediatric reconstructive urology (PURSUIT), presented in 2020 [26]. The paper provides clear criteria and design of the study, a template for the planned protocol, a roadmap, data and statistical analyses.

There are no completely original protocols of enhanced recovery among the included works. All the presented studies are based on the general concept of the ERAS strategy, consisting of 22 elements. The level of involvement in the protocol is described in some separate papers.

META-ANALYSIS OF LITERATURE DATA

The results of treatment were compared according to the principles of meta-analysis of data between groups of patients treated according to the standard protocol and according to the enhanced recovery program.

Among 15 clinical trials and 2,293 patients included in the meta-analysis, there were no significant differences in age ($p = 0.77$) or gender ($p = 0.63$) between the groups.

One of the basic advantages of enhanced recovery is a shorter hospital period, and the main disadvantage is the risk of readmission or re-operation, that is, re-hospitalization and repeated surgical intervention.

According to the results of the analysis (Fig. 4), a significant statistical difference in the duration of hospitalization was established: patients under the enhanced recovery protocol stay in the hospital less long ($p < 0.00001$). There is a very high heterogeneity of the results obtained ($I^2 = 97$ %) due to a significant difference in the timing of hospitalization in the included studies. When using the same treatment protocol and type of surgical intervention, the patient can stay in the hospital from one [12] to five days [17]. The mean difference in the length of stay is 2 days (95% CI: –2.56÷–1.36; $p < 00.00001$).

The reduction in the duration of hospitalization does not lead to an increase in the risk of re-treatment or re-operation when using ERP in urology ($p = 0.35$), which is shown in Fig. 5. The heterogeneity value for this test is considered insignificant ($I^2 = 0$ %), which indicates the general homogeneity of the data of different authors.

The risks of developing postoperative complications ≥ Class 2 according to the universal Clavien – Dindo classification were comparable in both groups ($p = 0.13$), which is shown in Figure 6. The heterogeneity of the combined results corresponds to an intermediate between moderate and high ($I^2 = 73$ %), since in several studies the predominance of complications in the ERP group was noted, however, most studies show that ERP is accompanied by a lower risk of complications. We should note the results of group analysis in reconstructive urology: a significantly lower risk of complications ($p = 0.02$) was established in the ERP group. In the subgroup of endourological operations – percutaneous puncture nephrolithotripsy and retrograde intrarenal surgery ("ЧПНЛ" and "РИРХ" in Fig. 7, respectively) – low heterogeneity was revealed, which increases the significance of the data obtained.

The use of ERP increases the expected success of the treatment by 1.74 times (OR = 1.74; 95% CI: 1.08–2.79; $p = 0.02$), which is shown in Figure 7. The results ob-

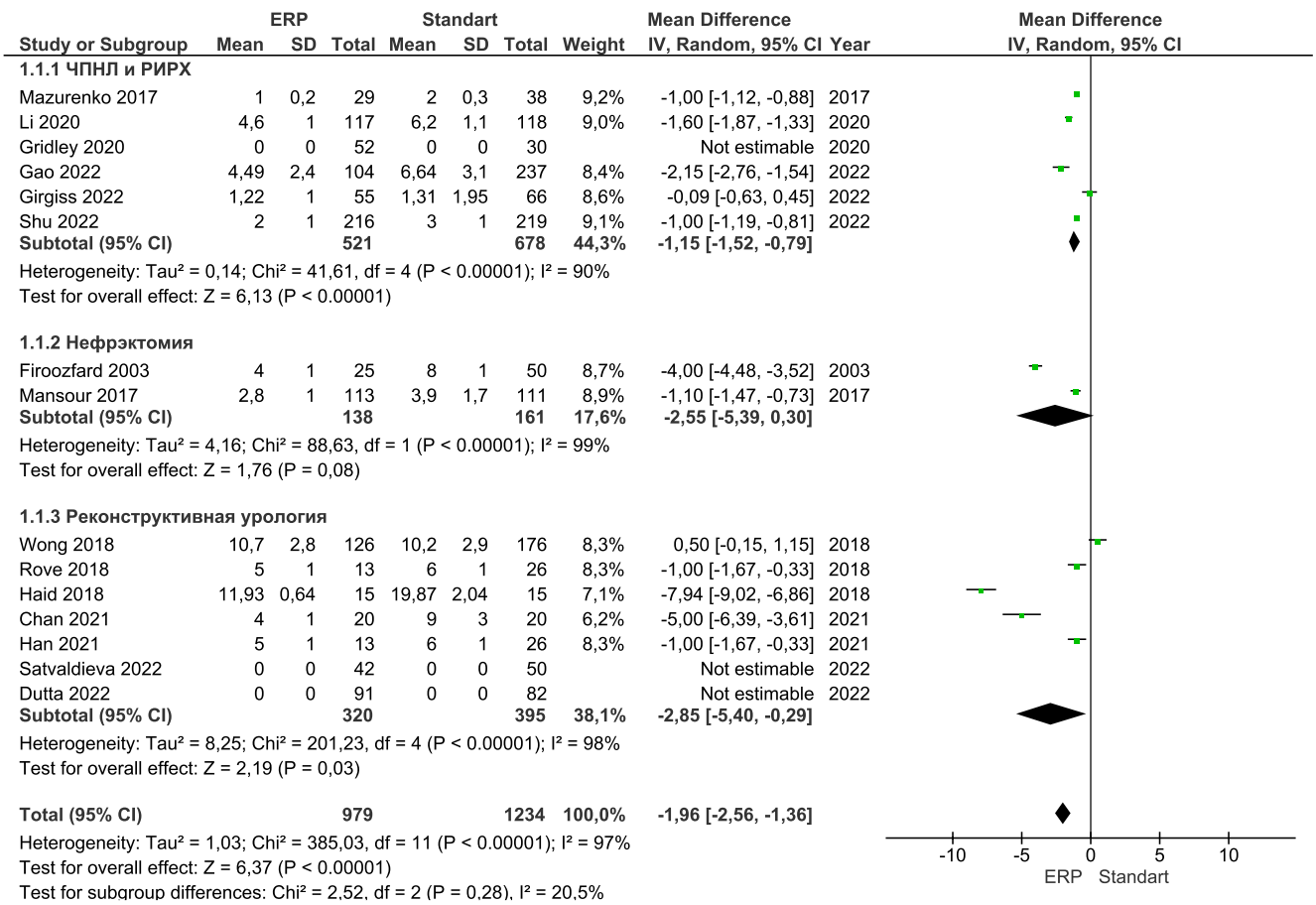

FIG. 4.

Forest diagram comparing the duration of hospitalization with the use of ERP and the Standard Treatment Protocol

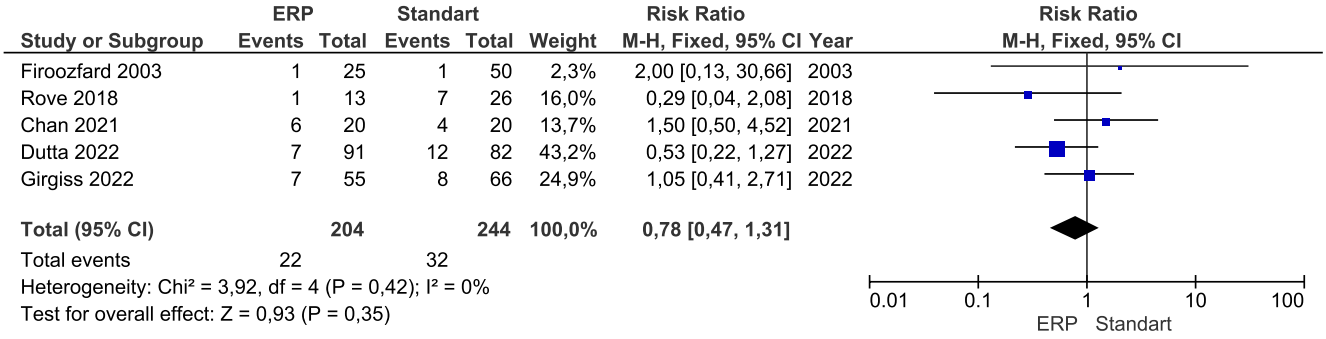

FIG. 5.

Forest diagram comparing the risk of readmission with the use of ERP and the Standard Treatment Protocol

tained have satisfactory heterogeneity of significance and (χ^2 = 10.82; I^2 = 54 %). The group analysis in the subgroups reconstructive urology and andrology ("ЧПНЛ" and "РИРХ") obtained homogeneous data (I^2 = 0 %), which significantly increases the value of the results and confirms the positive prognostic effect of ERP. Thus, when using ERP in reconstructive urology, the success rate of treatment increases by 3 times with high statistical reliability (OR = 3.21; 95% CI: 2.02–5.09; p < 0.00001; I^2 = 0 %).

Evaluation of the scientific validity and quality of meta-analysis

Figure 8 shows the analysis of the bias of the data presented by the authors in the included studies according to the RoBANS criteria. The results of the analysis of all 15 included papers indicate a low risk of bias.

The evaluation of the statistical heterogeneity of the results, presented in each forest diagram through the value of I^2, showed a general moderate heterogeneity of the data obtained by the authors, which is justified by the peculiarities of clinical practice and methodology of the work performed, as well as by different types of surgical techniques included in the meta-analysis (from endourological

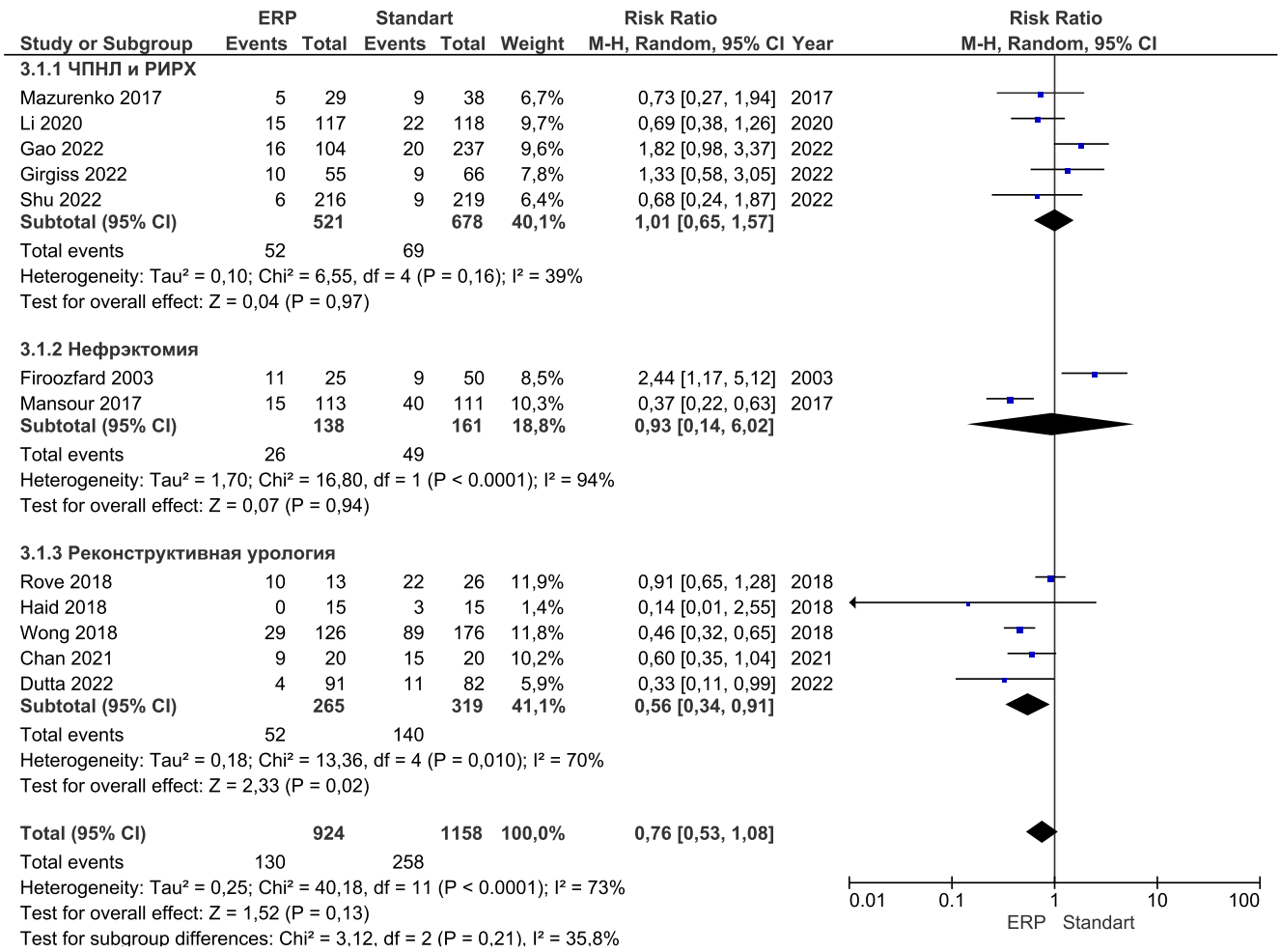

FIG. 6.

Forest diagram comparing the risk of developing complications of Clavien – Dindo ≥ Class 2 for the use of ERP and the Standard Treatment Protocol

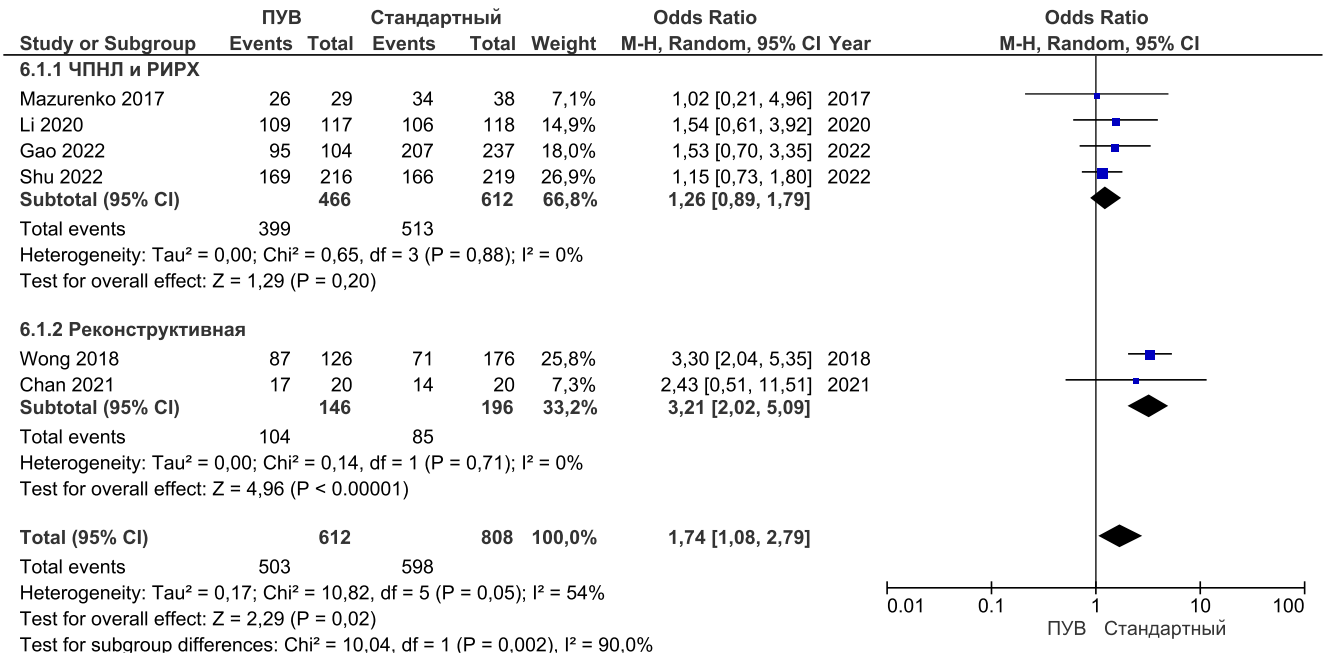

FIG. 7.

Forest diagram comparing the success of the treatment with the use of ERP and the Standard Protocol

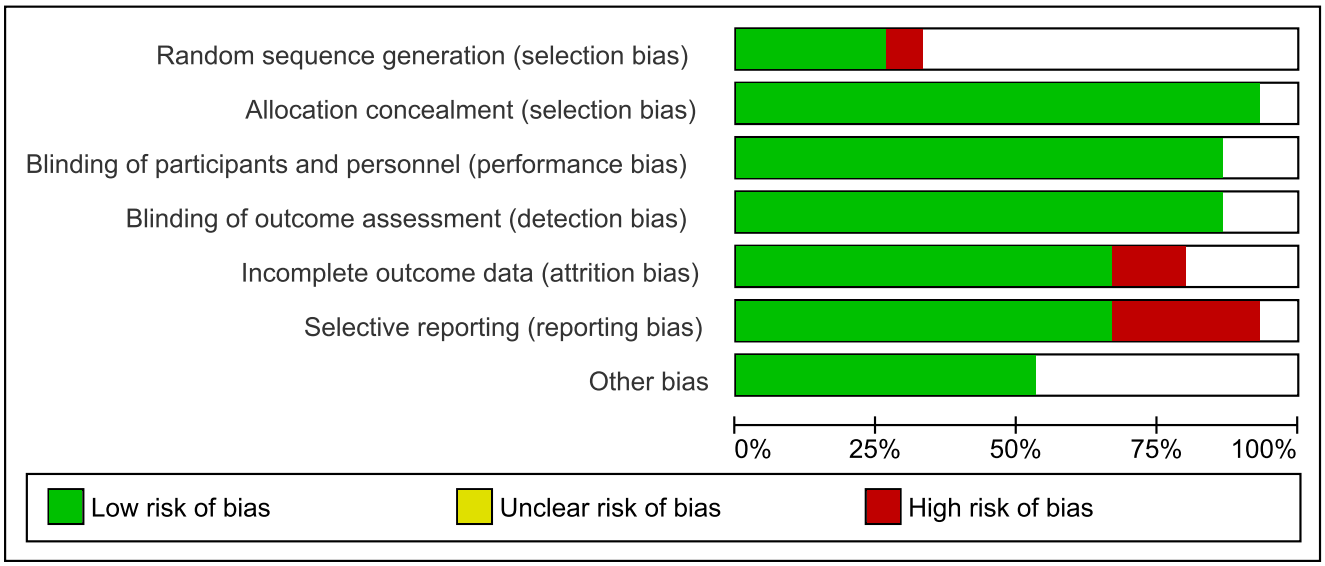

FIG. 8.
Analysis of publication bias

to reconstructive). When performing a subgroup analysis in therapeutic areas, the results are homogenized, which indicates the high significance of the data obtained. An unambiguous conclusion was obtained when analyzing the risk of re-hospitalization. The homogeneity of the initial data increases the reliability of the results obtained due to the absence of the influence of variation in values.

The stability of the final summary test to changes in the generalized sample and to the applied methods of analysis is analyzed. The value of the total value confirms the reliability of the results obtained and the absence of evidence of bias of the publications included in the meta-analysis.

DISCUSSION

The Enhanced Recovery Program (ERP) is an actual multimodal perioperative strategy aimed at improving the results and quality of treatment. Regardless of the surgical discipline, it is possible to develop and optimize the program for specific nosology. The application of ERP in coloproctology, thoracic and cardiac surgery, oncology, and neurosurgery is most studied. In the Russian Federation, there are a lot of monographs and several approved clinical recommendations on ERP in coloproctology. However, in a few disciplines, the scientific representation of ERP remains fragmented, both in Russia and in the world.

Several scientific papers devoted to the analysis of the use of enhanced recovery programs in urology demonstrate a relatively small involvement of the new multimodal strategy in the treatment of pathology of the urogenital tract. The authors agree that the use of even individual elements is promising, it will reduce the likelihood and significance of postoperative complications [27, 28]. At the same time, until May 2015, only 15 scientific papers published in English were directly devoted to ERP in urology [29]. By 2020, the number of publications has increased to 55, most of them are devoted to the ERP during radical cystectomy according to ERAS recommendations [30], including ones published in Russian [31–34]. The unequivocal conclusion is that there are no obstacles to expanding the use of ERP to other surgical procedures within the framework of the profile "urology".

The use of the Enhanced Recovery Program when performing endourological, reconstructive or organ removing interventions in urology with comparable risks of complications allows to achieve better treatment results, reduce the time of hospitalization, reduce the likelihood of repeated treatment and, as a result, reduce the total cost of treatment.

In general, a small number of clinical studies are presented on the subject of ERP as part of the search and inclusion strategy, in comparison with oncological urology, abdominal surgery and other disciplines. In total, PubMed presents 295 meta-analyses and systematic reviews on ERP, 9 of them – on oncological urology (the study of ERP during radical prostatectomy and cystectomy). Systematic reviews and meta-analyses on ERP in non-oncological urology have not been revealed.

The main conclusions of the meta-analysis obtained based on scientific evidence:

- Urological patients who are scheduled for surgical treatment should be included in the Enhanced Recovery Program according to an adapted protocol, which reduces the time and cost of treatment, reduces the likelihood of readmission and achieves better treatment results (evidence level – 1; recommendation level – A);
- The use of the Enhanced Recovery Program for urological patients when planning surgical treatment is not accompanied by increased risk of complications > *Class* 2 according to Clavien – Dindo (evidence level – 1; recommendation level – A).

Given the heterogeneity of surgical techniques and nosologies, technical bias should be considered when plan-

ning randomized clinical trials and subsequent meta-analyses.

The presented meta-analysis showed a statistically significant difference between ERP and the Standard Treatment Protocol ($p < 0.02$). The overall result of the performed analysis indicates the positive role of the Enhanced Recovery Program, regardless of the field of application in urology, which is consistent with the data of meta-analyses on the oncological urological profile [35, 36] and interdisciplinary analyses of ERAS programs [37].

Limitations of meta-analysis

A detailed systematic review and meta-analysis of the literature data was performed using standardized and recommended tools for evaluating the research methodology. When assessing the risk of systematic error, most of the included studies were of satisfactory or good quality, however, some were of poor quality.

Of the 15 clinical trials included in the meta-analysis, 12 (80 %) are non-randomized studies, which negatively affects the significance of the data obtained from these studies.

Most of the included studies clearly stated the objectives of the study, and although the selection of patients was generally acceptable, several studies did not clearly indicate the inclusion criteria, or a few statistics required when performing a meta-analysis. In addition, most studies do not provide a detailed description of the study design, the use of placebo control, types of randomizations, etc. There was a large methodological variability between the studies (for example, different protocols of enhanced recovery for similar urological pathology), as well as significant deviations from the ERAS recommendations for the implementation of the program (inclusion of <50 % of the program elements), which may explain the differences in the results obtained.

Since the purpose of this systematic review was to study the effectiveness of the use of enhanced recovery protocols in the treatment of urological diseases, a possible limitation is the excluding works on urological oncological diseases from the analysis. A meta-analysis of various outcomes was carried out with moderate heterogeneity of the results obtained. Therefore, the results should be evaluated and used as corresponding to a high level of evidence.

CONCLUSION

Based on a meta-analysis of data, with a high level of evidence and significance of recommendations (1-A), it was found that the use of the Enhanced Recovery Program allows better treatment results with comparable risks of complications.

Practical recommendations

In urological patients, when planning surgical treatment, the adapted Enhanced Recovery Program should be used, regardless of the nosological characteristics and type of intervention.

Conflict of interest

The authors declare no conflict of interest.

Research transparency

The study was not sponsored. The researchers are solely responsible for submitting the final version of the manuscript for publication.

Declaration on financial and other interactions

All authors participated in the development of the concept and design of the study and in writing the manuscript. The final version of the manuscript was approved by all authors. The authors did not receive a fee for the study.

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METHODS OF PHYSICAL REHABILITATION OF ELDERLY PEOPLE FOR THE PREVENTION AND TREATMENT OF SARCOPENIA

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ABSTRACT

The aim of the review *is to analyze the prevalence of sarcopenia in the elderly age group, the causes of its occurrence, and to present modern methods of prevention and physical rehabilitation.*
The study focuses on the relationship between exercise, training effects and physiological mechanisms, as well as the safety of various types of strength, anaerobic and multimodal training, which have a positive impact during the prevention and rehabilitation treatment of sarcopenia. Literature reviews, meta-analyses, and original studies are included that focus on older people in all settings, using validated assessment tools and methods. A literature search was conducted in four electronic databases – PubMed, Cochrane Library, Scopus, Springer, for the period from 2012 to June 30, 2022. There were no restrictions on the language bias of the publication.
Search strategy. *The keywords used to define the terms of participation in the review are "older/advanced age", "sarcopenia" and "sarcopenic obesity".*
Articles were included if they met the following criteria – cohorts with mean or median age ≥ 60 years and any of the following definitions of sarcopenia: European Working Group on Sarcopenia in the Elderly (EWGSOP), Asian Working Group on Sarcopenia (AWGS), International Working Group on Sarcopenia (IWGS). To ensure comparability of interventions, the review included studies that were conducted for at least 8 weeks, and the distribution of patients by study design was randomized. Also, articles involving hospitalized patients are excluded.

Key words: *sarcopenia, physical activity, rehabilitation, sarcopenic obesity, older adult, resistance training, aerobic exercise, exercise therapy, systematic review*

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РОЛЬ И СПЕЦИФИКА ФИЗИЧЕСКИХ НАГРУЗОК ПРИ САРКОПЕНИИ У ПОЖИЛЫХ ЛЮДЕЙ

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РЕЗЮМЕ

Цель обзора *– проанализировать распространённость саркопении в пожилой возрастной группе, причины её возникновения, представить современные методы профилактики и физической реабилитации.*
Исследование сосредоточено на взаимосвязи между физической нагрузкой, тренировочными эффектами и физиологическими механизмами, а также на безопасности различных видов силовых, анаэробных и мультимодальных тренировок, которые оказывают положительное влияние во время профилактики и восстановительного лечения при саркопении. Включены обзоры литературы, метаанализы и оригинальные исследования, которые акцентированы на людях пожилого возраста в любых условиях проживания, с применением проверенных инструментов и методов оценки. Был проведён поиск литературы в четырёх электронных базах данных (PubMed, Cochrane Library, Scopus, Springer) за период с 2012 г. по 30 июня 2022 г. Ограничений на языковой уклон публикации введено не было.
Стратегия поиска. *Ключевые слова, используемые для определения условий участия в обзоре: «пожилой/преклонный возраст», «саркопения» и саркопеническое ожирение».*
Статьи включались, если они соответствовали следующим критериям – когорты со средним или медианным возрастом ≥ 60 лет и любым из следующих определений саркопении: Европейская рабочая группа по саркопении у пожилых людей (EWGSOP), Азиатская рабочая группа по саркопении (AWGS), Международная рабочая группа по саркопении (IWGS). Для обеспечения сопоставимости вмешательств в обзор включены исследования, которые осуществлялись не менее 8 недель, а распределение пациентов по дизайну исследования было рандомизированным. Также, исключены статьи с участием госпитализированных пациентов.

Ключевые слова: *саркопения, физическая активность, реабилитация, саркопеническое ожирение, пожилой возраст, тренировки с отягощением, аэробные тренировки, лечебная физкультура, обзор литературы*

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Significant increases in life expectancy and declining birth rates are increasing the number of older and senile people worldwide. Due to declining functional capacity, health and well-being of older adults is a major focus of aging-related studies [1].

Sarcopenia is a geriatric disease with progressive loss of skeletal muscle mass and function [2], first described by Rosenberg [3]. In September 2016, sarcopenia entered the International Classification of Diseases, 10th Revision (ICD-10), under the code M62.84. This term has not yet been added to the 2019 ICD-11. Sarcopenia is one of the major health problems among older people and increases the risk of disability, falls, and injuries associated with falls, hospitalization, limitation of independence and mortality. Risk factors for sarcopenia include age, gender, physical activity level and chronic diseases [4]. Currently, there are several definitions of sarcopenia with no consensus, so its prevalence can vary greatly depending on the population surveyed (differences in gender, age, ethnicity), living conditions (hospitalization, senior centers), methods and assessment tools [5]. Although there are controversial opinions about sarcopenia, muscle strength, mass and physical functioning are its main diagnostic indicators [6, 7]. In particular, Japanese doctors estimate that the number of people aged 65 years and older has increased to 28.4 % in 2019, the highest in the world [1]. The number of people with sarcopenia in Izmir (Turkey) aged over 65 years is 5.2 % [8], in Brazil it is 4.5 % [9]. In China (PRC), it is 12.3 % among males and 7.6 % among females. In South Korea, the rate was 6.3–21.8 % among males and 4.10–22.1 % among females [10]. In Russia, the prevalence of sarcopenia reaches 22.1 % [11].

Research on sarcopenia has made little progress over a long period of time. Only in 2010 a clinical definition of sarcopenia was proposed for the first time by the European Working Group on Sarcopenia in Older People (EWGSOP) [2]. The diagnosis of sarcopenia required a decrease in both mass and function of human skeletal muscle (muscle strength and/or performance). In 2011 [12], the International Working Group for Sarcopenia (IWGS) presented a similar definition of sarcopenia, focusing on the assessment of physical function, including the ability to get up from a chair or a pace test. In 2014 [10], Asian Working Group for Sarcopenia (AWGS) and Foundation for the National Institutes of Health (FNIH) also presented their expert opinion on sarcopenia. In 2018, EWGSOP, based on the results of fundamental and clinical studies of sarcopenia in recent years, held a second meeting and updated its consensus [13]. The categories of sarcopenia have remained unchanged: "primary" (or age-related) when no other specific cause is apparent, and "secondary" when causative factors other than aging (or in addition to aging) are evident. EWGSOP2 defines sarcopenia as a muscle disease (muscle failure) with low muscle strength supplanting the role of low muscle mass as the primary factor. Moreover, EWGSOP2 has recently defined subcategories of sarcopenia: acute and chronic. Acute sarcopenia lasts less than 6 months, and chronic sarcopenia lasts more than six months. AWGS also updated its consensus on sarcopenia in 2019 [14]. The definition of sarcopenia given by EWGSOP2 is now more widely used in both practical clinical work and research area.

Currently, there is still a debate among scientists due to the lack of an unambiguous decision about primary (age-related) sarcopenia; whether it is a disease or a variant of the norm that goes alongside the aging process [4, 6, 10].

Sarcopenia is a multifactorial disease [13], with the main factors identified being low physical activity level, sedentary lifestyle, nutritional disorders and comorbidities that often accompany aging and act as preconditions for sarcopenia, senile asthenia, obesity and chronic diseases [15], contributing to decreased muscle mass, reduced calorie and protein intake, altered muscle metabolism, oxidative stress and degeneration of neuromuscular junctions [16].

Sarcopenia is one of the leading causes of disability among older people. Approximately 50 % of skeletal muscle mass (1–2 % of muscle mass per year) is lost between the ages of 50 and 80 [10, 13]. In other words, sarcopenia is one of the most important factors reducing the quality of life of the older adults and is associated with morbidity and mortality.

RESISTANCE TRAINING

Current clinical guidelines include resistance training (RT) as a primary treatment strategy for sarcopenia [17]. During RT, patients exercise with gradually increasing resistance using weight-bearing training, free weights and bodyweight exercises [18]. RT programs improve muscle strength, mass and physical performance of older people [19]. It is proved that resistance training as a recognized treatment for muscle atrophy has been shown to reduce hospital stay by increasing indicators of hand grip strength test and muscle cross-sectional area among older adults [20]. Although exercise cannot completely prevent neuromuscular aging, resistance training has great potential to mitigate age-related changes [21]. But despite this, RT programs are not usually used in clinical practice for the rehabilitation of patients with sarcopenia [22].

Exercise programs that have attracted the attention of specialists as a measure to combat sarcopenia are common (Table 1). Their safety and positive results for the treatment of this condition among older people have been studied.

It is clear that high-load resistance training (H-RT) induces muscle hypertrophy among older adults [23], but, due to comorbidities such as musculoskeletal disorders, coronary artery disease and diabetes, it should be performed with caution and under constant medical supervision [24]. Moreover, H-RT is known to cause joint pain due to high loads, in which case low- and moderate-load resistance training is recommended to the patient [1].

Therefore, although this type of resistance training is an effective method of sarcopenia prevention, the number of older people who will be able to perform is very limited.

M-RT (moderate-load resistance training) is usually distinguished from H-RT by a lighter weight (up to 75 % of 1RM) and identical frequency of exercise [25]. For example, Wake

TABLE 1

CHARACTERISTICS OF EACH TYPE OF STRENTH TRAINING FOR INCREASING SKELETAL MUSCLE MASS IN THE ELDERLY

Resistance training	Training features			
	Resistance, % of 1RM	Exercises per week	Sets and repetitions	Author, publication year
H-RT	70–85 % of 1 RM	2–3	1–3 × 8–15	Fragala M.S. et al. (2019)
L-BFR	10–50 % of 1 RM	2–3	1–4 × 15–30	Thiebaud R.S. et al. (2013) Yasuda T. et al. (2017) Cook S.B. et al. (2017) Centner C. et al. (2019) Rodrigo-Mallorca D. et al. (2021)
L-ST	30–50 % of 1 RM	2–7	1–3 × 5–15	Watanabe Y. et al. (2014) Kanda K. et al. (2018) Takenami E. et al. (2019)
L-FAIL	20 % of 1 RM	3	1 × 80–100	Van Roie E. et al. (2013)
M-RT	50–75 % of 1 RM	2–4	3 × 8–12	Michel J.M. et al. (2022) Vasconcelos K.S. et al. (2016)

Note. 1RM – one-repetition maximum; H-RT – high-load resistance training; L-BFR – low-load resistance training with blood flow restriction by an elastic designed cuff belt; L-FAIL – low-load resistance exercise to volitional fatigue; L-ST – low- or moderate-load resistance training without blood flow restriction; RT-ML – moderate-load resistance training.

Forest University conducted a 10week experiment on participants aged ≥ 60 years with alternating exercise intensity ranging from 50–75 % of 1RM for 10 weeks and found consistent increases in muscle volume, strength and endurance among most participants regardless of gender [26]. A study conducted by K.S. Vasconcelos et al. (Brazil), involving 31 women aged 65 to 80 with sarcopenic obesity (bidirectional pathogenic interaction between visceral fat accumulation in the body and loss of skeletal muscle mass, strength and function), proved a statistically significant increase in knee extensor muscle strength and quadriceps femoris muscle strength after a 10-week resistance training programme with 60-minute sessions twice a week, but, according to the authors, the effectiveness of the training for muscle mass gain was insignificant [27].

As a practical application, it is suggested that resistance training among older and frailer individuals should be started by performing 8–10 repetitions in a series with a weight with which they could perform at least 20 maximal repetitions, and no more than 4–6 repetitions in a series with a weight that would allow them to perform 15 repetitions [21]. Since sarcopenia affects muscles throughout the body [13], it is recommended to perform comprehensive exercises involving all muscle groups [18].

There is currently much scientific evidence that low-load resistance training with blood flow restriction by an elastic designed cuff belt (L-BFR), also known as Kaatsu training [28, 29], is used as a countermeasure against sarcopenia among older adults [28, 29]. Reviews focusing on older adults have reported that L-BFR can induce similar muscle mass gains compared to H-RT, but has less effect on muscle strength [30]. A study on L-BFR safety was conducted at Seirei Christopher University (Japan), and symptoms of subcutaneous bleeding, numbness and dizziness among subjects were decribed, but no serious abnormalities were detected [28]. Despite the favorable effects of the training on skeletal muscle, there are some serious concerns about performing L-BFR for treating individuals with cardiovascular and endocrine diseases [31]. It is recommended to use L-BFR according to current guidelines and under regular medical supervision [32, 33].

This method may be quite effective in preventing sarcopenia among older adults who can only perform low-load strength training due to health-related reasons.

Low- or moderate-load resistance training without blood flow restriction (L-ST) is a popular exercise technique. It is characterized by a relatively slow movement that restricts muscle blood flow and creates a tonic force (3 seconds in a downward or upward movement without relaxation or pause). During the training, intramuscular pressure of upper and lower limb muscles increases, inhibiting both blood flow and outflow from the muscle [34]. A sustained increase in strength at a maximal load of 40–50 % of 1RM has been proven [35, 36]. Moreover, L-ST (knee extension, 30 % of 1RM, twice a week for 12 weeks) resulted in increased quadriceps muscle strength and hypertrophy among older people, increasing muscle strength and muscle size not only among young but also among older people [35]. Also, S. Usui et al. found that L-ST inreased muscular strength and skeletal muscle mass, but had very little effect on energy production during dynamic explosive exercise [37].

Thus, L-ST is an accessible alternative as well as an effective way for older people to increase muscle size and strength.

Low-load resistance exercise to volitional fatigue (L-FAIL) is very similar to L-ST in terms of technique and maximum weight capacity. In case of L-FAIL, the training approach is completed only after the person cannot technically correctly lift a weight that is 20–30 % of 1RM [38]. This technique is also called "training to failure".

Similar to the observations of L-BFR, stimulation of muscle protein synthesis by L-FAIL will occur independently of exercise, as long as resistance exercise was performed before volitional fatigue [39]. R. Ogasawara et al. reported in their study that L-FAIL induces muscle gain comparable to that induced by normal H-RT among healthy young adults [40].

Most studies on L-FAIL involved young and middle-aged people. Studies involving older people are very limited. In their study, E. Van Roie et al. conducted training with older adults and found that L-FAIL (20 % of 1RM, 80–100 repetitions, one set) causes muscle hypertrophy comparable to H-RT (80 % of 1RM, 10–15 repetitions, two sets) [41]. A more recent study involving 56 older adults (68.0 ± 5.0 years) assigned to leg press exercises with different loads (80 %, 40 % and 20 % of 1RM) conducted by E. Van Roie et al. proved that muscle volume returns to baseline after 24 weeks of detraining regardless of the loads in the exercise [42].

No results of studies on the effects of L-ST and L-FAIL on the risk of falls or osteoarthritis exacerbation among older adults were found. Safety and side effects of these exercise methods are poorly understood.

L-FAIL-based training methods involve a higher number of repetitions and require a high level of motivation over a longer period of time than other training programs.

AEROBIC TRAINING

Aerobic (cardio) training increases aerobic endurance, reduces systolic and pulse blood pressure and blood lipid levels [43], resulting in increased cardiovascular endurance [44] and is another important form of physical activity (Table 2).

M.P. Harber et al. found in their study that aerobic training during 12 weeks of bicycle ergometer induced skeletal muscle hypertrophy and age-related adaptation of myofibril function among older men (mean age 74.3 years). Post-exercise aerobic capacity was 13.3 % higher and quadriceps muscle volume determined by MRI has increased by 6.1 % ($p < 0.05$) [45]. Also, at the University of Thessaly (Greece) Z. Bori et al. concluded that 12 weeks of aerobic exercise have an effect on the enhancement of mitochondrial biogenesis, i.e. the number of mitochondrial copies in skeletal muscle cells increases to ensure the production of more ATP against the background of increased tissue energy demand during exercise among older people [46].

L.F. Ferreira et al. in their study (115 women aged 60 years and older) concluded that aerobic training does not have any statistically significant effect, unlike weight training [47]. On the other hand, a study by N.T. Chen et al. conducted at Taipei Medical University (Taiwan) proved an increase in muscle mass and a decrease in total fat mass among patients with sarcopenia aged 65–75 years after a course of aerobic training, but it was also confirmed that the effect of the training in the group engaged in a strength protocol was statistically significantly higher [48].

T. Morat et al. studied the effect of Nordic walking on older men and women. As a result, cholesterol levels decreased by 12 %, endurance and speed of overcoming distance increased (week 1 – 5.45 km/h, week 12 – 6.51 km/h) [49].

TABLE 2

THE EFFECT OF AEROBIC EXERCISE ON THE ELDERLY

Gender	Age	Training protocol	Result	Author, publication year
Male/female	74 ± 3	Bicycle ergometer, 20–45 min, 3–4 days per week, 12 weeks	↑ muscle size ↑ endurance ↑ MB	Harber M.P. et al. (2012) Bori Z. et al. (2012)
Male/female	65–75	Dance, 60 min, 2–3 days a week, 8 weeks	↑ muscle size ↑ spinal extensor muscle strength	Chen H.T. et al. (2017)
Male/female	≤ 65	Aerobic exercise, 60 min, 3 times a week, 26 weeks	↓ body fat mass ↑ PP = strength	Villareal D.T. et al. (2017)
Male/female	69.9 ± 5	Nordic walking, 60 min, twice a week, 12 weeks	↑ endurance ↓ body fat mass	Morat T. et al. (2017)

Note. ↑ – increase; ↓ – decrease; «=» – no changes; PP – physical performance; MB – mitochondrial biogenesis.

When properly approached, aerobic exercises improve muscle hypertrophy and strength among older people.

MULTIMODAL TRAINING

Currently, preference is given to complex, multimodal (combined) forms of exercise. This is because standard forms of physical therapy do not meet the criteria for therapeutic exercise for age-related sarcopenia treatment [50]. Multimodal exercise includes a combination of resistance training, cycling, aerobic training, balance training and other activities (Table 3). Furthermore, in addition to this effect, the combination of aerobic and resistance training can also promote fat mass loss, which is of great importance for sarcopenic obesity treatment [51].

There is no general opinion yet on the duration and frequency of multimodal exercises for older adults. If L.Y. Zhu et al. [52] used protocols of 40–50 minutes 3 times a week, L.Z. Wang et al. believe that 20 minutes 2 times a week is sufficient to obtain a positive effect [53].

D.T. Villareal et al. compared strength, aerobic and combined training in their study (160 participants, ≤ 65 years) and concluded that maximal strength increased statistically significantly in the resistance and combined groups (19 and 18 %, respectively; aerobic – 4 %). The time required to complete the obstacle course decreased more in the combined group than in the aerobic group (13 and 7 %, respectively). Gait speed increased more in the combined group (by 14 %) than in the aerobic group (by 9 %) [45].

Y.Q. Zhu et al. in their parallel study [54] used tai chi exercises and HIIT, proving the benefits of this method for older people with sarcopenia (2.4 ± 0.43 % increase in hand grip strength, 15.0 ± 3.2 % increase in quadriceps muscle strength). M.Y. Lee et al. reported that 12 weeks of combined training (resistance training and active recovery) improved walking and balance skills as well as isokinetic muscle contraction [55]. Many experts found important effects of multimodal programs on all indicators of sarcopenia among older people [56], focusing on sustained increases in muscle strength and physical performance [57–59].

There are many studies on high levels of performance following sessions of high-intensity interval training (HIIT), which provides intense cycles alternating with periods of low intensity for rehabilitation, providing physiological effects in less time, in contrast to normal training [60]. According to studies, HIIT showed similar or even higher effects compared to aerobic exercise in improving muscle strength, enhancing physical performance and increasing muscle mass among older adults [61].

The results of studies related to the benefits of HIIT for patients with sarcopenia are relatively recent. For example, at Shiraz University (Iran), Z. Hooshmandi et al. conducted a study on older women with sarcopenia using a HIIT

TABLE 3

VARIETIES OF MULTIMODAL SETS OF EXERCISES

Training method	Duration and frequency of training per week	Result	Author, publication year
AE + RT	20–50 min, 3–7 times a week, 3–6 months	↑ lean body mass ↓ body mass ↓ SPPB	Gudlaugsson J. et al. (2013) Jung W.S. et al. (2019) Zhu L.Y. et al. (2019)
RT + AR	80 min (RT – 20 min, AR – 60 min), 3 times a week, 12 weeks	↑ SPPB ↑ endurance	Li et al. (2020)
AE + RT + FT	60 min, week 1–8 – 3 times a week, week 9–24 – twice a week	↑ SPPB ↑ gait speed ↑ lean body mass	Liu C.K. et al. (2014)
RT + BCE	50–60 min, twice a week, 12 weeks	↑ muscle mass ↑ gait speed ↑ strength	Kim H. et al. (2013)
Tai chi + PE	20 min, 5 times a week, 8 weeks	↑ muscle mass ↑ hand grip strength	Zhu Y.Q. et al. (2019)
HIIT	5–10 min, 3 times a week, 2–4 months	↑ strength ↑ endurance	Hooshmandi Z. et al. (2021)

Note. ↑ – increase; ↓ – decrease; AE – aerobic exercise; RT – resistance training; AR – active recovery; FT – flexibility training; BCE – balance and coordination exercises; PE – passive exercises; SPPB – series of physical performance tests; HIIT – high-intensity interval training.

protocol. The study showed a significant decrease in body fat percentage, increase in hand grip strength and appendicular skeletal muscle mass ($p < 0.001$) in the experimental group compared to the control group [62]. In their publications, G. Panayiotou et al. reported that after two sessions of HIIT older people can perform various physical and even high-intensity exercises without prolonged adaptation and negative effects on muscle function [63]. The result is of great importance because these exercises may induce health-promoting effects that can improve quality of life of older adults.

Therefore, HIIT might become a promising method in combating age-related loss of muscle mass and function. However, it is worth noting that the benefit of HIIT for patients with sarcopenia is not yet fully studied, due to high-intensity exercises and questionable safety.

Multimodal training is an effective method of treatment for age-related sarcopenia. It combines different types of exercises and allows the individual characteristics of a particular person to be taken into account.

PASSIVE EXERCISES

The use of passive exercise is justified when patients with sarcopenia are unable to exercise. Whole-body vibration (WBV) for older adults has been shown to significantly improve various physical indicators including isometric leg strength, dynamic knee strength, number of repetitions in a squat and jump height [64]. Improvement in isokinetic testing performance among older adults during knee extension is achieved at an average frequency of 40 Hz for 360 seconds [65]. In addition, a 12-week course of WBV for older adults with sarcopenia aged ≥ 65 years (3 times a week for 60 seconds, 10 repetitions) can improve skeletal muscle mass, physical fitness and quality of life [66].

WBV may be a promising treatment to improve muscle strength, physical performance and skeletal muscle mass index among older people with sarcopenia [66]. However, after conducting a meta-analysis (7 studies, 223 participants) S. Wu et al. (China) believe that long-term course of WBV is not recommended because side effects such as premature wear-and-tear degeneration of tissues that form the vertebral articulations may occur, and the fact that the increase in serum testosterone and growth hormone levels does not have a firm evidence. The authors insist that more thorough studies with a larger sample size are needed to further investigate and confirm the benefits of WBV for older and senile people [67].

The results of literature reviews can also be contradictory. For example, L. Vlietstra et al. [68] found that older people with sarcopenia can significantly improve muscle mass with exercise, while W. Bao et al. [69] did not reach this conclusion in their study.

Sarcopenia is a disease that affects almost exclusively the older population and does not discriminate on the basis of gender, as men and women are equally affected. The obtained data suggest that exercise is significant for treatment and prevention of sarcopenia, effectively improving muscle function and physical performance of older adults [4, 14]. It is strongly recommended to exercise in groups, under the constant supervision of a specialist and/or health care professional. This is especially important during high-load and high-intensity resistance training and intensity, due to frequent chronic diseases and comorbid conditions among older and senile people [13, 70].

CONCLUSION

An increasing number of scientists realize the clinical importance of sarcopenia. Despite impressive advances in science and technology, effective treatments for sarcopenia are still unavailable, and the pathophysiological specificity of age-related loss of muscle mass is still being studied.

Nowadays, given the results of many studies and the fact that with proper medical supervision and self-monitoring, physical activity can be practiced throughout life. Lo/moderate-intensity resistance training and multimodal (complex) training are seen as a particularly effective countermeasure against sarcopenia.

Based on the results of the studies presented, it is important to determine the training method that is appropriate for the individual and prevent sarcopenia as early as possible.

Conflict of interest

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

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CARDIOLOGY

ANALYSIS OF CORONARY ARTERY LESION DEGREE AND RELATED RISK FACTORS IN PATIENTS WITH CORONARY HEART DISEASE

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ABSTRACT

Background. *The study of the association of risk factors and atherosclerotic burden assessed by coronary angiography is promising in terms of both understanding the pathogenesis of the disease and predicting its development.*

The aim of the study *was to investigate the relationship between traditional risk factors and the severity of coronary atherosclerosis in patients with stable CHD.*

Materials and methods. *Risk factors were studied in 100 patients who underwent angiography. Based on the Gensini (GS) score, participants were divided into groups: patients with moderate lesion of coronary arteries (GS = 8–39), with severe lesion (GS ≥ 40), and control group (GS = 0). To verify the association between the variables, Pearson's chi-square test was used. The results were presented as relative risk (RR) and the confidence interval (95% CI).*

Results. *It was found that in patients with GS score less than 40 points, statistically significant factors were hypertension (RR = 2.6; 95% CI: 1.023–10.09; p = 0.018), family history (RR = 2.94; 95% CI: 1.501–5.762; p < 0.001), depression (RR = 1.81; 95% CI: 1.202–2.738; p = 0.028), In patients with GS ≥ 40, the most important factors were diabetes (RR = 1.72; 95% CI: 1.187–2.511; p = 0,017), family history (RR = 2.02; 95% CI: 1.233–3.315; p = 0.002), inactivity (RR = 1.85; 95% CI: 1.219–2.824; p = 0.005). The GS scores were significantly higher in smokers compared non-smokers (44.0 vs. 32.0; p = 0.043).*

Conclusion. *The most significant influence on the development of coronary atherosclerosis is exerted by a family history and physical inactivity. Arterial hypertension and depression are associated with moderate coronary artery disease. Severe atherosclerosis is associated with diabetes mellitus, long smoking history, low levels of high-density lipoprotein cholesterol.*

Key words: *coronary heart disease, coronarography, Gensini score, risk factors*

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ФАКТОРЫ РИСКА И СТЕПЕНЬ ПОРАЖЕНИЯ КОРОНАРНЫХ АРТЕРИЙ У БОЛЬНЫХ С ИШЕМИЧЕСКОЙ БОЛЕЗНЬЮ СЕРДЦА

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РЕЗЮМЕ

Обоснование. *Изучение взаимосвязи факторов риска со степенью поражения коронарного русла по данным шкалы Gensini является ещё одним подходом в понимании как патогенеза атеросклероза коронарных артерий (КА), так и прогнозирования заболевания.*

Цель исследования. *Проанализировать связь факторов риска с тяжестью поражения КА у больных ИБС и определить ведущие факторы, влияющие на выраженность атеросклероза. Материалы и методы. Изучены факторы риска у 100 больных, которым была выполнена плановая коронароангиография. Количественная оценка атеросклероза проведена с использованием шкалы Gensini (GS). Обследуемые разделены на группы по медиане GS = 40 баллов: умеренного поражения КА (GS = 8–39), тяжёлого поражения (GS ≥ 40), интактные сосуды (GS = 0, n = 30). Сравнение качественных признаков проводилось с помощью критерия χ^2 Пирсона. Для оценки влияния факторов на индивидуальный риск рассчитывались показатель относительного риска (ОР) и 95%-й доверительный интервал (95% ДИ).*

Результаты. *Установлено, что у лиц с GS менее 40 баллов статистически значимыми факторами были артериальная гипертензия (ОР = 2,6; 95% ДИ: 1,023–10,09; p = 0,018), семейный анамнез (ОР = 2,94; 95% ДИ: 1,501–5,762; p < 0,001), депрессия (ОР = 1,81; 95% ДИ: 1,202–2,738; p = 0,028), а у пациентов с GS более 40 баллов – сахарный диабет (ОР = 1,72; 95% ДИ: 1,187–2,511; p = 0,017), семейный анамнез (ОР = 2,02; 95% ДИ: 1,233–3,315; p = 0,002), гиподинамия (ОР = 1,85; 95% ДИ: 1,219–2,824; p = 0,005). Показатели GS были выше у лиц, имевших длительный стаж курения, по сравнению с никогда не курившими (Me 44,0 против 32,0; p = 0,043).*

Заключение. *Наиболее значимое влияние на развитие коронарного атеросклероза оказывают семейный анамнез и гиподинамия. Артериальная гипертензия и депрессия взаимосвязаны с умеренным поражением коронарных артерий. С тяжёлым атеросклерозом ассоциированы сахарный диабет, длительный стаж курения, пониженный уровень холестерина липопротеидов высокой плотности.*

Ключевые слова: *ишемическая болезнь сердца, коронароангиография, шкала Gensini, факторы риска*

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INTRODUCTION

Cardiovascular diseases (CVDs) are the leading cause of death worldwide, the most common of which is coronary heart disease (CHD). As reported by the World Health Organization (WHO), mortality from CHD has increased 4-fold since 2000, reaching 8.9 million cases in 2019. [1]. In Russia, mortality from CVDs keeps its leading position despite improvements in therapeutic and surgical treatment.

The morphologic basis of CHD is atherosclerotic narrowing of the coronary arteries (CA). Stenosis degree, localization, extent of atherosclerotic plaque and the number of affected arteries influence the severity of angina pectoris. Nevertheless, according to the literature, no significant CA lesions were found in 20 % of patients with typical angina, indicating a microvascular form of angina [2].

In order to prevent the development and progression of CHD, risk factors (RF) continue to be actively studied. Lifestyle, environmental exposures and genetic factors are known to influence the onset and development of CHD. The association of such factors as smoking, arterial hypertension (AH), diabetes mellitus (DM), obesity and hypercholesterolemia with the development of CHD was established as early as the 1960s in the Framingham Heart Study. Currently, there are more than 200 cardiovascular RFs, among which modifiable and non-modifiable RFs are distinguished. The main modifiable factors are dyslipidemia, smoking, AH, obesity, psychosocial stress and DM. Non-modifiable factors include male gender, age, and family history of CVD. High prevalence of RFs is registered in Russia, and often one person has several of them, with varying degrees of severity. According to national studies, the incidence of AH in working-age people is 47.3 %, obesity – 26.9 % [3]. Smoking, despite decreasing rates in recent decades, remains at a high level and is, according to some data, 39 % in men and 13.6 % in women [4]. Low physical activity leading to increased CVDs is found in 38.8 % of the population [5]. Such RF as dyslipidemia occurs in half of the population [6], DM in 5.4 %, and carbohydrate tolerance disorders in 19.3 % of the population [7]. In recent decades, the psychological status influence problem on the course of CVD has attracted much attention. A significant number of patients with CHD have symptoms of anxiety and depression, which negatively affects the course and prognosis of the disease. According to the Russian KOMETA (Comet) study, in patients with CHD and/or AH, clinically significant anxiety is diagnosed in 25.5 %, pronounced depressive symptoms – in 16.3 % [8]. At the same time, there are studies in the literature that indicate that 20 % of cardiovascular events occur in the absence of traditional RFs [9].

Despite widespread preventive work among the population and the use of drug therapy, including statins, in patients with CHD, morbidity and mortality from CVD remain at a high level. In this regard, the study of independent predictors of obstructive lesion development in epicardial arteries remains relevant [10, 11].

THE AIM OF THE STUDY

To analyze the relationship between cardiovascular risk factors and obstructive coronary artery disease in CHD patients and to determine the leading factors affecting the severity of coronary atherosclerosis.

MATERIALS AND METHODS

The study was performed at the Medical Center of the Far Eastern Federal University (Vladivostok) in the design of a prospective comparative study in the period from January to November 2021. The study included 100 patients with CHD who underwent routine coronary angiography (CAG) to confirm the diagnosis and decide on surgical methods of myocardial revascularization. The study population included 61 males and 39 females and the mean age was 60.88 ± 7.59 years. According to the CAG results, the main group consisted of 70 patients with CA lesions, the comparison group – 30 patients without atherosclerotic vascular changes. The main and control groups did not differ in age and gender.

Inclusion criteria: patients diagnosed with stable CHD, with indications for diagnostic CAG, who signed informed consent.

Patients with acute coronary syndrome in the last 6 months, severe valvular heart disease, patients with chronic heart failure, low left ventricular ejection fraction according to echocardiography (< 35 % as per Simpson), with signs of severe hepatic and renal failure, oncological and inflammatory diseases were not included in the study.

All patients underwent clinical, laboratory and instrumental examinations in accordance with the standards of CHD diagnostics [12]. Cardiovascular risks were assessed according to the criteria of the national recommendations of the Society of Cardiology of Russian Federation [13]. The following factors were taken into account: gender, age, obesity (BMI ≥ 30 kg/m^2), family history of CVD (myocardial infarction or unstable angina in men aged < 55 years, in women < 60 years), smoking, presence of arterial hypertension (blood pressure ≥ 140/90 mm Hg or constant intake of antihypertensives), diabetes mellitus (fasting glucose > 6.1 and 7.0 mmol/l in capillary and venous blood, respectively), physical activity < 3.5 hours/week, psychosocial factors (anxiety-depressive symptoms), hypercholesterolemia (total cholesterol (TC) > 5 mmol/l or taking statins). The Hospital Anxiety and Depression Scale (HADS) questionnaire was used to identify anxiety and depressive symptomatology. The degree of symptom severity was expressed in scores: a total score within 8-10 points indicated subclinical anxiety/depression, more than 10 points – clinically pronounced anxiety/depression.

Coronary angiographic study was performed using radial access according to the Judkins technique and a Philips Allura Xper FD 20 machine (Philips Healthcare, USA). Angiograms were analyzed by two independent physician (X-ray surgeons) visually and automatically using Xcelera software (Philips Healthcare, USA). Hemodynamically signifi-

cant stenoses were considered to be narrowing of ≥ 50 % of the lumen diameter of the basilar arteries and/or the left main artery.

The Gensini score (GS) was used to quantify coronary atherosclerosis. The GS score was calculated according to the degree and localization of stenosis. Vessel diameter stenosis of 25 %, 50 %, 75 %, 90 %, 99 %, and complete occlusion were graded as 1, 2, 4, 8, 16, and 32 points, respectively. Further, the scores were multiplied by a coefficient calculated depending on the stenosis localization: the left main artery – 5; proximal segment of the left anterior descending artery (LAD) and proximal segment of the circumflex artery (Cx) – 2.5; middle segment of the left anterior descending artery – 1.5; right coronary artery, distal segment of the left anterior descending artery, posterior descending artery (PDA) and obtuse marginal artery – 1.0; other segments – 0.5. The GS index was calculated as the sum of severity productions of each stenosis expressed in points multiplied by the coefficient calculated for each CA segment [14].

Laboratory studies included determination of lipid spectrum parameters and glucose level in blood serum using Randox enzyme kits (Ireland) and Sapphire-500 biochemical analyzer.

The study was conducted in accordance with the requirements of the WMA Declaration of Helsinki (revision 2013), approved by the local ethical committee (minutes No. 10 of 28.12.2020). All subjects signed a voluntary informed consent.

Statistical processing of materials was performed using IBM SPSS Statistics 26.0 software (StatSoft Inc., USA). Quantitative variables are presented in the text as: $M \pm \sigma$, where M is the mean, σ is the standard deviation (in case of normal data distribution), and *Me* (Q1; Q3), where *Me* is the median, Q1 is the lower quartile, Q3 is the upper quartile (in case of non-normal distribution). Normality of distribution of quantitative signs was assessed using Kolmogorov – Smirnov, Shapiro – Wilk tests and graphical representation of histograms. Nominal (qualitative) values are given in absolute numbers (*n*) and percentages (%). The Kruskal – Wallis test was used for comparison of three independent samples of quantitative indicators, and the Bonferroni-corrected Mann – Whitney test was used for pairwise post hoc groups comparisons. Differences between the qualitative attributes of the two groups were determined using Pearson's chi-square test (χ^2). Assessment of the studied factors influence on the individual risk of coronary lesions was calculated as relative risk score (RR) and 95 % confidence interval (95% CI). Informative features with a CI value greater than 1.0 were considered to be the most statistically significant factors. Differences were considered statistically significant at $p < 0.05$.

RESULTS

In the study cohort, coronary atherosclerosis was assessed using the GS score and the number of affected vessels was considered. Hemodynamically significant stenoses were diagnosed in 70 patients. The nature of CA lesions in the subjects is shown in Table 1.

The lesion in one artery system was detected in 23 (32.9 %) patients, two arteries – in 21 (30.0 %) and three arteries – in 26 (37.1 %) patients. The GS index ranged from 8 to 160 points, the median was 40.0 (20.0; 62.5) points, the value obtained was taken as a cut-off point to divide patients into moderate and severe coronary atherosclerosis groups. According to the obtained angiographic data, three study groups were formed: Group 1 (comparison group) – GS = 0 points ($n = 30$), Group 2 (group of moderate CA lesions) – GS = 8–39 points ($n = 33$), group 3 (group of severe CA changes) – GS ≥ 40 points ($n = 37$).

The moderate CA lesion group (GS = 8-39) was mainly represented by patients with single- and double-vessel changes, having stenoses of 50–90 %. The group with severe lesions (GS ≥ 40) included patients with two- and three-vessel lesions, with ≥ 91 % stenoses.

The incidence of cardiovascular factors in the subjects is presented in Table 2. Comparative analysis showed a high

TABLE 1

CORRELATION BETWEEN THE DEGREE OF CORONARY ARTERY STENOSIS, THE SEVERITY OF CORONARY ATHEROSCLEROSIS ACCORDING TO THE GENSINI SCORE, AND THE NUMBER OF AFFECTED ARTERIES

Coronary artery stenosis degree, %	Gensini index, Me (Q1; Q3)			
	40.0 (20.0; 62.5)	16.0 (12.0; 24.0)	35.5 (24.5; 42.5)	63.0 (47.5; 84.5)
	Main cohort ($n = 70$)	Single-vessel disease ($n = 23$)	Double vessel disease ($n = 21$)	Three-vessel disease ($n = 26$)
50–75	1 (1.4 %)	1 (4.3 %)	–	–
76–90	51 (74.3 %)	21 (91.3 %)	14 (66.7 %)	16 (61.5 %)
≥ 91	18 (24.3 %)	1 (4.3 %)	7 (33.3 %)	10 (38.5 %)

TABLE 2

THE PREVALENCE OF RISK FACTORS IN PATIENTS WITH CORONARY ARTERY DISEASE DEPENDING ON THE SEVERITY OF CORONARY ARTERIES LESION

Risk factors	GS index, points			Statistical significance level, *p*
	Group 1: GS = 0 (*n* = 30)	Group 2: GS = 8–39 (*n* = 33)	Group 3: GS ≥ 40 (*n* = 37)	
Male gender, *n* (%)	18 (60 %)	18 (54.5 %)	26 (70.3 %)	$p_{1-2} = 0.490$ $p_{1-3} = 0.945$ $p_{2-3} = 0.177$
Age, $M \pm \sigma$	60.5 ± 9.78	61.48 ± 5.63	60.32 ± 7.78	$p_{1-2} = 0.490$ $p_{1-3} = 0.945$ $p_{2-3} = 0.319$
Obesity, *n* (%)	9 (30.0 %)	11 (33.3 %)	15 (40.5 %)	$p_{1-2} = 0.111$ $p_{1-3} = 0.371$ $p_{2-3} = 0.536$
Family history of CVD, *n* (%)	9 (30 %)	25 (75.8 %)	25 (67.6 %)	**$p_{1-2} < 0.001$** **$p_{1-3} = 0.003$** $p_{2-3} = 0.452$
Smoking, *n* (%)	6 (20.0 %)	8 (24.2 %)	6 (16.2 %)	$p_{1-2} = 0.796$ $p_{1-3} = 0.690$ $p_{2-3} = 0.405$
Previously smoking, *n* (%)	8 (26.7 %)	7 (21.2 %)	18 (48.6 %)	$p_{1-2} = 0.615$ $p_{1-3} = 0.068$ **$p_{2-3} = 0.022$**
Low physical activity, *n* (%)	6 (20 %)	15 (45.9 %)	20 (54.1 %)	**$p_{1-2} = 0.026$** **$p_{1-3} = 0.005$** $p_{2-3} = 0.474$
AH, *n* (%)	20 (66.7 %)	30 (90.9 %)	32 (86.5 %)	**$p_{1-2} = 0.018$** $p_{1-3} = 0.055$ $p_{2-3} = 0.564$
DM, *n* (%)	3 (10 %)	5 (15.2 %)	13 (35.1 %)	$p_{1-2} = 0.543$ **$p_{1-3} = 0.017$** $p_{2-3} = 0.058$
Anxiety, *n* (%)	6 (20 %)	8 (24.2 %)	9 (24.3 %)	$p_{1-2} = 0.688$ $p_{1-3} = 0.675$ $p_{2-3} = 0.994$
Depression, *n* (%)	2 (6.7 %)	9 (27.3 %)	5 (13.5 %)	**$p_{1-2} = 0.028$** $p_{1-3} = 0.366$ $p_{2-3} = 0.153$
RF number, *Me* (Q1; Q3)	4.0 (3.0; 4.25)	6.0 (5.0; 6.5)	6.0 (4.5; 7.0)	**$p_{1-2} < 0.001$** **$p_{1-3} < 0.001$** $p_{2-3} = 0.772$

Note. Statistical significance of differences between groups was performed by Mann – Whitney and Pearson's chi-square test (χ^2); p_{1-2}, p_{1-3}, p_{2-3}, statistical significance of differences between groups.

prevalence of factors in patients with obstructive vascular lesions. In Group 2 (GS = 8–39), the occurrence of CVD family history was 2.5 times ($p < 0.001$), hypodynamia – 1.5 times ($p = 0.026$), AH – 1.4 times ($p = 0.018$), and depressive disorders – 4.1 times ($p = 0.028$) higher than in the experimental (comparison) group. In Group 3 (GS ≥ 40), there was a 2.25-fold ($p = 0.003$) increase in the prevalence of family history, 2.7-fold ($p = 0.005$) increase in hypodynamia, and 3.5-fold ($p = 0.017$) increase in DM relative to the comparison group. The number of RFs per patient in the groups with CA lesions (Groups 2 and 3) was 1.5 times higher than in the experimental group ($p < 0.001$).

RF analysis of Groups 2 and 3 revealed statistical differences in the number of patients who quit smoking. Thus, the group with a GS score of ≥ 40 had 2.3 times more individuals who quit smoking relative to the group with GS = 8–39 points ($p = 0.022$). In addition, patients with a history of smoking had statistically significantly higher GS index scores than never smokers (median GS score – 44.0 vs. 32.0; $p = 0.043$).

In the study, there were no statistically significant differences between groups in the prevalence of a factor such as obesity. Nevertheless, the incidence of this factor was high, in the groups with CA lesions (Groups 2 and 3) it was 33.3 % and 40.5 %, respectively, and in the comparison group it was 30 %.

Evaluation of blood biochemical parameters in the subjects revealed differences in blood glucose and high-density lipoprotein (HDL) cholesterol levels. Glucose levels were higher in Group 3 relative to the comparison group ($p < 0.001$) and Group 2 ($p = 0.008$). HDL cholesterol values were statistically significantly lower in Group 3 than in Group 2 ($p = 0.025$) and the comparison group ($p = 0.003$). Lipid profile and carbohydrate metabolism data of patients are given in Table 3.

To identify the most statistically significant factors in CHD patients influencing the development of coronary atherosclerosis, the relative risk (RR) was calculated. Table 4 shows only the RRs with lower and upper confidence interval values greater than 1.

In patients with CHD the risk of moderate atherosclerosis increases 2.9 times in the presence of family history of CVD, 2.6 times in the presence of AH, 1.7 times in the presence of low physical activity, 1.8 times in the presence of depression in relation to individuals without these factors. In addition to family history and hypodynamia, DM increases the risk of severe CA lesions by 1.7 times. In addition, the combination of ≥ 5 RFs in one patient indicates a high probability of obstructive coronary lesions.

TABLE 3

LIPID PROFILE AND BLOOD GLUCOSE LEVEL IN PATIENTS WITH CORONARY ARTERY DISEASE DEPENDING ON CORONARY ARTERIES LESION, *ME* (Q1; Q3)

Blood values	GS index, points			Statistical significance level, *p*
	Group 1: GS = 0 (*n* = 30)	Group 2: GS = 8–39 (*n* = 33)	Group 3: GS ≥ 40 (*n* = 37)	
Glucose, mmol/L	5.2 (4.9; 5.8)	5.9 (5.25; 6.65)	6.7 (5.65; 9.05)	**$p_{1-2} = 0.018$** **$p_{1-3} < 0.001$** ***$p_{2-3} = 0.008$***
TC, mmol/L	4.9 (4.4; 5.72)	4.59 (3.77; 5.07)	4.23 (3.47; 5.27)	$p_{1-2} = 0.205$ $p_{1-3} = 0.064$ $p_{2-3} = 0.533$
TG, mmol/L	1.35 (1.05; 2.43)	1.3 (0.94; 1.85)	1.26 (0.89; 1.87)	$p_{1-2} = 0.397$ $p_{1-3} = 0.228$ $p_{2-3} = 0.737$
HDL cholesterol, mmol/L	1.26 (1.11; 1.53)	1.36 (1.02; 1.46)	1.09 (1.0; 1.25)	$p_{1-2} = 0.804$ **$p_{1-3} = 0.003$** **$p_{2-3} = 0.025$**
LDL cholesterol, mmol/L	2.9 (2.26; 3.57)	2.64 (2.12; 3.1)	2.58 (1.93; 3.28)	$p_{1-2} = 0.259$ $p_{1-3} = 0.177$ $p_{2-3} = 0.707$
The Atherogenic Index (AI), c.u.	3.90 (3.4; 4.72)	3.59 (2.77; 4.07)	3.23 (2.47; 4.19)	$p_{1-2} = 0.173$ **$p_{1-3} = 0.034$** $p_{2-3} = 0.437$

TABLE 4

INFLUENCE OF RISK FACTORS ON VARYING DEGREES OF CORONARY ARTERY DISEASE

Risk factors	GS index, points	Statistical significance level, p	RR	95% CI
Family history of CVD	8–39	< 0.001	2.94	1.501–5.762
	≥ 40	0.002	2.02	1.233–3.315
Low physical activity	8–39	0.026	1.72	1.095–2.711
	≥ 40	0.005	1.85	1.219–2.824
Arterial hypertension	8–39	0.018	2.6	1.023–10.09
Depression	8–39	0.028	1.81	1.202–2.738
Diabetes mellitus	≥ 40	0.017	1.72	1.187–2.511
Number of risk factors	8–39	< 0.001	3.28	1.50–7.14
	≥ 40	< 0.001	3.47	1.55–7.78

Note. Only statistically significant associations between factors and outcome are presented (confidence interval of at least 1).

DISCUSSION

To study the relationship between CVD factors and the severity of coronary atherosclerosis lesions, CAG data were analyzed using GS score and determination of cut-off point by the median of the study sample, which allows more accurately distinguishing patients with different degrees of coronary lesions. This approach of dividing groups by median is widely used in clinical trials [15].

According to the data obtained, independent predictors of hemodynamically significant CA stenoses were determined in CHD patients. One of the main factors is family history of CVD, which increases the probability of coronary atherosclerosis in CHD patients more than 2 times. According to various sources, the contribution of genetic factor to the development of CVD is from 30 to 80 % [16]. However, its role is not fully clear. A number of researchers have pointed out the need to use genetic risk scales to predict the development of CHD. Researchers' opinions on this issue are contradictory. For example, it is known that in individuals with increased genetic risk of CVD development prophylactic correction of traditional modifiable RFs (smoking, AH, dyslipidemia) reduces the total risk of CHD. The study of hereditary factors is of practical interest in terms of individualized prevention.

One of the most common risk factors in the world is AH, which is associated with a high risk of cardiovascular complications: myocardial infarction and cerebral stroke. According to the study, it was found that patients with AH have a 2.5-fold increased risk of developing CA lesions. Persistently elevated BP is known to contribute to the development of atherosclerosis through autonomic dysregulation of vascular endothelial function. The presence of common pathogenetic mechanisms has a mutual influence and leads to the progression of CHD and the development of cardiovascular complications. For instance, myocardial hypertrophy and elevated BP cause coronary insufficiency even in moderate atherosclerotic lesions of CA.

The study found that individuals with low physical activity have more than a 1.5-fold increased risk of developing CHD. In modern living conditions, physical activity is drastically reduced, especially in the developed countries. Hypodynamia is associated with obesity, impaired carbohydrate and lipid metabolism. The issue of considering the inclusion of low physical activity as a significant RF in prognostic scores for risk stratification is relevant.

Among the investigated factors with moderate atherosclerotic lesions of CA, statistically and clinically significant were found to be an aggravated family history of CVD, AH and hypodynamia. DM and hyperglycemia are asso-

ciated with severe coronary lesions. As is known, against the background of chronic hyperglycemia there is a violation of oxidation process in cells, accumulation of free radicals leading to endothelial dysfunction, which leads to a more severe course of CHD. Numerous studies support the association between chronic hyperglycemia and the development of adverse cardiovascular outcomes. According to the results of this study, patients with DM are 1.7 times more likely to develop severe vascular damage to the heart.

Smoking is one of the key RFs influencing the development of multivessel coronary artery disease. Nicotine is known to negatively affect the sympathoadrenal system, increases platelet aggregation, and raises blood lipids. Smoking cessation is the most effective secondary prevention intervention that leads to reduced risk of CVD progression and mortality. According to M.S. Duncan et al., 5 years after quitting smoking, the risk is significantly reduced compared to those who continue to smoke, but approaches the level of never smokers only after 10–15 years. [17]. According to the results of this study, the GS index scores of past smokers were statistically significantly higher than those of never smokers. Despite smoking cessation, a long history of the habit was associated with multivessel severe arterial heart disease.

Dyslipidemia plays a key role in CA atherosclerosis development. When analyzing the blood lipid spectrum in the study in patients with severe CA lesions, HDL cholesterol indices were statistically significantly lower than in the group with moderate lesions. Low HDL levels are known to contribute to accelerated development of atherosclerosis and are associated with high cardiovascular risk. Their favorable role in protecting the endothelium from damage has been shown. However, the influence of such factors as smoking, AH, DM, age, and hypercholesterolemia leads to reduction of their protective properties [18]. The further investigation of factors leading to HDL biological function changes shall help in the field of prognostic evaluation of cardiovascular risks.

A high prevalence of anxiety-depressive symptomatology in CHD patients was determined. However, only depression has an impact on the development of moderate severity coronary atherosclerosis. In this study, depressive disorders were more common in women over 65 years of age and were associated with low physical activity and obesity. The results can be compared with the Russian KOMETA (Comet) study data, in which a high prevalence of depression, mostly in women, has been shown. Unfavorable psychological background was associated with such RF as hypodynamia and higher BMI values.

One important finding of the study is the association between the presence of coronary atherosclerosis and the number of combined RFs. The presence of five or more RFs in one patient has the greatest impact on coronary lesions. RF combination should be considered as a cumulative effect that has an unfavorable impact on the CHD course and prognosis. Various scores for calculation total risk are used nowadays, with different prognostic value for risk stratification in patients with CHD.

CONCLUSION

Thus, the cardiovascular risk factors analysis in CHD patients showed that family history of CVD and low physical activity are independent predictors of obstructive coronary atherosclerosis development. AH and depression are correlated with moderate coronary lesions. The main factors that have the strongest influence on the development of diffuse severe coronary atherosclerosis are DM, hyperglycemia, a long history of smoking, and a reduced HDL cholesterol level. The cumulative effect of several cardiovascular risk factors increases 3-fold the likelihood of developing obstructive coronary atherosclerosis. The obtained data indicate that the risk of CHD should be reduced both with the use of drug therapy and preventive measures taking into account cardiovascular and psychosocial factors.

Conflict of interest

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

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LECTURES

PREVENTION OF PERIOPERATIVE ISCHEMIC STROKE AFTER NON-CARDIAC AND NON-NEUROSURGICAL OPERATIONS IN THE LIGHT OF THE SCIENTIFIC STATEMENT AND GUIDELINES FOR THE SECONDARY PREVENTION OF ISCHEMIC STROKE AND TRANSIENT ISCHEMIC ATTACK AHA/ASA 2021. PART 1: DEFINITION, RISK FACTORS, PATHOGENESIS, PROGNOSIS, PRINCIPLES OF PRE- AND INTRAOPERATIVE PREVENTION

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ABSTRACT

Perioperative ischemic stroke is a potentially fatal complication that greatly increases the risk of poor outcome in surgical patients. Despite the relatively low prevalence among patients undergoing non-cardiosurgical and non-neurosurgical interventions (about 0.1–1.0 %), the total number of annually developing perioperative ischemic strokes in patients of this profile is high due to the large number of operations performed in the world. Since the publication in 2014 of the last fundamental work on the prevention of perioperative stroke, approaches to primary and secondary prevention, diagnosis, conservative and reperfusion treatment of ischemic stroke have been seriously modified. The numerous changes that have taken place have created the prerequisites for revising existing approaches to providing care for perioperative ischemic stroke. In 2021, updated documents of foreign researchers/associations on the problem of perioperative ischemic stroke in non-cardiac and non-neurosurgical patients were published. This review, which consists of two parts, presents current data that summarizes the most relevant information on this topic. The first part of the review outlines the general provisions on perioperative ischemic stroke (definition, risk factors, pathogenesis, predictive models), strategies for pre- and intraoperative prevention.

Key words: *perioperative stroke, non-cardiac and non-neurological surgery, perioperative complications, preoperative preparation, asymptomatic cerebral infarcts, intraoperative prevention*

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ПРОФИЛАКТИКА ПЕРИОПЕРАЦИОННОГО ИШЕМИЧЕСКОГО ИНСУЛЬТА ПОСЛЕ НЕКАРДИОХИРУРГИЧЕСКИХ И НЕНЕЙРОХИРУРГИЧЕСКИХ ОПЕРАЦИЙ В СВЕТЕ НАУЧНОГО ЗАЯВЛЕНИЯ И РЕКОМЕНДАЦИЙ ПО ВТОРИЧНОЙ ПРОФИЛАКТИКЕ ИШЕМИЧЕСКОГО ИНСУЛЬТА И ТРАНЗИТОРНОЙ ИШЕМИЧЕСКОЙ АТАКИ AHA/ASA 2021 г. ЧАСТЬ 1: ОПРЕДЕЛЕНИЕ, ФАКТОРЫ РИСКА, ПАТОГЕНЕЗ, ПРОГНОЗИРОВАНИЕ, ПРИНЦИПЫ ПРЕД- И ИНТРАОПЕРАЦИОННОЙ ПРОФИЛАКТИКИ

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РЕЗЮМЕ

Периоперационный ишемический инсульт является потенциально смертельным осложнением, многократно увеличивающим риск неблагоприятного исхода у пациентов хирургического профиля. Несмотря на относительно низкую распространённость среди пациентов, подвергающихся некардиохирургическим и ненейрохирургическим вмешательствам (около 0,1–1,0 %), общее число ежегодно развивающихся периоперационных ишемических инсультов у пациентов данного профиля является высоким ввиду большого числа выполняемых в мире операций. С момента опубликования в 2014 г. последней фундаментальной работы на тему профилактики периоперационного инсульта подходы к первичной и вторичной профилактике, диагностике, консервативному и реперфузионному лечению ишемического инсульта были серьёзно модифицированы. Произошедшие многочисленные изменения создали предпосылки к пересмотру существующих подходов к оказанию помощи при периоперационном ишемическом инсульте. В 2021 г. в свет вышли обновлённые документы зарубежных исследователей/ассоциаций, посвящённые проблеме периоперационного ишемического инсульта у пациентов некардиохирургического и ненейрохирургического профиля. В настоящем обзоре, состоящем из двух частей, представлены современные данные, обобщающие наиболее актуальную информацию по данной теме. В первой части обзора изложены общие положения о периоперационном ишемическом инсульте (определение, факторы риска, патогенез, модели прогнозирования), стратегии пред- и интраоперационной профилактики.

Ключевые слова: *периоперационный инсульт, некардиохирургическое и ненейрохирургическое оперативное вмешательство, периоперационные осложнения, предоперационная подготовка, асимптомный церебральный инфаркт, интраоперационная профилактика*

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INTRODUCTION

Perioperative ischemic stroke (PS) is a potentially fatal complication in patients undergoing any surgical intervention, multiplying the risk of unfavorable outcome many times over. For example, after non-cardiac and non-neurosurgical surgery, the 30-day risk of mortality increases 8–10-fold with absolute mortality rates of 21 to 26 % [1–3]. Despite the fact that historically the problem of PS became the most obvious in cardiovascular surgery due to the relatively high prevalence of this complication in cardiac surgery patients (2–10 % depending on the type of surgery [4]), at present the task of PS prevention and treatment is extremely relevant for patients of any surgical profile. Although the proportion of PSs in patients undergoing non-cardiac and non-neurological interventions is relatively low (0.1 % to 1.0 % depending on the type of surgery; see Table 1), the total number of PSs developing annually in this category of patients is high due to the large number of operations performed worldwide and represents a significant public health burden. More than 250 million surgical procedures are performed annually worldwide [3]; major surgeries are performed annually in 4 % of the world's total population [5]. Given a total European population of more than 500 million, the annual number of major interventions is estimated at 19 million, with 5.7 million surgeries (30 %) performed in patients at increased risk of cardiac complications. In the United States, more than 5 million patients over 45 years of age undergo non-cardiac surgery each year, with an estimated number of PSs of more than 25,000 cases per year [3]. In Russia, there has also been a steady increase in the number of surgeries performed annually over the last few years: in 2005 – 8,735 thousand, in 2010 – 9,277 thousand, in 2015 – 9,882 thousand, in 2016 – 9,974 thousand, in 2017 – 9,943 thousand, in 2018 – 10,020 thousand [6]; and the estimated number of PSs in Russia may be as high as 15,000–20,000 per year. PSs increase the length of stay and the probability of discharge to a long-term care facility [2, 7, 8].

PS prognosis, prevention, and treatment are currently receiving considerable attention. The greatest focus has traditionally been on investigating the issues and reducing the risks of PS in cardiac surgical patients. As of publishing the article, the main document on PS prevention among patients in this category is Scientific Statement from the American Heart Association 2020: Considerations for Reduction of Risk of Perioperative Stroke in Adult Patients Undergoing Cardiac and Thoracic Aortic Operations [9]. The basic document covering the problem of PS in non-cardiac and non-neurological patients for a long time was Perioperative Care of Patients at High Risk for Stroke during or after Non-Cardiac, Non-Neurologic Surgery: Consensus Statement from the Society for Neuroscience in Anesthesiology and Critical Care edited by G.A. Mashour et al. [10]. In 2021, a new American Heart Association/American Stroke Association (AHA/ASA) scientific statement, «Perioperative Neurological Evaluation and Management to Lower the Risk of Acute Stroke in Patients Undergoing Noncardiac, Nonneurological Surgery» was published summarizing current thinking about the problem of PS in patients after non-cardiac and non-neurological surgeries. Furthermore, new AHA/ASA Guidelines for the Secondary Prevention of Ischemic Stroke and Transient Ischemic Attack [11] were published in 2021, complementing the AHA/ASA Scientific Statement 2021 in terms of PS prevention issues. The multi-part review presents data summarizing information from these two documents. The first part of the review outlines the general background of PS (definition, risk factors, pathogenesis, prediction models, preoperative and intraoperative prevention strategies). The following parts of the review will outline the current principles of antithrombotic therapy in the perioperative period, diagnosis and treatment of PS.

Definition and risks of perioperative ischemic stroke. Perioperative stroke can be defined as any embolic, thrombotic, or hemorrhagic cerebrovascular event with motor, sensory, or cognitive dysfunction lasting at least 24 hours, occurring during surgery or within 30 days of surgery. As with non-perioperative cerebrovascular events, most PSs are ischemic rather than hemorrhagic. The incidence of PS in patients undergoing non-cardiac, non-neurosurgical surgeries ranges from 0.1 % to 1.0 %, according to the following retrospective studies [10] (Table 1).

Using the US National (Nationwide) Inpatient Sample (NIS) from 2004 to 2013, N.R. Smilowitz et al. reported that despite an overall decrease in the composite of major adverse cardiovascular and cerebrovascular events in non-cardiac surgery, the incidence of PS increased during this period from 0.52 % in 2004 to 0.77 % in 2013. A trend toward increased PS risk was evident for both men and women and among different races and ethnic groups. Of note, all of these studies did not independently clinically evaluate patients with PS, nor did they always use magnetic resonance imaging (MRI) to accurately temporally assess the ischemic event, so the true number of PSs may be higher or lower than in the data reported by the investigators [12].

Common risk factors for perioperative stroke. Numerous studies have consistently identified elderly age, kidney disease, and prior transient ischemic attack/stroke as key risk factors for PS. Risk factors such as myocardial infarction within the previous 6 months, atrial fibrillation, arterial hypertension, chronic obstructive pulmonary disease, current smoking, female gender, and diabetes mellitus were also identified as independent, further increasing the risk of PS. There is evidence that patients who underwent emergency surgery or certain types of surgery (head and neck, thoracic, intra-abdominal, vascular, transplant, orthopedic surgeries) were at higher risk of developing PS [12].

Asymptomatic cerebral infarct. Asymptomatic cerebral infarcts, sometimes referred to in the English-language literature as covert or silent strokes, are acute cerebral ischemic events without clinical manifestations. Cerebral infarctions are usually detected by neuroimaging techniques

TABLE 1

THE INCIDENCE OF PERIOPERATIVE ISCHEMIC STROKE AFTER NON-CARDIAC AND NON-NEUROSURGICAL OPERATIONS

Types of surgeries	PS, all patients, % (*n*)	PS, patients of or over 65 years of age, % (*n*)
Bateman B.T. et al. (2009); Nationwide Inpatient Sample		
Hip replacement surgery (*n* = 1,568)	0.4 (6)	0.5 (5)
Lung resection (*n* = 1,484)	0.3 (5)	0.7 (5)
Colon resection (*n* = 33,426)	0.4 (130)	0.7 (100)
Mashour G.A. et al. (2011); American College of Surgeons–National Surgical Quality Improvement Program		
Bile duct surgery (*n* = 43,289)	0.1 (36)	0.2 (23)
Breast removal surgery (*n* = 36,793)	0.0 (16)	0.1 (11)
Hernia surgery (ventral/umbilical/postoperative/other) (*n* = 32,638)	0.1 (28)	0.3 (21)
Inguinal hernia surgery, including usage of surgical mesh made from plastic (*n* = 26,448)	0.1 (17)	0.1 (10)
Appendectomy (*n* = 26,046)	0.0 (6)	0.2 (4)
Bariatric esophageal and gastric surgeries (*n* = 23,766)	0.0 (5)	0.0 (0)
Extracerebral and neck tumors (*n* = 20,057)	0.0 (7)	0.1 (3)
Thoracic/femoral small vessel surgery (*n* = 5,883)	0.0 (2)	0.1 (1)
Small intestine – resection/stoma (*n* = 5,860)	0.5 (27)	0.6 (14)
Small intestine – lysis of adhesions, others (*n* = 5,683)	0.3 (17)	0.7 (14)
Diagnostic studies of abdominal organs (*n* = 5,760)	0.5 (26)	0.9 (18)
Surgical management of pancreatitis (*n* = 4,832)	0.3 (15)	0.5 (10)
Limb amputation (*n* = 4,800)	0.8 (37)	1.1 (29)
Gastric surgeries (*n* = 4,749)	0.3 (16)	0.7 (12)
Esophageal surgeries (*n* = 4,635)	0.0 (1)	0.1 (1)
Hysterectomy (*n* = 4,454)	0.1 (3)	0.2 (2)

TABLE 1 (*continued*)

Types of surgeries	PS, all patients, % (*n*)	PS, patients of or over 65 years of age, % (*n*)
Arthroscopy (*n* = 4,255)	0.0 (0)	0.0 (0)
Spinal surgeries (*n* = 3,480)	0.1 (4)	0.3 (3)
Abdominoperineal resection (*n* = 3,169)	0.0 (0)	0.5 (5)
Knee surgeries (*n* = 2,970)	0.1 (4)	0.2 (4)
Anorectal abscess (*n* = 2,508)	0.0 (0)	0.0 (0)
Simple skin and soft tissue surgeries (*n* = 2,383)	0.3 (6)	0.6 (4)
Coloanal anastomosis (*n* = 2,293)	0.2 (4)	0.2 (2)
Liver surgeries (*n* = 2,144)	0.3 (6)	0.8 (6)
Anorectal resection (*n* = 2,103)	0.0 (1)	0.0 (0)
Surgeries for bone fractures (*n* = 2,065)	0.1 (3)	0.3 (3)
Skin and soft tissue biopsies (*n* = 2014)	0.1 (2)	0.2 (1)

and are catamnestically associated with the development of cognitive impairment, dementia, increased risk of recurrent stroke, and increased mortality. The incidence of perioperative asymptomatic cerebral infarcts varies according to the type of surgery and is probably higher in patients undergoing vascular or cardiac surgery. The detection rate of asymptomatic cerebral infarcts after carotid endarterectomy (CEA) can be as high as 17 %, and 30–50 % after internal carotid artery (ICA) stenting or cardiac surgery. The development of asymptomatic cerebral infarcts after ICA stenting was also associated with an increased risk of recurrent cerebral ischemic events, with this risk increasing with the number of areas of silent infarctions. According to a prospective multicenter pilot study by M. Mrkobrada et al. (2016), in 100 patients over 65 years of age after non-cardiac surgery, the incidence of asymptomatic cerebral infarcts based on postoperative MRI was 10 %. In the larger multicenter prospective Neurovision study, asymptomatic cerebral infarct was diagnosed in 7 % of patients based on routine MRI scans of 1,114 patients performed on days 2–9 after elective non-cardiac surgery. Among these patients, the risk of cognitive decline during 1-year follow-up was almost 2-fold higher compared with patients without evidence of asymptomatic cerebral damage. The incidence of perioperative delirium was also higher in the perioperative asymptomatic cerebral infarct group [12].

Pathogenesis of perioperative ischemic stroke. In patients undergoing cardiac surgery, nearly 2/3 of ischemic strokes are the result of emboli from proximal sources: either as a result of direct manipulation of the heart/major arteries or intraoperative performance of the bypass pump, or as a result of the development of delayed complications such as atrial fibrillation or myocardial infarction (MI). In patients who have undergone non-cardiac surgery or intervention on great vessels, the cause of PS is less clear. Stroke subtypes were not established in most studies because many of the earlier studies did not include advanced diagnostic tests such as MRI or vascular imaging. PS mechanisms suggested for patients undergoing non-cardiac and non-neurosurgical operations may include hypotension/low blood flow states, previously undetected large artery stenosis, tissue hypoxia associated with anemia, thromboembolism (including cardiac and transcardiac), fat embolism, and increased coagulation/thrombosis in the setting of systemic inflammation, endothelial dys-

function, and discontinuation of antithrombotic drugs preoperatively [12].

Perioperative stroke risk stratification. Numerous cardiovascular risk stratification tools have been used to predict perioperative complications, including the Revised Cardiac Risk Index (RCRI), the MI or Cardiac Arrest Calculator, and the American College of Surgeons Surgical Risk Calculator (ACS-SRC), but all of these tools have not been developed specifically for PS risk prediction. Two other risk assessment scales, CHADS2 and CHA2DS2-VASc, were originally developed and validated to predict the annual risk of stroke in patients with nonvalvular atrial fibrillation; however, it has been shown that they predict the perioperative risk of stroke in cardiac surgery patients even in the absence of atrial fibrillation.

The effectiveness of these tools in PS risk stratification was retrospectively evaluated by ACS investigators in a large cohort of patients ($n = 540,717$) undergoing noncardiac surgery using the US National Surgical Quality Improvement Program registry. The proportion of patients with PS in the studied sample was 0.27 %, and the highest frequency was observed in patients undergoing vascular surgery or neurosurgery. The ACS surgical risk calculators and the MI/cardiac arrest risk calculator demonstrated greater high predictive accuracy than other risk prediction models, even though they were not designed to predict stroke risk. The AHA/ASA 2021 Scientific Statement recommends a uniform approach for identifying patients at increased risk of PS using the ACS web-based surgical risk calculator [13]. It would be advisable to discuss the results of the prognosis with patients to inform them about the risks and to make joint decisions about surgery, with the caveat that this calculator, although not directly predicting the risk of stroke, can identify patients at high risk of serious complications in general, including those with a high probability of stroke in the perioperative period [12].

Timing of surgical interventions after previous stroke. Patients with previous ischemic stroke are at increased risk of PS. In this case, the risk of PS depends on the length of time between the history of stroke and the surgery performed. In 2014, M. Jørgensen et al. analyzed the data of the Danish National Patient Registry and reported an increased risk of ischemic stroke and other serious adverse cardiovascular events (MI and death due to other cardiovascular causes) in patients who underwent non-cardiac surgery after a previous stroke (Table 2) [12].

Patients who underwent elective non-cardiac surgery within 3 months of stroke had the highest risk of ischemic stroke. Although the risk of serious adverse cardiovascular and cerebrovascular events, 30-day mortality, and ischemic stroke was higher in patients undergoing non-cardiac surgery within 12 months of a previous stroke overall, the increased odds ratio for each of these end points leveled off by about month 9. Using the same data, these authors also showed that patients who underwent emergency non-cardiac surgery within 3 months of a previous stroke were more than 20 times more likely to develop PS. Similar temporal trends in PS risk have been observed after stroke in patients who did not undergo surgery, but the absolute event rates in these studies of perioperative patients were higher than in observational studies or clinical trials among unoperated patients with recent transient ischemic attack or nondisabling stroke. Although evidence of an association between timing of surgery and stroke risk is limited

TABLE 2

ADJUSTED ODDS RATIO OF PERIOPERATIVE ISCHEMIC STROKE STRATIFIED BY TIME BETWEEN HISTORY OF ISCHEMIC STROKE AND SURGERY

Indicators	Number of strokes, *n*	Number of follow-ups, *n*	Stroke rate, %	Odds ratio (95 % CI)
No history of ischemic stroke	368	474,046	0.078	1
Ischemic stroke (age undetermined)	210	7,137	2.94	16.24 (13.23–19.94)
Ischemic stroke < 3 months ago	103	862	11.95	67.60 (52.27–87.42)
Ischemic stroke from 3 to 6 months ago	21	469	4.48	24.02 (15.03–38.39)
Ischemic stroke from 6 to 12 months ago	16	898	1.78	10.39 (6.18–17.44)
Ischemic stroke ≥ 12 months ago	70	4,908	1.42	8.17 (6.19–10.80)

Note. 95 % CI – 95 % confidence interval.

to only these two studies, the AHA/ASA, to reduce the risk of perioperative stroke in patients undergoing non-cardiac surgery, suggests delaying elective non-cardiac surgery for at least 6 months and, if possible, even 9 months after a previous stroke [12].

Extracranial carotid artery stenosis. The AHA/ASA Guidelines for the Secondary Prevention of Ischemic Stroke and Transient Ischemic Attack recommend that patients with a high degree of extracranial stenosis (> 70 %) and ipsilateral symptoms of ischemic stroke or transient ischemic attack within the past 6 months undergo CEA or ISA stenting. Patients with moderate symptomatic stenosis (50–69 %) are also considered for revascularization surgery if the surgical risk is < 6 %. It is important to consider that performing carotid artery stenting and CEA is itself associated with cardiovascular risks. For example, carotid artery stenting is associated with a slightly higher risk of stroke and CEA with a slightly higher risk of MI. Recommendations for patients with known asymptomatic high-grade ISA stenosis who are to undergo non-cardiac and non-neurosurgical interventions are ambiguous. However, patients with known asymptomatic high-grade ISA stenosis (> 70 % by ultrasound or > 60 % by selective angiography) should be considered candidates for CEA and ISA stenting if the perioperative risks of stroke, MI, and death are < 3 %, according to the 2021 AHA/ASA guidelines. Planned surgical procedures may be delayed if carotid revascularization treatment is planned (for symptomatic or asymptomatic high-grade carotid stenosis), but the optimal duration of this delay is unknown and may be determined predominantly by the timing of the most recent cerebrovascular event (no earlier than 6–9 months after a previous stroke) [12].

The European Stroke Organization (ESO) guidelines published in the European Stroke Journal in 2021 regarding stroke prevention in patients with carotid atherosclerosis, in accordance with meta-analyses of randomized controlled trials on primary stroke prevention, recommend surgical intervention on the ISA when asymptomatic stenosis is 60–99 % [14]. CEA is favored as the surgical intervention. The relative risk (RR) of stroke ipsilateral stenosis after CEA compared with optimal medical therapy is 0.79 (95 % confidence interval (95% CI): 0.59–0.90), equivalent to a reduction of 19 cases per 1,000 patients. There was also evidence that post-CEA reduced the risk of stroke in any cerebral blood supply basin (RR = 0.74; 95% CI: 0.59–0.92). Perioperative safety of surgical interventions is currently of special concern, and European guidelines define the borderline rate of perioperative stroke or death for patients with asymptomatic stenosis as 2 % [15]. For secondary stroke prevention, there is strong evidence regarding the need for surgery at 70–99 % ISA stenosis (RR = 0.37; 95% CI: 0.27–0.50, corresponding to a reduction of 169 cases per 1,000 patients) and moderate strength evidence regarding the efficacy and safety of interventions at 50–69 % stenosis (RR = 0.82; 95% CI: 0.58–1.15, corresponding to a reduction of 29 cases per 1,000 patients). CEA is recommended as surgery for all patients having 50–99 % stenosis. For the group of patients younger than 70 years of age, stenting with angioplasty is recommended as an alternative. Patients with ISA stenoses < 50 % are not currently recommended to undergo surgery in routine practice. The timing of interventions is determined by the following decisions: the minimum is within the next 2 weeks after stroke (i. e., at the first medical center where the stroke patient is located); the maximum is up to 6 months, and the patient's disability should not exceed a mRS score of 3 [14].

Intracranial stenosis. Asymptomatic intracranial stenosis (50–99 % as measured by selective angiography) carries a 15 % risk of recurrent stroke within the first year after the event, but the overall risk of PS in this patient population remains unknown. Stroke prevention in a patient with intracranial stenosis is non-surgically managed with antithrombotic therapy and careful modification of risk factors. Intracranial stenosis stenting is accompanied by an increased risk of hemorrhagic complications, and the experience of using these operations among patients who did not receive conservative medical treatment is limited by a small number of follow-ups. A small retrospective study by D. Blacker et al. (2003) of patients with severe intracranial stenosis of vertebral or basilar arteries who underwent surgical intervention (vascular, cardiac or general) showed that the PS rate in the studied group was 6 % [12].

Patent foramen ovale. Patent foramen ovale (PFO) is present in 25 % of the population and is not associated with any morbidity among most people. However, data from three recent clinical trials (Søndergaard L. et al., 2017; Mas J. et al., 2016; Saver J. et al., 2017) support the potential association between PFOs and cryptogenic stroke in patients aged < 60 years and the benefit of PFO closure in selected patient groups. In a retrospective study of more than 150,000 patients undergoing non-cardiac surgery under general anesthesia, Ng et al. (2018) found that a diagnosis of PFO established preoperatively significantly increased the likelihood of PS. These patients had more severe PSs and were more often accompanied by involvement of large vascular territories with the formation of extensive zones of ischemic damage. In a large study of patients undergoing a total hip replacement; from the US National (Nationwide) Inpatient Sample, the risk of PS was 29 times higher (7.14 % vs. 0.26 %; $p < 0.001$) among patients with atrial septal defect/PFO compared with controls. Thus, there is a need for further research to develop optimal approaches for PS prevention in the population of individuals with PFO. If there is evidence of the need for surgery to close the PFO, it should be considered before elective surgery is performed; and urgent and emergent surgical interventions should not be delayed to address the PFO [12].

β-blockers. In 2008, a large randomized controlled trial regarding the use of β-blockers in POISE perioperative conditions (Effects of extended-release metoprolol succinate in patients undergoing non-cardiac surgery) revealed a tendency to increase the number of PSs, possibly associated with arterial hypotension against the back-

ground of metoprolol administration, while no such dependence was shown for other β-blockers. However, in 2017, M. Jørgensen et al. based on a large cohort study found no difference in the risk of overall mortality or serious adverse cardiac events when using different subtypes of β-blockers.

The AHA/ASA 2021 Scientific Statement on Perioperative Ischemic Stroke Prevention in Patients after non-Cardiac and non-Neurosurgical Operations, citing the 2014 ACC/AHA Guideline on Perioperative Cardiovascular Evaluation And Management Of Patients Undergoing Noncardiac Surgery: A Report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines) supports the continuation of β-blockers in patients who have already been taking β-blockers for an extended period of time prior to surgery. These guidelines also suggest that β-blockers should be offered to patients with established high cardiac risk based on preoperative evaluation or in the presence of ≥ 3 risk factors according to RCRI, but even in these cases β-blockers should not be started on the day of surgery [12].

Statins. To reduce the risk of cardiovascular events, patients already taking statins are advised to continue perioperative therapy prior to non-cardiac surgery. Multiple cohort studies (Berwanger O. et al., 2016; London J. et al., 2017; Ma B. et al., 2018) and the results of a recent meta-analysis of patients undergoing non-cardiac surgery (study groups included vascular surgery and neurosurgery) show that the use of statins in the perioperative period leads to significant reductions in postoperative MI, death from cardiac causes, de novo atrial fibrillation, and all-cause mortality, including myocardial injury and stroke. However, none of these studies showed a significant reduction in the risk of PS [12].

Arterial blood pressure. Maintaining appropriate perfusion of the heart, brain, and other vital organs is the cornerstone of anesthesia management. A significant number of anesthesiologists commonly use a mean arterial pressure (MAP)[1] of 60 mmHg and a systolic BP of 100 mmHg as the threshold, assuming that in healthy individuals without cerebrovascular disease, cerebral blood flow is maintained within a range of 60 to 150 mmHg. Intraoperative drop in BP is a very common event, with episodes of drop in MAP below 20 % of baseline occurring in 90 % of surgical procedures. There are currently about 140 different definitions of intraoperative hypotension, but the most common are: a decrease in systolic BP < 80 mmHg and a decrease in systolic BP more than 20 % below baseline.

Hypotension can be considered a modifiable risk factor for PS. However, most studies that have examined the causal relationship between intraoperative hypotension and perioperative strokes have inconclusive evidence, with the exception of the results of the POISE study (2008).

Currently, there is insufficient evidence defining target values of intraoperative BP levels that reliably prevent the development of cerebral ischemia. At the same time, there is evidence of BP thresholds associated with target organ damage and mortality. A 2018 systematic review reported a modest increase in the risk of target organ damage and mortality (RR = 1.4–2.0) with a decrease in MAP < 65 mmHg lasting > 10 min, but no statistically significant associations between MAP thresholds and stroke were found. In 2017, the multicenter randomized controlled trial (INPRESS) (Effect of individualized vs standard blood pressure management strategies on postoperative organ dysfunction among high-risk patients undergoing major surgery: A randomized clinical trial) found that maintaining systolic BP fluctuations within 10 % of baseline was associated with a 30 % reduction in postoperative internal organ dysfunction. However, this study has been criticized because the BP targets in the control group were much lower than those used by most anesthesiologists. Nowadays, the Perioperative Quality Initiative Consensus Statement on Intraoperative Blood Pressure, Risk and Outcomes for Elective Surgery 2019 guidelines (concerning that systolic BP values < 100 mmHg and MAP < 60–70 mmHg may be associated with myocardial and renal damage) are the most convincing.

Recognizing the current lack of data to establish accurate intraoperative BP targets to reduce the risk of perioperative strokes, the AHA/ASA 2021 Scientific Statement suggests considering maintaining intraoperative MAP above 70 mm Hg. Although, there are no data on upper thresholds for intraoperative MAP in non-cardiac surgery, it is recommended to avoid excessive hypertension, which may cause myocardial ischemia, cerebral edema, or damage to other target organs. Also, the AHA/ASA 2021 Scientific Statement supports the recommendations of G.A. Mashour et al. (2014) that the BP difference between the brachial artery and the brain should be taken into account when performing surgery in the sitting position (e. g., shoulder, cervical spine surgery) [12].

Perioperative targeted (directed) therapy. Thirty years ago, W.C. Shoemaker et al. first outlined the principles of targeted therapy aimed at maintaining optimal target organ perfusion among high-risk surgical patients. Despite the controversy over its benefits, targeted therapy continues to be widely used in clinical practice. A 2013 Cochrane meta-analysis based on 31 randomized trials involving 5,092 participants found that administration of fluids and vasoactive agents aimed at increasing total blood flow did not significantly reduce mortality, but did reduce overall complication rates by 32 %, including 29 % reduction in the rate of renal failure and 49 % reduction in the rate of respiratory distress/acute respiratory distress syndrome. In 2014, the multicenter, randomized, controlled OPTIMISE (Effect of a perioperative, cardiac output–guided hemodynamic therapy algorithm on outcomes following major gastrointestinal surgery) trial reported a 6.8 % reduction in the postoperative absolute risk of complications and 30-day mortality (95% CI: 0.38–13.9 %; $p = 0.07$) in 734 high-risk patients undergoing major gastrointestinal surgery who received targeted therapy aimed at maintaining cardiac output. Although

[1] Calculation formula is as follows:
MAP = 1/3 × systolic BP + 2/3 × diastolic BP

the results of this study were not statistically significant on their own, their inclusion in the updated meta-analysis established the overall benefit of targeted perioperative hemodynamic support techniques (hazard ratio, 0.77 [95% CI: 0.71–0.83]). So far, no study of perioperative targeted therapy has demonstrated its effectiveness in preventing the development of perioperative stroke, which may be explained by the relatively low incidence of PS and insufficient sample size [12].

Transfusion of blood and its components in the perioperative period. Anemia is known to increase the risk of stroke in non-surgical patients and is associated with a higher risk of poor functional outcomes and mortality in patients with acute stroke. However, two large observational studies of patients undergoing non-cardiac surgery have not shown an increased incidence of adverse central nervous system outcomes among patients with preoperative anemia. Using data from more than 227,000 patients, K.M. Musallam et al. (2011) found that patients with moderate to severe anemia were 44 % more likely to experience mortality, 52 % more likely to experience cardiac complications, and 41 % more likely to experience respiratory complications compared to patients without anemia. However, no differences in central nervous system complications, including PS, were found. L. Saager et al. (2013) also reported similar findings using data on nearly 575,000 patients. Collectively, these studies form a somewhat confusing picture of anemia being a risk factor for stroke in the general population but not in patients undergoing non-cardiac surgery.

Although anemia is associated with higher rates of mortality and morbidity among patients undergoing non-cardiac surgery, observational studies have consistently demonstrated that patients who received blood transfusion in the perioperative period have worse outcomes compared to the group without hemotransfusion (Bernard A. et al., 2009; Glance L. et al., 2011; Ferraris A. et al., 2012; Karkouti K. et al., 2012; Aquina C. et al., 2017). However, the pooled results of randomized trials did not support this information, revealing an overestimation of the risks associated with performing hemotransfusion. A 2018 meta-analysis based on 37 randomized controlled trials involving > 19,000 patients showed that the risk of stroke, MI, congestive heart failure, renal failure, and 30-day mortality were not significantly influenced by hemoglobin thresholds before starting transfusion. The authors of the study believe that a major limitation of this meta-analysis is that it did not include enough information on patients with traumatic brain injury, acute coronary syndrome, or congestive heart failure to extend these recommendations to very high-risk patient populations. Patients with increased risk of PS are considered appropriate to be added to this same group by the AHA/ASA.

The AHA/ASA 2021 Scientific Statement, citing recommendations from the American Association of Blood Banks, suggests that a hemoglobin threshold of 80 g/L should be used to decide whether to initiate blood transfusions in patients with recent stroke or existing cerebrovascular disease. In light of the current uncertainty in guideline documents regarding the treatment and prevention of perioperative ischemic stroke, the AHA/ASA 2021 Scientific Statement suggests considering a transfusion threshold of 80 g/L for most patients with increased risk of stroke and a threshold of 90 g/L for patients with acute PS or a history of a cerebrovascular event due to severe carotid stenosis or occlusion. When considering initiating hemotransfusion in patients at high risk of stroke with higher hemoglobin values, the risks of noninfectious complications such as transfusion-associated circulatory overload (may occur in up to 5 % of transfusions) should be weighed against the unproven benefit of using a higher transfusion threshold [12].

Choice of anesthetic technique. The neuroprotective effects of anesthetics have been studied intensively over the past 50 years, but there is currently no evidence that anesthetics are neuroprotective, even though anesthetics have been found to reduce cerebral metabolic rate and mimic the effects of ischemic preconditioning. In 2018, representatives of the Perioperative Neurotoxicity Working Group, based on work sponsored by the American Society of Anesthesiology Brain Health Initiative, published a set of recommendations and stated that there is a lack of evidence for an increased risk of perioperative neurocognitive disorders with anesthetic gases. Current evidence suggests that anesthetics are neither neuroprotective nor neurotoxic, and that the choice of anesthetic is unlikely to affect the risk of stroke in the perioperative period [12].

A 2016 Cochrane meta-analysis of 31 randomized controlled trials (3,231 patients) compared neuraxial (spinal or epidural) versus general anesthesia in patients undergoing surgery for hip fractures. This meta-analysis did not report differences in 30-day mortality, heart attack or stroke rates, but concluded that the quality of evidence was too low and the sample size too small to draw any definitive conclusions. With an estimated stroke rate < 1 %, it may not be possible to design randomized controlled trials to reach conclusions about the superiority of local anesthesia over general anesthesia. Two large (528,495 and 182,307 patients) retrospective studies (Chu C. et al., 2015; Memtsoudis S. et al., 2013) did not demonstrate an advantage of neuraxial anesthesia over general anesthesia in the prevention of PS among patients undergoing hip surgery. In a 2017 meta-analysis, L.M. Smith et al. found no differences in 30-day mortality between groups of patients in whom neuraxial, combined (neuraxial and general), and general anesthesia were used for major surgery and limb surgery (appr. 1.1 million follow-ups). Neuraxial anesthesia was associated with a 60 % reduction in pulmonary complications, but no difference in cardiac complication rates compared with general anesthesia alone. Combined (neuraxial and general) and general anesthesia did not differ in the incidence of pulmonary or cardiac complications. However, this meta-analysis did not examine the association between local anesthesia and stroke. Thus, at present, the advantages of local anesthesia over general an-

esthesia in reducing the perioperative risk of ischemic stroke have not been proven [12].

Strategies for artificial lung ventilation (ALV). Given that hypocapnia can exacerbate cerebral ischemia, the injured brain may be particularly susceptible to its effects. These data suggest that it is prudent to avoid hypocapnia in patients at high risk of PS, and that hypocapnia may be exceptionally harmful for patients with PS. Artificial lung ventilation (ALV) using lower tidal volumes reduces mortality among patients with acute lung injury and acute respiratory distress syndrome. The use of ventilation protective modes has become a best practice for the care of critically ill patients. The use of ALV protective modes results in a lower incidence of serious pulmonary complications, sepsis, and death among patients undergoing non-cardiac surgery. Although the preventive effect of protective ALV regimens on the risk of PS has not been studied separately, it is believed that prevention of pulmonary complications may lead to fewer episodes of hypoxemia in the perioperative period and reduce the risk of stroke among high-risk surgical patients. In this regard, it is reasonable to apply protective ALV modes as part of the implementation of a general strategy to improve perioperative outcomes [12].

CONCLUSION

Thus, to reduce the risk of perioperative ischemic stroke, key risk factors (such as age, renal disease, history of transient ischemic attack/stroke), overall cardiovascular risk, and the type of elective surgery should be assessed before surgical intervention. It is possible to establish individual PS risk using the ACS-SRC online surgical risk calculator. In this case, a web interface should be used [13]. In patients who have had a recent ischemic stroke, elective surgery should be delayed for at least 6 months (preferably 9 months) from the date of the stroke. ISA revascularization (type of intervention is determined individually) should be performed in patients with symptomatic (stroke or transient ischemic attack within the last 6 months) carotid stenosis (>70 %) before elective surgery. Should evidence of the need for a PFO closing operation be obtained, consideration should be given to conducting it before the elective surgery is performed. It is necessary to continue previously prescribed therapy with antihypertensive drugs, statins in order to reduce the incidence of cardiovascular complications and overall mortality. One should refrain from prescribing β-blockers on the day of surgical intervention, provided that the patient has not taken them before.

When performing surgical intervention to reduce the risk of PS, it is necessary to: maintain intraoperative mean BP > 70 mmHg (especially in patients at moderate or high risk of PS); perform hemotransfusion at a threshold hemoglobin level of 80 g/L in patients with recent stroke or significant cerebrovascular disease (e. g., carotid or intracranial stenosis > 70 %); avoid hypocapnia during ALV and use protective ALV modes with lower tidal volumes (as part of an overall strategy to reduce postoperative complications); taking into account the lack of evidence on the benefits of different types of anesthesia for the prevention of PS, the choice of anesthesia should be based on the type of surgical intervention, the skills of anesthesiologists and the individual characteristics of the patient.

Conflict of interest

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

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MICROBIOLOGY AND VIROLOGY

EXPRESSION OF THE *soxRS* REGULON IN BACTERIAL CELLS EXPOSED TO VARIOUS STRESS FACTORS

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ABSTRACT

Background. *Some stress responses contribute to the formation of bacterial antibiotic resistance, including the soxRS oxidative defense regulon. Elevation of reactive oxygen species production and oxidative stress was detected in bacterial cells exposed to various environmental stresses. It can be supposed that a stress-mediated increase in the level of reactive oxygen species will activate the expression of the soxRS regulon genes, which may provide pre-adaptation to antibiotics.*
The aim. *To study changes in the expression of soxRS regulon genes in Escherichia coli cells exposed to NaCl, acetic acid, and heating.*
Materials and methods. *Gene expression was measured in cells bearing reporter gene fusions (soxS::lacZ, nfo::lacZ). An overnight broth culture was diluted in fresh LB broth to OD600 = 0.1 and cultivated at 37 °C without stirring until OD600 = 0.3, then the stressors were applied.*
Results. *Exposure to NaCl and acetic acid activated the expression of soxRS regulon genes, while heating caused a decrease in gene expression. An increase in the expression level was observed in cells subjected to stresses of low intensity (which did not cause a decrease in the number of colony-forming units (CFU) by the 4th hour of exposure compared to the beginning of the stress exposure) and medium intensity (which caused a 10-fold decrease in the number of CFU), whereas high-intensity stresses (which caused a decrease in the number of CFU by more than 10 times), regardless of their nature, were accompanied by a decrease in the expression of the soxRS regulon genes.*
Conclusion. *Under the conditions studied, only the osmotic stress caused by the addition of NaCl was accompanied by a significant activation of the soxRS regulon genes. Sublethal exposure to NaCl, causing an increase in the expression of soxRS regulon genes by 2–2.5 times, may provide pre-adaptation of bacteria to the factors that this regulon is aimed at counteracting, including antibacterial drugs.*

Key words: *osmotic shock, acid stress, heat shock, oxidative stress, antibiotics, soxS*

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ЭКСПРЕССИЯ ГЕНОВ *soxRS*-РЕГУЛОНА В КЛЕТКАХ БАКТЕРИЙ, ПОДВЕРГНУТЫХ ДЕЙСТВИЮ РАЗЛИЧНЫХ СТРЕСС-ФАКТОРОВ

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РЕЗЮМЕ

Актуальность. *В формирование устойчивости бактерий к антибиотикам вносят вклад различные адаптивные механизмы, в том числе гены защитного ответа на окислительный стресс, объединённые в soxRS-регулон. В стрессовых условиях в клетках бактерий происходит повышение продукции активных форм кислорода и развитие окислительного стресса. Можно предположить, что повышенный уровень активных форм кислорода будет активировать экспрессию генов soxRS-регулона, что может обеспечить преадаптацию бактерий к воздействию антибиотиков.*

Цель. *Исследовать изменение экспрессии генов, входящих в soxRS-регулон, в клетках Escherichia coli, подвергнутых действию NaCl, повышенных температур и уксусной кислоты.*

Материалы и методы. *Уровень экспрессии генов определяли с использованием штаммов E. coli, несущих репортерные генные слияния промотора исследуемого гена (soxS, nfo) со структурной частью гена lacZ, в условиях периодического культивирования в бульоне LB без перемешивания.*

Результаты. *Активацию экспрессии генов soxRS-регулона вызывало воздействие NaCl и уксусной кислоты, а тепловой шок сопровождался снижением генной экспрессии. Увеличение уровня экспрессии наблюдалось в клетках, подвергнутых стрессам низкой (не вызывавшим снижения количества колониеобразующих единиц в культуре к четвёртому часу воздействия по сравнению с началом стрессового воздействия) и средней интенсивности (вызывавшим снижение количества колониеобразующих единиц на порядок), а стрессовые воздействия высокой интенсивности (вызывавшие снижение количества колониеобразующих единиц более чем на порядок) вне зависимости от их физико-химической природы сопровождались снижением экспрессии генов soxRS-регулона.*

Заключение. *В исследованных условиях только осмотический стресс, вызванный внесением NaCl, сопровождался значимой активацией генов, входящих в soxRS-регулон. Сублетальное воздействие NaCl, вызывая повышение экспрессии генов soxRS-регулона в 2–2,5 раза, может обеспечивать преадаптацию бактерий к факторам, на противодействие которым направлен данный регулон, в том числе к антибактериальным препаратам.*

Ключевые слова: *осмотический шок, кислотный стресс, нагревание, окислительный стресс, антибиотики, soxS*

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The formation of resistant forms of microorganisms is the reason for the reduced effectiveness of antibiotic therapy. The mechanisms underpinning drug resistance include target alteration or protection, modification and inactivation of the antimicrobial compound, rearrangement of metabolic pathways, or restriction of antibiotic accumulation in the microbial cell (by reducing the transport of the drug into the cell and increasing its active release from the cell) [1–3].

Various mechanisms of defense responses to natural stress factors may be involved in the adaptation of bacteria to antibiotic drugs [4, 5]. In particular, in response to antibiotic exposure, the expression of *soxRS* regulon genes that protect bacteria from oxidative stress is activated. The increased baseline level of expression of this regulon in some cases results in clinically relevant antibiotic resistance in bacteria [6–9].

SoxRS regulon is a two-stage control system. The SoxR protein enters the active form and triggers the expression of the *soxS* gene; the newly synthesised SoxS protein then activates the expression of other genes within this regulon. The SoxR protein is activated by one-electron oxidation of its [2Fe-2S] clusters or their nitrosylation by reactive nitrogen species [10–13]. The *soxRS* regulon includes genes encoding superoxide dismutase that neutralizes superoxide anions (*sodA*), endonuclease involved in DNA repair (*nfo*), isoforms of enzymes resistant to oxidative damage (*fumC*, *acnA*), iron transport regulator (*fur*), proteins limiting the accumulation of hydrophilic xenobiotics in the cell (*tolC*, *micF*, *acrAB*), proteins presumably involved in the maintenance of the reduced form of iron-sulfur sites of enzymes (*fldAB*, *fpr*), and other proteins with unknown functions [14].

It is known that exposure to antibacterial drugs and natural stress factors of different nature causes increased production of free radicals and development of oxidative stress in bacterial cells. While the role of reactive oxygen species and their contribution to the death of cells exposed to various stress factors remains a debatable issue, the accumulation of free radicals caused by stress factors not directly related to their production has been confirmed by numerous publications [15–20]. Many of these stressors, e. g. high osmolarity of the medium, heating, exposure to ethanol and short-chain fatty acids, are used as antimicrobial treatments or preservatives. If these stressors cause induction of *soxRS* regulon, their sublethal effects may contribute to the pre-adaptation of bacteria to antibiotic exposure.

In this study, the expression of the *soxRS* regulon genes was studied in *Escherichia coli* cells exposed to sodium chloride, elevated temperatures and acetic acid (CH3COOH) using the gene fusion method.

MATERIALS AND METHODS

Objects of the study and cultivation conditions. *Escherichia coli* strains carrying transcriptional gene fusions were used as study objects. *E. coli* EH40 strain (GC4468, but *soxS::lacZ*) was kindly provided by B. Demple [21], *E. coli* N9213 strain (GC4468, but *nfo::lacZ* Δmar *rob::kan*) was kindly provided by R.G. Martin [22].

Bacteria maintained on LB slant agar were transferred to 5 ml of LB broth and cultured without agitation at 37 °C for 5–6 h. The grown cells were transferred into 50 ml of LB broth and cultured at 37 °C for 14–16 h. The bacterial culture was then diluted in fresh nutrient medium to an optical density measured at a wavelength of 600 nm (OD600) of 0.1 and cultured under the conditions described above. Once the bacterial culture reached OD600 = 0.3, it was exposed to stressors. Sodium chloride and acetic acid were added to the bacterial culture and the culture was placed on a water bath with appropriate temperature to reproduce heat shock.

The gene expression level was determined using reporter gene fusions of the promoter of the studied gene and the structural part of the *lacZ* gene encoding β-galactosidase. It is assumed that the amount (activity) of the reporter protein is directly proportional to the expression level of the studied gene. β-galactosidase activity was measured in cells pretreated with a mixture of sodium dodecyl sulfate and chloroform using *o*-nitrophenyl-β-D-galactopyranoside as a substrate. β-galactosidase activity was determined and calculated (in Miller units) according to the standard protocol proposed by J. Miller [23].

Bacterial culture density was estimated by measuring its OD600 using a UV1280 spectrophotometer (Shimadzu, Japan) and a cuvette with 10 mm optical path.

The number of colony-forming units (CFUs) was determined by plating on the surface of LB agar in Petri dishes. The number of colonies formed was counted after incubation at 37 °C for 16–18 h.

Statistical data processing was performed using Statistica 6.0 software package (StatSoft Inc., USA). Data are presented as mean and standard error of the mean calculated from at least three independent experiments. The statistical significance of the differences between the mean values of the compared groups was determined using unpaired t-test at $p \leq 0.050$.

RESULTS AND DISCUSSION

Osmotic stress was caused by addition of sodium chloride, acid shock was induced by addition of acetic acid, and heat stress was induced by heating from 37 to 42–55 °C. The effect of these stresses of different intensities on the expression of the *soxS* gene, which encodes a transcriptional regulator responsible for the activation of genes of the regulon, and its target gene *nfo*, which encodes a DNA repair enzyme, was studied. The intensity of stress was assessed by the change in the number of colony-forming units by the fourth hour of stress exposure relative to the moment of the onset of stress exposure (Table 1). Several levels of stress strength were distinguished: subinhibitory exposure (the number of CFUs in the stressed culture increased during the cultivation time); mild stress (inhibitory exposure, the number of CFUs in the culture remained at the same level as at the time

of stressor application); moderate stress (the number of CFUs decreased by about one order of magnitude) and severe stress (the number of CFU decreased by more than one order of magnitude).

TABLE 1

THE NUMBER OF COLONY-FORMING UNITS IN *E. COLI* CULTURE AFTER FOUR-HOUR EXPOSURE TO STRESSORS

Conditions	lgCFU/ml
Control, unstressed	8.3 ± 0.4*
30 mg/ml of NaCl	8.1 ± 0.3*
50 mg/ml of NaCl	7.6 ± 0.1
70 mg/ml of NaCl	6.9 ± 0.6
100 mg/ml of NaCl	6.1 ± 0.4*
200 mg/ml of NaCl	2.8 ± 1.9*
0.125 mg/ml of CH_3COOH	8.4 ± 0.5*
0.25 mg/ml of CH_3COOH	7.5 ± 0.4
0.5 mg/ml of CH_3COOH	7.3 ± 0.1
2 mg/ml of CH_3COOH	5.7 ± 1.2*
42 °C	8.2 ± 0.3*
45 °C	8.1 ± 0.2*
55 °C	0

Note. The number of CFU/ml at the time of stressor application was 7.4 ± 0.3; * – statistically significant difference from that at the time of stressor application ($N \geq 3$; T-test; $p \leq 0.050$).

Subinhibitory exposure had no effect on the expression level of the *soxRS* regulon genes (data not shown). In response to exposure of 50–100 mg/ml sodium chloride (mild and moderate stress), the level of *soxS* gene expression increased in *E. coli* cells in a dose-response manner; more intense osmotic stress did not induce changes in gene expression (Fig. 1b).

Under mild osmotic stress, the change in expression occurred in two stages: the gene expression level decreased after an increase in the initial stage of sodium chloride exposure and then began to increase again after the third hour of cultivation. An increase in *soxS* gene expression after addition of acetic acid to the concentrations that did not reduce the number of CFUs in the culture (0.25–0.5 mg/ml) was observed in the first 15 min from the onset of exposure; more intense acid stress was accompanied by a decrease in gene expression (Fig. 1g). The expression of *soxS* was lower in cells subjected to heating compared to cells grown under optimal conditions (37 °C) regardless of the severity of heat stress (Fig. 1e).

Changes in *nfo* gene expression under stress factors were similar to changes in *soxS* gene expression: mild and moderate osmotic shock caused an increase in gene expression, acid shock, which did not decrease the number of CFUs, slightly increased gene expression (Fig. 2), and more severe acid stress and heat exposure led to a decrease in gene expression (data not shown).

Therefore, under the conditions studied, activation of *soxRS* regulon gene expression was induced by exposure to sodium chloride and, to a lesser extent, acetic acid, while heat shock was accompanied by a decrease in gene expression. An increase in the expression level was observed in cells subjected to mild and moderate stresses, while severe stresses, which caused the death of a significant number of bacterial culture cells regardless of their physicochemical nature, were accompanied by a decrease in *soxRS* regulon gene expression. A decrease in gene fusion expression does not appear to be a specific response, but rather a consequence of a general metabolic suppression and inhibition of protein synthesis, including the reporter β-galactosidase.

The data obtained are consistent with the results of transcriptome analysis, which demonstrated an increase in the expression of the *soxRS* regulon genes (*soxS*, *fumC*, *fpr*, *acnA*) in *E. coli* cells when exposed to 0.3 M (17.5 mg/ml) sodium chloride [24]. Activation of *soxS* gene expression was also observed in *E. coli* cells subjected to osmotic shock induced by exposure to 0.4 and 0.9 M sucrose [25].

An increase in *sodA* mRNA synthesis in *Bacillus cereus* cells grown in media with pH = 5.4–4.5 and an increase in superoxide dismutase activity in *Staphylococcus aureus* cells grown in medium with pH = 4.0 and pH = 2.0, compared to cultivation in medium with neutral pH, have been previously shown [26, 27], suggesting activation of the *soxRS* regulon under conditions of acid stress. In this study, we demonstrated a slight increase in *soxRS* regulon gene expression during the initial stages of development of acid stress induced by acetic acid exposure.

Our results showed a decrease in the level of gene expression in cells grown at temperatures higher than optimal (37 °C). Earlier studies showed an increased level of *soxS* gene expression in cells grown at 43 °C compared to cells grown at 30 °C, which is regarded as an activation of expression in response to heat [24]. On the other hand, decreasing the cultivation temperature relative to the optimal level could cause a decrease in gene expression, which could also explain the observed differences in *soxS* expression level.

Thus, only osmotic stress induced by sodium chloride application, out of the three stress conditions investigated (exposure to acetic acid, sodium chloride, or heating), was accompanied by a significant activation of *soxRS*-regu-

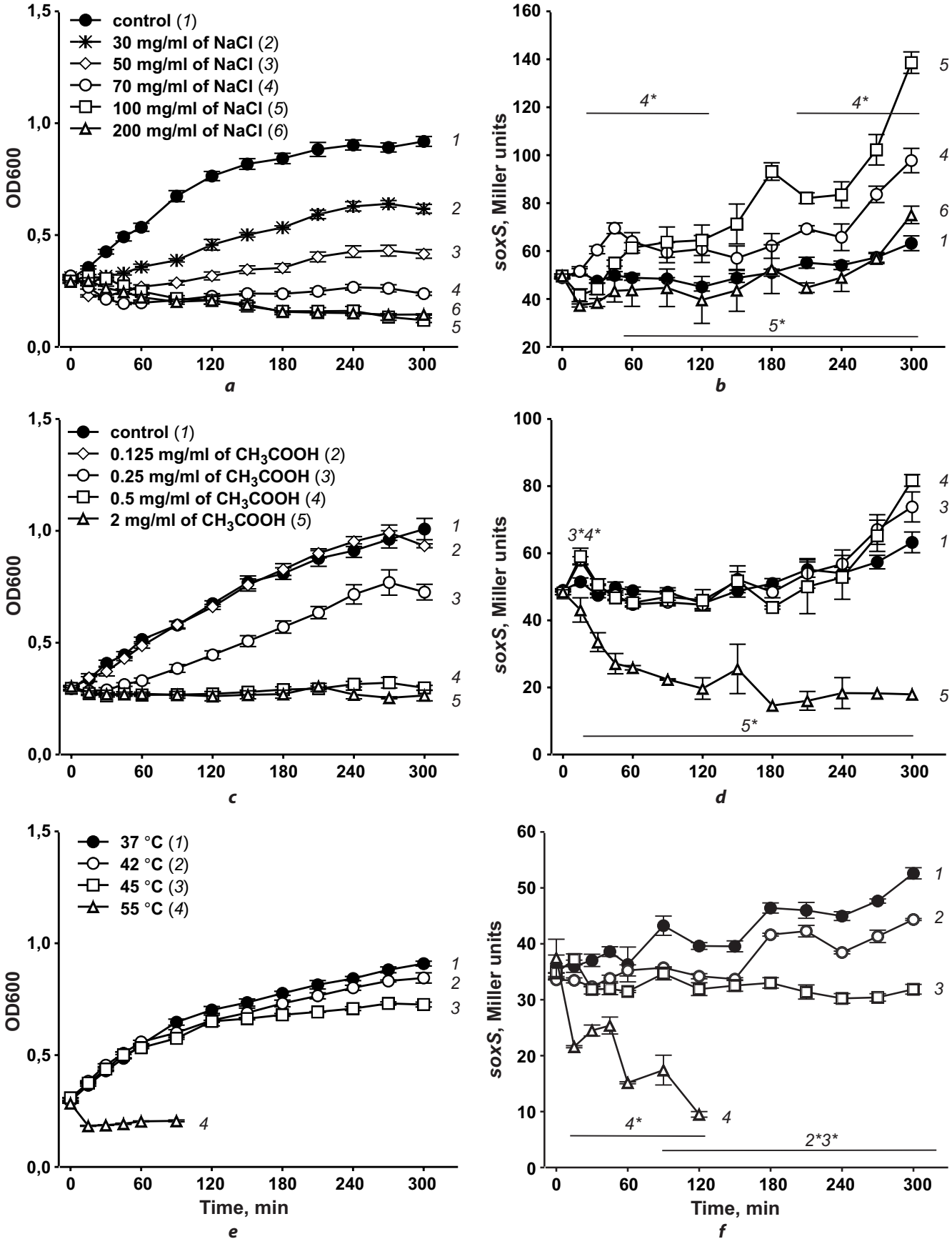

FIG. 1.

*Changes in the optical density (OD600) of E. coli culture and soxS gene expression in E. coli EH40 cells in response to osmotic (**a**, **b**), acid (**c**, **d**), and heat stress (**e**, **f**): * – statistically significant difference from the unstressed culture (control (1)) (N ≥ 3, T-test; p ≤ 0.050)*

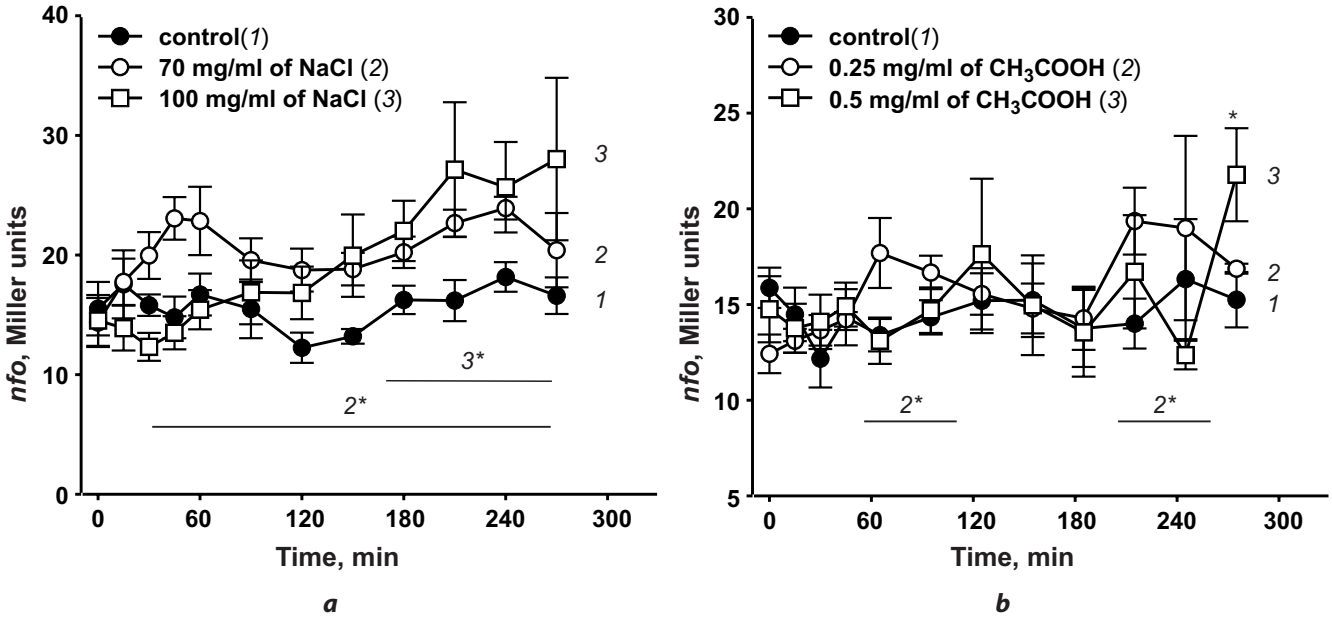

FIG. 2.

*Changes in nfo gene expression in E. coli N9213 cells in response to osmotic (**a**) and acid (**b**) stress: * – statistically significant difference from the unstressed culture (control (1)) (N ≥ 3; T-test; p ≤ 0.050)*

lon genes of antioxidant defence. Sublethal exposure to sodium chloride, causing a 2–2.5-fold increase in the expression of *soxRS* regulon genes, may provide pre-adaptation of bacteria to the factors that this regulon is aimed at counteracting, including antibacterial drugs.

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Conflict of interest

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

The studies were conducted without the use of animals and without using humans as test subjects.

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MORPHOLOGY, PHYSIOLOGY AND PATHOPHYSIOLOGY

CIRCADIAN RHYTHM OF CARBOHYDRATE METABOLISM IN HEALTH AND DISEASE

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ABSTRACT

The article presents a review of the main circadian mechanisms regulating carbohydrate metabolism and their role in maintenance of energy homeostasis; the molecular genetic structure of the circadian system is also discussed. The role of adipose tissue and other organs and systems in the maintenance of circadian rhythm of carbohydrate metabolism, both in health and in obesity and diabetes, is highlighted. Particular attention is paid to diurnal rhythms of endocrine factors responsible for metabolic patterns of hormones such as cortisol, growth hormone and melatonin. Gender differences in the circadian regulation of energy and carbohydrate metabolism are also discussed, as well as their changes in different age periods. Article provides detailed review of the mechanisms of glucose utilization, reactivity of the pancreatic islets and peripheral insulin sensitivity shifts at different time periods of the day in people with normal body weight, android and gynoid types of obesity, both in women and men. Protective factors of energy metabolism circadian regulation structure preventing the development of diabetes mellitus and cardiovascular disease in individuals with so-called "metabolically healthy" obesity type are discussed. Article provides a review of various pathways of circadian rhythm disturbances, mechanisms of their development, as well as exogenous and endogenous factors leading to carbohydrate metabolic circadian rhythm misalignment, such as shift work, untiming of natural and artificial lighting, jet lags, sleep disorders. Represented data contribute to a new look at the pathogenesis of obesity and carbohydrate metabolism disorders in various types of obesity in men and women, that provides basis for searching for new effective methods of prevention and treatment of these conditions, elaboration of evidence-based diets and physical activity recommendations, as well as approaches to their medical treatment.

Key words: *carbohydrate metabolism, circadian rhythms, insulin, glucose, diabetes mellitus, obesity, adipose tissue*

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ЦИРКАДНЫЙ РИТМ УГЛЕВОДНОГО ОБМЕНА В НОРМЕ И ПРИ ПАТОЛОГИИ

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РЕЗЮМЕ

В статье представлен обзор сведений об основных механизмах циркадной регуляции углеводного обмена, а также её роли в поддержании энергетического гомеостаза, рассмотрена молекулярно-генетическая структура циркадной системы. Освещена роль жировой ткани и других органов и систем в циркадном ритме углеводного обмена, как в норме, так и при ожирении и сахарном диабете 2-го типа. Особое внимание уделено суточной ритмике эндокринных факторов, определяющих метаболические паттерны таких гормонов, как кортизол, соматотропный гормон, мелатонин. В статье отдельно обсуждаются гендерные различия циркадной регуляции энергетического и углеводного метаболизма, а также их изменения в различные возрастные периоды. Проведён подробный обзор механизмов изменения утилизации глюкозы, реактивности инсулярного аппарата поджелудочной железы и чувствительности периферических тканей к инсулину в разное время суток у лиц с нормальной массой тела, андроидным и гиноидным типами ожирения, как у женщин, так и у мужчин. Обсуждены защитные факторы в структуре циркадной регуляции энергетического метаболизма, препятствующие развитию сахарного диабета и сердечно-сосудистых заболеваний у лиц с так называемым «метаболически здоровым» типом ожирения. Рассмотрены различные варианты нарушений циркадных ритмов, механизмы их возникновения, а также экзогенные и эндогенные факторы, приводящие к нарушениям циркадного ритма углеводного обмена, такие как сменная работа, нарушение естественного и искусственного освещения, смена часовых поясов, расстройства сна. Приведённые сведения способствуют формированию нового взгляда на патогенетические механизмы развития нарушений углеводного обмена при различных типах ожирения у мужчин и женщин, что даёт основания для поиска эффективных методов профилактики и лечения этих заболеваний, определения научно-обоснованных режимов питания и физических нагрузок, а также подходов к их медикаментозной терапии.

Ключевые слова: *углеводный обмен, циркадные ритмы, инсулин, глюкоза, сахарный диабет, ожирение, жировая ткань*

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INTRODUCTION

There is more and more information concerning the essential role of not only central but also peripheral oscillators acting in metabolically active organs in the regulation of glucose homeostasis to ensure circadian coordination of key metabolic processes. Integral work of central and peripheral oscillators allows the organism to predict the development of events related to the day-night cycle, including processes related to the periodization of sleep-wakefulness, hunger-satiety cycles of the organism. In this regard, obtaining new knowledge related to blood glucose homeostasis as a key energy substrate is one of the fundamental tasks for understanding the mechanisms of metabolism regulation in the human body in health, as well as for determining pathogenetic approaches to the treatment of such diseases as obesity, dyslipidemia, atherosclerosis, type 2 diabetes mellitus and others.

Circadian rhythms are periodically repeating patterns of physiologic processes every 24 hours. Cyclic processes occurring in the human body determine the equilibrium both within the organism and its equilibrium with the environment, which provides adaptation to the environmental conditions. Circadian rhythm is generated endogenously by a genetically encoded molecular clock with a periodicity of about a day [1].

Currently, more than 300 physiological functions and processes are known to have circadian rhythm, including: body temperature, motor performance, sensitivity of the organism to environmental factors; different levels of biologically active substances in body tissues and organs, as well as in biological fluids; intensity of metabolic processes, as well as providing cells, tissues and organs with energy and plastic resources [2, 3]. A circadian clock, influencing the expression of synthesis and hormone secretion involved in the metabolism regulation, contributes to the maintenance of body weight adequate to external conditions [4, 5].

One of the most striking examples of circadian rhythms are the stable circadian and ultradian rhythms of blood glucose level dynamics, which have developed in the course of evolutionary development under the influence of organism's peculiarities of functioning in the environment. Glucose homeostasis represents a model of energy metabolism circadian control, enhancing the efficiency of this substrate usage. So, while during activity, blood glucose is predominantly of dietary origin, during rest, glucose is gradually recruited from glycogen in the liver and maintains the required level in the blood within a relatively narrow range of concentrations [6]. During this process, liver glycogen content undergoes large daily fluctuations necessary to maintain blood glucose levels, as glycogen synthesis and breakdown change during periods of wakefulness/feeding and rest/starvation, respectively [7, 8].

The circadian model of carbohydrate metabolism neurohumoral regulation formed during phylo- and ontogenesis is highly reliable because it is a multilevel and self-regulating system [3]. This system regulating glucose homeostasis is represented by both a central biological clock located in the hypothalamus and a peripheral circadian clock in organs and tissues such as muscle, adipose tissue, liver, and pancreas.

Discordance in the circadian clock can lead to significant disorders in the endocrine glands rhythms, which play a leading role in the realization of most physiological functions. The resulting hormonal disorders affect a range of metabolic responses, and the effects on glucose and lipid homeostasis lead to the development of such disorders as metabolic syndrome, obesity and type 2 diabetes [4, 6]. Adipose tissue accumulation leads to changes in daily fluctuations in body temperature, heart rate, blood pressure, fasting glycemia. Further disorders of carbohydrate metabolism in the form of type 2 DM development contribute to the aggravation of metabolic disorders, which leads to even greater changes in the structure of circadian rhythms in the body [9].

CIRCADIAN CONTROL OF ENERGY METABOLISM

Endogenous circadian rhythms of metabolism are reproduced by a multi-oscillatory system consisting of a central clock located in the suprachiasmatic nucleus (SCN) of the hypothalamus, as well as the peripheral clocks represented in virtually all organs, tissues, and cells of the human body [10]. SCN functioning is triggered primarily by light signals via the retinohypothalamic tract. Further, through nerve and/or hormonal pathways, the SCN transmits temporal signals to other brain regions, particularly the epiphysis, as well as to peripheral organs such as the adrenal glands, muscles, adipose tissue, pancreas, liver, and gastrointestinal tract [10]. The central clock uses hormones such as cortisol, melatonin, STH, leptin, and synaptic projections, particularly the autonomic nervous system, as signals that regulate metabolism [11, 12].

Peripheral tissues, by integrating SCN signals with environmental factors and behavior (including nutrition, light, sleep, and physical activity), as well as with their own autonomous rhythms, maintain the circadian rhythm of the body's energy metabolism [13]. It has been shown in experimental models that (almost) all cells in the body express the molecular mechanism of the circadian clock, and the hunger-satiety cycle is one of the main synchronization timers for the peripheral clocks [14]. Thus, the rhythm of food intake largely controls the circadian expression of liver genes [15].

Molecular regulation of energy metabolism circadian rhythms is provided by a set of genes that trigger and maintain the clock mechanism of the organism as a whole. Autonomous intracellular rhythms are maintained at the molecular level by circadian genes and proteins that form a transcriptional-translational feedback loop (TTFL). The main negative transcriptional-translational feedback loop includes core clock genes such as *CLOCK, BMAL1* (also known as *ARNTL*), *PER* and *CRY* [16]. TTFL operates in a ~ 24-hour cycle, activating a rhythmic cascade of transcriptional and post-transcriptional events involving thousands of tar-

get genes [17]. In total, about 10 % of gene transcripts show circadian periodicity and, moreover, even more proteins undergo oscillations due to circadian rhythms at the post-transcriptional and post-translational levels [17]. Thus, circadian rhythms are generated endogenously in the body and persist for quite a long time even in the absence of external time cues [1]. Since different stimuli determine the rhythm of the central and peripheral clocks, both systems can be desynchronized whenever their respective timers are out of sync. In this regard, synchronization of photic (light) and non-photic (non-light) stimuli is necessary for the circadian system to work more accurately and coherently.

CIRCADIAN RHYTHMS OF CARBOHYDRATE METABOLISM IN NORMAL

The circadian rhythms of carbohydrate metabolism are fairly well studied in healthy individuals. Numerous studies have found diurnal variation in glucose tolerance with a maximum in the morning hours and reduced glucose tolerance in the evening [18–21]. The above diurnal variations are independent of the route of glucose entry into the body and are characteristic of both oral and intravenous glucose or insulin tolerance tests [22, 23] and mixed-feeding conditions [24]. Daily fluctuations in glucose tolerance are largely determined by the diurnal rhythm of β-cell reactivity, insulin secretion and its clearance. It has been shown that β-cell reactivity is higher in the morning than at other times of the day [18, 22], while secretion rate and insulin levels in response to glucose or food intake are most significant in the afternoon and evening [25]. Insulin clearance also varies by day: its extraction by the liver has been shown to be lower in the morning, relative to the evening [25].

The diurnal rhythm of carbohydrate metabolism is also determined by the peripheral sensitivity of tissues to insulin, primarily muscle [26, 27], liver [28], and adipose [24, 29]. Regarding the latter, only subcutaneous adipose tissue has been shown to undergo a circadian rhythm of insulin sensitivity with the highest amplitude of insulin sensitivity, 54 % higher at midday relative to midnight [29]. The results of our own studies with the oral glucose tolerance test (OGTT) at different times of the day indicate that in both men and women with normal body weight, evening time is characterized by a physiological increase in insulin resistance, manifested by a decrease in the rate of glucose utilization compared to the morning hours. Gender features of the carbohydrate metabolism circadian rhythm consisted in lower glucose tolerance in the morning in men relative to women. The explanation for this may be the preferential accumulation of visceral adipose tissue in men compared to the proportion of subcutaneous adipose tissue, as determined by the ratio of waist circumference to hip circumference [30]. It is visceral adipose tissue that largely determines the level of free fatty acids (FFAs) in the blood. The predominant alternate use of carbohydrates and fats in energy metabolism is confirmed by the fact that FFA levels are also subject to diurnal fluctuations and are consistent with the diurnal rhythms of glucose homeostasis [18, 24].

As mentioned above, effective regulation of carbohydrate-fat metabolism is supported by a number of counterinsulatory hormones, such as somatotropic hormone, the level of which is in a pronounced relationship with glycemic levels during nighttime sleep (10:00 PM – 2:00 AM) [31]. Cortisol is also responsible for circadian fluctuations in glycemia and insulinemia. Hydrocortisone infusion is known to dramatically suppress insulin secretion and increase peripheral insulin resistance in about 4–6 hours, and maintains these effects up to 12–16 hours after administration [32]. The effects of melatonin are also reflected in the circadian balance of regulators in the form of increased secretion of counterinsulatory hormones, predetermining increased insulin resistance and glucose tolerance in the evening and at night [33]. Conversely, increased daytime insulin sensitivity and increased pancreatic glucose sensitivity coincide with an anabolic orientation of energy metabolism. It has also been suggested that melatonin may directly affect the expression of clock genes [34]. For example, one of the melatonin effects is not only the regulation of the expression of circadian genes of the transcription-translation feedback loop in cells of the central nervous system and β-cells of the pancreas, but also an increase in their sensitivity to the action of Glucagon-like peptide-1 (GLP-1), which in turn stimulates insulin secretion [35].

Analyzing the changes in glucose tolerance and insulin sensitivity in the circadian rhythm, it can be assumed that the physiological significance of the phenomenon of insulin resistance lies in the activation of lipid oxidation in peripheral tissues in the evening and night hours, which is necessary for the functioning of muscle and fat cells and is also aimed at reducing lipotoxicity [36]. Thus, the increase in lipid utilization and the switch of energy metabolism to fat oxidation in the evening is the presumed cause of hyperglycemia and determines the daily rhythm of glucose levels. The biological meaning of switching to fat metabolism in the late phase of the circadian cycle lies in the expediency of restoring the structure, reserves and cell function of peripheral tissues, as well as in getting rid of unspent excess fat stores to avoid fat degeneration and lipotoxicosis. The oxidation products accumulated during the day inhibit glycolysis and the delivery of glucose into the cells. Perhaps one of the key roles in this process is played by leptin, the secretion of which is characterized by a cosine curve with a peak in the middle of the night [37]. It is known that leptin is able to induce in somatic cells the synthesis of enzymes involved in the non-oxidative metabolism of FFAs. In turn, leptin deficiency or developing resistance to it leads to a switch of FFA metabolism to the pathway of synthesis of long-chain fatty acids and their esterification into triglycerides. The resulting increased lipid content in peripheral tissues can lead to adipose degeneration, lipotoxicosis, and cellular apoptosis [38]. We believe that it is this protective function of leptin against excessive

fat accumulation that defines its basic biological meaning. Based on the above, it can be assumed that it is leptin that plays a key role in regulating the switch from carbohydrate to fat metabolism during the transition from light to dark time. Thus, the accumulation of adipose tissue and insulin resistance are elements of the system of energy metabolism regulation, formed in the course of evolution and contributing to the survival and reproduction of offspring in extreme conditions.

FACTORS LEADING TO DISORDERS OF CIRCADIAN RHYTHM OF CARBOHYDRATE METABOLISM

Shiftwork

There is increasing evidence that shifts in circadian rhythms caused by an inappropriate combination of key external factors, such as shift work, exposure to bright light at night, sleeping during the day, disordered eating, low motor performance during the day, lead to metabolic disorders and the formation of pathological conditions in the form of increased levels of glucose, insulin, triglycerides, the development of obesity and type 2 diabetes mellitus (DM), and accelerated aging [39–41].

A meta-analysis of observational studies showed that people who work shifts have a 9 % increased risk of developing type 2 DM compared with those without a history of shift work [42]. In a longitudinal study using cohorts of nurses, this risk also depended on the length of shift work, increasing by 5 % for every 5 years of shift work [43]. It has also been shown that workers with rotation shifts were even more likely to develop type 2 DM than workers with a fixed night schedule [42].

Natural and artificial light disturbance and change of time zones

Another risk factor for circadian rhythm disorder is irregularities in lighting, both natural and artificial. Epidemiologic evidence suggests that exposure to bright light in the evening or at night increases the risk of metabolic disease. In a cross-sectional study of more than 100,000 women, bright room lighting during sleep was strongly associated with higher BMI, waist circumference, and waist to hip circumference ratio [44]. In addition, in a prospective cohort study, older adults exposed to light at night (≥ 3 lux) demonstrated a 10 % increase in body mass index (BMI) over 10 years [45]. In another study, increased exposure to light in the evening (18–38 lux) was associated with a 51 % increased risk of developing type 2 DM [46], while a phase delay of every hour in light above 500 lux was associated with a 1.3 kg/m^2 increase in BMI [47]. In a cohort study involving 43,722 women, artificial light at night during sleep significantly influenced the risk of weight gain and the development of obesity, especially in women who had a light or TV on in the room during sleep [48]. Insolation disorder is associated with impaired melatonin production. Exposure to bright light during the daytime has been shown to increase melatonin secretion at night [49]; therefore, lack of bright light during the daytime may weaken the central clock rhythm and, hence, lead to metabolic disorders. In contrast, evening or nighttime exposure to bright light suppresses melatonin production, resulting in significant changes in hormonal balance [12]. Decreased melatonin levels can lead to the development of food addiction, manifested by increased appetite, episodes of compulsive eating behavior, and elements of night eating syndrome. In modern society, the influence of social factors on the timing of meals and the maintenance of circadian rhythms of sleep and wakefulness is great. Clear examples include nighttime eating with shift work schedules and postponing meals when time zones change. An increase in caloric intake in the evening and night hours is often accompanied by an increase in total daily caloric intake, as well as a shift in preference toward foods rich in rapidly digestible carbohydrates. For example, one study showed a prevalence of refined carbohydrates and high-calorie meals in the diet of individuals working the night shift [39]. Another study (n = 98) showed decreased levels of melatonin and serotonin and an inverse relationship of their levels with all types of eating disorders in individuals with metabolic syndrome [50]. The results of another study including 100 patients with metabolic syndrome demonstrated a shift in the nocturnal peak of melatonin without a confirmed decrease in its levels [51].

Another common type of circadian rhythm disorders is jet lag, quantified as a discrepancy between sleep and wakefulness times on weekdays and weekends with discrepancies in social and biological time [52]. It has been found that people experiencing jet lag (approximately 69 % of the population [52]) have a 1.75-fold higher incidence of type 2 DM and prediabetes compared to individuals who do not change time zones [53]. Moreover, individuals with an evening chronotype were at a 2–2.5-fold higher risk of developing type 2 DM compared to those with a morning chronotype [54].

Sleep disorders

Another of the mechanisms explaining metabolic changes in circadian misalignment are sleep disorders. According to a number of studies, excess body fat is associated with a number of circadian sleep rhythm disorders [55]. It has been shown that episodes of late falling asleep can lead to circadian misalignment and exacerbate insulin resistance [56]. Phase shifts in sleep timing, even when sleep duration is kept constant, also cause circadian shifts leading to metabolic dysfunction. Sleep deprivation worsens glycemic outcomes in patients with and without DM [57]. Not only shorter (< 6 h) but also longer (> 9 h) sleep duration has been shown to be unfavorably associated with insulin resistance. Although the association between insufficient sleep and DM is more or less understood, little is known about how excessive time spent sleeping or hypersomnia (10–12 hours) increases the risk of developing diabetes [58]. The link between sleep disturbances and diabetes is two-way: chronic sleep dis-

turbances increase the risk of developing insulin resistance, and diabetes worsens sleep quality. When the diurnal rhythm of going to sleep is shifted, melatonin peak shifts to the beginning of awakening, the total duration of sleep decreases, and due to insulin resistance in the morning hours, there is an increase in postprandial glycemia at lunchtime. This is because the circadian system influences phase I insulin secretion through the SCN and melatonin receptors (MT1 and MT2). Consequently, the increase in fasting and postprandial hyperglycemia on the background of circadian misalignment is mainly due to the increase in insulin resistance, rather than due to a decrease in β-cell function [59].

Diet violation

An important factor that meaningfully affects circadian rhythm is food intake. There is now no doubt that meal timing plays one of the key roles in maintaining daily homeostasis of glycemic levels [60]. Several studies have reported that delaying the meal phase has adverse metabolic consequences, even when food intake is limited to daytime hours. Changing lunch time from 01:00 PM to 04:30 PM increased glucose increment by 46 %, and decreased carbohydrate oxidation in the fasting state [61]. Late dinner induces nocturnal glucose intolerance and reduces fatty acid oxidation and mobilization, especially in those who fall asleep early [62]. Another study showed that an abrupt shift in dinner time from 07:00 PM to 10:30 PM increased post-breakfast glucose levels the next morning by 7–8 % and increased 24-h glucose levels by 4 mg/dL, although it had no effect on 24-h energy expenditure [63]. A nighttime meal, even if it consists of low glycemic index foods, is associated with a more significant increase in glycemia and insulinemia compared with an equivalent meal in the morning hours [61].

In a randomized cross-over study, it was shown that late dinner time led to increased nighttime melatonin concentrations and decreased glucose tolerance in *MTNR1B* melatonin receptor gene carriers [64]. The melatonin role in this process is supported by the observation that significant impairment of glucose tolerance at late dinner was observed only in carriers of the rs10830963 SNP allele variant of the *MTNR1B* gene of the melatonin receptor [64], associated with a high risk of developing type 2 DM [65]. This conclusion is further supported by placebo-controlled trials demonstrating that administration of exogenous melatonin in the morning and evening reduces glucose tolerance [66], and that this effect is six times more pronounced in carriers of the rs10830963 SNP allele of the *MTNR1B* gene than in non-carriers [64, 67].

Improper timing of meals can negatively affect the course of type 2 DM. Patients with type 2 DM who consumed more than 25 % of their daily energy in the evening hours had worse glycemic control, higher levels of glycosylated hemoglobin, and more complications of diabetes [68]. Late meal timing may impair glucose tolerance for a number of reasons: (1) eating during an unfavorable circadian phase; (2) eating concurrently with increased melatonin concentrations; and (3) late meal timing causes internal misalignment (main working hypothesis). According to the latter hypothesis, misalignment of food intake may lead to dissociation of central and peripheral clocks in metabolically active tissues [69], which has been confirmed experimentally [70]. However, to date, there is no direct evidence that internal desynchrony per se negatively affects glucose control [71], suggesting that the first two mechanisms may also be important. Anyway, late meal time can be considered as a risk factor for carbohydrate metabolism disorders [10]. These data may serve as a basis for recommending that patients with carbohydrate metabolism disorders eat earlier in the day and refrain from eating later in the day.

Changing the distribution of calories between meals (even if meal times have not changed) also affects metabolic risk factors. According to various authors, increased consumption of caloric food at lunchtime and evening contributes to the accumulation of visceral adipose tissue, the development of liver steatosis, abdominal obesity and disorders of carbohydrate metabolism [71]. G.K.W. Leung et al. suggested that daily glycemia fluctuations also depend on the distribution of consumed carbohydrates with different glycemic index during the day. Indeed, diurnal fluctuations in blood glucose concentrations were maximized when a higher carbohydrate meal followed a lower carbohydrate meal [71]. This fact is important to take into account in the training and organization of nutrition of persons with carbohydrate metabolism disorders.

Thus, the most significant risk factors for the development of carbohydrate metabolism disorders associated with circadian dysfunction include irrational distribution of caloric intake during the day, late breakfast and dinner, shift in bedtime, shortened sleep duration, exposure to artificial light in the evening, as well as too short and long intervals between meals.

CIRCADIAN RHYTHMS OF CARBOHYDRATE METABOLISM IN PATHOLOGY

Obesity

Most studies that have focused on carbohydrate metabolism rhythms have not considered obesity types and gender differences [5, 9]. At the same time, these factors are among the key determinants that determine the pathogenesis of metabolic syndrome and carbohydrate metabolism disorders. It is well known that when it comes to obesity, it is largely gender that determines the regional distribution of adipose tissue, in turn influencing cardiometabolic risk factors [72].

The accumulation and distribution of adipose tissue in different depots differs significantly between men and women, which is reflected in the development timing of metabolic and associated disorders. A comparative analysis of gender differences in fat accumulation in ontogenesis has allowed us to establish that in all age periods, subcutaneous distribution of fatty tissue predominates in wom-

en, while in men, in most cases, its accumulation in the abdominal region is greater [73, 74]. However, the dynamics of adipose tissue accumulation still has a dependence on age. In the case of overweight and obesity at a younger age, for example, the gynoid type dominates in males. In older age groups (by the end of the first period of adulthood) in males, active accumulation of fat in the abdominal region leads to an equal incidence of gynoid and android types of obesity, after which the android type of obesity begins to predominate [73].

In women, the gynoid type predominates in the presence of overweight and obesity until the end of the second period of adulthood, and thereafter, as age increases (including in old age), there is an increase in the occurrence of the android type of obesity [74]. What is interesting is that the age at which the android type of obesity begins to predominate occurs about two decades later in women than in men. This largely determines the fact that obese men are characterized by an earlier onset of carbohydrate metabolism disorders and cardiovascular diseases, while in women these disorders begin to occur much more frequently at the end of the reproductive period. This ultimately has an overall impact on longer life expectancy in women [73].

Recently, more and more attention has been paid to the study of so-called "metabolically healthy obesity", in which individuals with a characteristic phenotype lack metabolic abnormalities [75, 76]. The properties of "metabolically healthy" were found to be more characteristic of gynoid obesity with a high ratio of subcutaneous to abdominal fat. These individuals with a lower type of fat distribution are less characterized by hyperglycemia, hypoadiponectinemia, and insulin resistance, which are precursors to the development of diabetes mellitus and cardiovascular disease [21].

Women with different types of obesity were found to have different diurnal rhythms of glycemia and insulinemia. For example, women with gynoid type of obesity are more characterized by functional hyperinsulinemia provoking postprandial hypoglycemia. The latter, as we have shown earlier, occurs due to increased glucose utilization in peripheral tissues, which suggests that in this phenotype glucose is the main source of energy and substrate of lipogenesis in adipose tissue during light and dark hours of the day [21].

In women with android type obesity, the pattern of the glycemic curve during OGTT is similar to that of women with normal body weight, both in the morning and in the evening. However, comparable glucose levels in this type of obesity are achieved at the cost of significant postprandial hyperinsulinemia. During OGTT, android-type obese women had 4-fold higher levels of immunoreactive insulin (IRI) at the 60th minute of the test in the morning compared with normal-weight women, and 2-fold higher levels in the evening. This significant difference is the result of insulin resistance due to the high metabolic activity of visceral adipose tissue. An interesting fact is that in the group of women with android type of obesity, a more pronounced increase in blood glucose levels at the 60th minute of the test in the evening occurred in the setting of lower insulin levels than when a similar OGTT was performed in the morning. Apparently, this phenomenon is indicative of the developing functional exhaustion of the insular apparatus in the afternoon [77], which is a precursor to type 2 DM.

Thus, in both types of obesity there are disorders in the diurnal rhythms of carbohydrate metabolism, but they are mediated by different mechanisms. In gynoid obesity carbohydrate load provokes hyperinsulinemia and hypoglycemia, and in android obesity – insulin resistance, hyperglycemia and compensatory hyperinsulinemia, which determines the peculiarities of the pathogenesis of the obesity different types and carbohydrate metabolism disorders development.

Type 2 diabetes mellitus

A large number of studies have found that the rhythms of glucose tolerance, insulin levels, and insulin sensitivity characteristic of healthy individuals may be impaired, inverted, or absent in individuals with type 2 DM [23, 29]; these patterns may also change in older age [25]. Studies with hyperglycemic clamp in individuals with type 2 DM have demonstrated an inverted glucose tolerance profile, with glucose tolerance improving throughout the day while awake [25].

In a study with 24-hour glucose infusion to adult patients with type 2 DM and obese non-diabetic individuals with comparable BMI, glucose levels were found to be highest in the morning and lowest in the evening [78]. Moreover, the amplitude of glycemic fluctuations was approximately 2-fold higher in individuals with type 2 DM compared with those with obesity. While increased glycemia at night correlated with increased cortisol levels, increased insulin secretion at night corresponded to increased glucose levels only in obese subjects but not in those with type 2 DM. In fact, no temporal rhythms of insulin secretion rate were detected in subjects with type 2 DM. These differences in the rhythms of glycemia and insulinemia fluctuations may be due in part to differences in the timing and amplitude of the cortisol rhythm in individuals with diabetes compared to healthy ones [28].

Two other studies of patients with type 2 DM have shown a lack of rhythmicity in muscle glycogen stores [25] and peripheral insulin sensitivity [79]. Nevertheless, adults with type 2 DM show distinct rhythms of hepatic glycogen accumulation and hepatic insulin sensitivity [78]. As a result, overall insulin sensitivity in individuals with type 2 DM reaches a maximum at about 7:00 PM. And a minimum in the morning. This rhythm of liver sensitivity to insulin may explain the well-known "dawn phenomenon" (fasting hyperglycemia). Increased endogenous glucose production during the night hours also contributes to the fasting/morning hyperglycemia observed in type 2 DM [79].

Thus, circadian rhythm disorders are also accompanied by carbohydrate metabolism disorders, playing a role in the pathogenesis of metabolic diseases. The present data demonstrate that shifts in circadian rhythms caused by untimely exposure to light, sleep, and meals impair glycemic

control and increase the risks of obesity and type 2 DM. Whether interventions that restore normal circadian rhythm can actually prevent or have a favorable effect on the course of metabolic diseases remains completely unclear.

Perhaps the differences between glucose metabolism in individuals with type 2 DM and without carbohydrate metabolism disorders may be related to impaired functioning of the central biological clock of the SCN in type 2 DM. In particular, the number of arginine-vasopressin-immunoreactive neurons (AVIN), VIP neurons (VIPN) and glial fibrillary acidic protein immunoreactive (GFAP-ir) astroglial cells are significantly reduced in the SCN among type 2 DM patients compared to healthy individuals [80].

CONCLUSION

Thus, circadian rhythms of carbohydrate metabolism are determined by diurnal variations in a large number of metabolic processes, including β-cell sensitivity, peripheral insulin sensitivity, insulin clearance, and the amount of fat and its ratio in various depots. Circadian rhythms of carbohydrate metabolism were formed in phylogenesis under the influence of natural factors of the human environment, which determine physiological needs and functional energy expenditure necessary for the realization of life activity processes. The circadian rhythm of carbohydrate metabolism, first of all, predetermines the phasicity of glucose usage as an energy substrate. So, in the morning and afternoon, its usage is determined by: light, awakening, hunger, hormonal regulators (cortisol, insulin), motor performance, food intake and other regulatory factors. In the evening and at night, physiologic insulin resistance promotes a switch to fat metabolism to rid somatic cells of excess lipids and prevent lipotoxicity.

In current conditions, when a person is characterized by hypodynamia; excessive and extended practically for the whole time of day consumption of nutrients, and, above all, refined carbohydrates; light stress; psychoemotional stress associated with the release of glucocorticoids, both central and molecular mechanisms of circadian rhythms maintenance are disturbed, which, in turn, increases the negative impact of exogenous factors on human metabolism parameters, forming a vicious circle of the pathological process risk factor formation. In this regard, the question arises, what is primary: disorders of circadian rhythms, which contribute to the development of metabolic pathology, or they are secondary and only strengthen the metabolic disorders formed under the influence of exogenous factors? The answer to this question will largely help to find pathogenetic approaches to dietary and drug correction of carbohydrate metabolism in the treatment of diseases and conditions such as obesity, DM, dyslipidemia, atherosclerosis and other endocrine-exchange disorders.

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Conflict of interest

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

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NEUROLOGY AND NEUROSURGERY

BIOLOGICAL AND PHYSICAL MECHANISMS OF CEREBRAL ANEURYSMS FORMATION, GROWTH AND RUPTURE

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ABSTRACT

According to various researchers, the prevalence of unruptured cerebral aneurysms (CAs) in the general population varies from 2 to 5 %. In the vast majority of cases, CAs do not have clinical and neurological manifestations and are discovered incidentally during routine neuroimaging studies. CAs can cause intracranial hemorrhage. As a rule, hemorrhages of this type occur in patients aged 40–60 years. It has been established that about 10–15 % of patients die from an aneurysmal hemorrhage before they receive specialized medical care. Recurrent aneurysmal intracranial hemorrhage is the main cause of high mortality and disability in this group of patients. The search for literature sources in the scientific databases PubMed/Medline, EMBASE, Cochrane Library and eLibrary demonstrated the existence of numerous studies devoted to the study of molecular biology and biophysical mechanisms of formation, growth and rupture of CAs. Combining the results of these studies was the motivation for writing this literature review. The paper reflects in detail the role of inflammation and molecular genetic factors in the growth and rupture of the CAs, and presents the biophysical factors of the rupture of the CAs. The authors pay special attention to the shape, size and coefficient of the CAs as the most important geometric risk factors for the formation and rupture of the CAs. This review presents current data on mathematical modeling of various types of CAs with an assessment of the risk of rupture of the latter, which has found its application in wide clinical practice. The authors also attempted to describe the hemodynamic features in various types of CAs. In turn, the type of blood flow in the CAs cavity largely depends on the size and shape of the latter and the geometry of the carrier artery, which is the basis for preoperative planning and the choice of tactics for surgical treatment of patients with unruptured CAs.

Key words: *cerebral aneurysms, formation, growth, rupture, inflammation, biology, biophysics, mathematical model*

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БИОЛОГИЧЕСКИЕ И БИОФИЗИЧЕСКИЕ МЕХАНИЗМЫ ФОРМИРОВАНИЯ, РОСТА И РАЗРЫВА ЦЕРЕБРАЛЬНЫХ АНЕВРИЗМ

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РЕЗЮМЕ

По данным различных исследователей, распространённость неразорвавшихся церебральных аневризм (ЦА) в общей популяции варьирует от 2 до 5 %. В подавляющем большинстве случаев ЦА не имеют клинико-неврологических проявлений и обнаруживаются случайно при выполнении плановых нейровизуализационных исследований. ЦА может явиться причиной внутричерепного кровоизлияния. Как правило, кровоизлияния такого типа встречаются у пациентов в возрасте 40–60 лет. Установлено, что около 10–15 % пациентов умирают от аневризматического кровоизлияния до оказания им специализированной медицинской помощи. Повторное аневризматическое внутричерепное кровоизлияние выступает основной причиной высокой летальности и инвалидизации указанной группы пациентов. Проведённый поиск литературных источников в научных базах данных PubMed/Medline, EMBASE, Cochrane Library и eLibrary продемонстрировал наличие многочисленных исследований, посвящённых изучению молекулярной биологии и биофизических механизмов формирования, роста и разрыва ЦА. Объединение результатов указанных исследований и явилось побудительным моментом к написанию данного литературного обзора. В работе детально отражена роль воспаления и молекулярно-генетических факторов в росте и разрыве ЦА, представлены биофизические факторы разрыва ЦА. Особое значение авторами уделено форме, размерам и коэффициенту ЦА как важнейшим геометрическим факторам риска формирования и разрыва ЦА. В настоящем обзоре представлены современные данные о математическом моделировании различных типов ЦА с оценкой степени риска разрыва последних, что нашло своё применение в широкой клинической практике. Также авторами предпринята попытка описания гемодинамических особенностей в различных типах ЦА. В свою очередь тип кровотока в полости ЦА во многом зависит от размера, формы последней и геометрии несущей артерии, на чём основано предоперационное планирование и выбор тактики хирургического лечения пациентов с неразорвавшимися ЦА.

Ключевые слова: *церебральные аневризмы, формирование, рост, разрыв, воспаление, биология, биофизика, математическая модель*

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INTRODUCTION

According to autopsy data, the prevalence of cerebral aneurysms (CAs) is 1–5 % of all deaths [1]. Morphologically, CAs are characterized by fragmentation of the internal elastic lamina with damage to the endothelial lining of the vessel, which eventually leads to changes in all layers of the vascular wall in the form of aneurysmal bulge formation, which can be considered as a pathological formation and, at the same time, a compensatory mechanism to reduce the local hemodynamic load on the vascular wall [2, 3]. In the vast majority of cases, CAs do not have clinical and neurological manifestations and are discovered incidentally during routine neuroimaging studies. However, CA can be a cause of intracranial hemorrhage. As a rule, hemorrhages of this type occur in patients aged 40–60 years [4]. The incidence of CA rupture has been shown to increase from 3 per 100,000 population in the group under 30 to 30 per 100,000 population among people over 60 [5]. It has also been established that about 10–15 % of patients die from an aneurysmal hemorrhage before they receive specialized medical care. The mortality rate during the first 3 weeks after CA rupture is 20–30 %, within 30 days it reaches 46 %, and more than 30 % of the population is deeply disabled [6, 7]. It is important to emphasis that recurrent aneurysmal intracranial hemorrhage is the main cause of high mortality and disability in this group of patients [7, 8].

The search for literature sources in the scientific databases PubMed/Medline, EMBASE, Cochrane Library and eLibrary demonstrated the existence of numerous studies devoted to the study of molecular biology and biophysical mechanisms of formation, growth and rupture of CAs. Undoubtedly, knowledge of these mechanisms will allow optimizing existing and developing new methods of treatment for patients with CA in the near future.

THE AIM OF THE STUDY

Analysis of current literature data devoted on the study of biological and biophysical mechanisms of formation, growth and rupture of cerebral aneurysms.

MOLECULAR BIOLOGY OF CA

Role of inflammation

Some studies have shown that vascular wall inflammation plays a crucial role in the formation and growth of CA [9]. Thus, N. Chalouhi et al. [10] noted in their study that constant pronounced hemodynamic impact on the vascular wall leads to the activation of inflammatory process in the latter with the participation of matrix metalloproteinases (MMPs), smooth myocytes, macrophages and the development of oxidative stress. Endothelial dysfunction resulting from a number of modifiable and non-modifiable risk factors (smoking, arterial hypertension, local blood flow disturbance in cerebral vessels, genetic factors) represents the initial stage of CA formation. Oxidative stress initiates the process of vascular wall destruction due to the accumulation of free radicals and destruction of structural elements of the endothelial lining [10, 11].

The next stage of vascular wall destruction is activation of the inflammatory process that involves macrophages, mast cells, T-lymphocytes and a number of proinflammatory mediators and cytokines [12]. A long-lasting inflammation process leads to changes in the phenotype of smooth muscle cells and remodeling of the vascular wall towards the synthesis of extracellular matrix components in the middle coat of the vessel [13]. Changes in the phenotype of smooth muscle cells contribute to the degradation of the internal elastic lamina, impaired collagen synthesis and dysregulation of the synthesis of extracellular matrix components [13]. Vascular wall remodeling is directly related to the degree of nitric oxide (NO) production [13, 14]. It is well known that NO is the most important angioprotector, but only if it is synthesized in adequate amounts. Inadequate NO content in the vascular endothelium may determine the development of endothelial dysfunction and a number of pathological conditions [14]. Thus, NO hyperproduction may result from excessive activation of endothelial NO synthase (eNOS). NO, formed as a result of this process, when interacting with superoxide anion radical can be converted into very toxic substances (peroxynitrite, nitrotyrosine), which have a number of pathogenic effects on the vascular wall (increased oxidation of proteins and lipids, inactivation of enzymes, including mitochondrial enzymes, increased permeability of cytoplasmic membranes, damage to nucleic acids and activation of apoptosis). According to modern concepts, impaired NO bioavailability is the main cause of endothelial dysfunction in the presence of risk factors (arterial hypertension, coronary heart disease, diabetes mellitus, metabolic syndrome) [15].

The final stage of CA formation is apoptosis of smooth muscle cells, which leads to thinning of middle coat of the vessel and increased risk of vessel rupture [16]. In addition, macrophages, attracted by proinflammatory mediators and cytokines into the vascular wall thickness, begin to produce large amounts of MMPs, which break down collagen and other components of the extracellular matrix [17]. All this inevitably leads to additional thinning of the vascular wall, potential formation of CA with its subsequent rupture and development of intracranial hemorrhage. A schematic representation of the role of inflammatory factor in CA growth and rupture is shown in Figure 1.

Role of genetic factors

The genetic disposition to CA formation is well studied. The association of CA with various hereditary nosological forms has been proved, genes responsible for the synthesis of structural components of the vascular wall have been identified, and mutations in the latter among patients with CA have been analyzed in detail [18]. There is a high incidence of CA in some families in the absence of evidence of any systemic pathological conditions [18, 19]. Thus, the presence of the following chromosome loci is statistically significantly associated with familial CA:

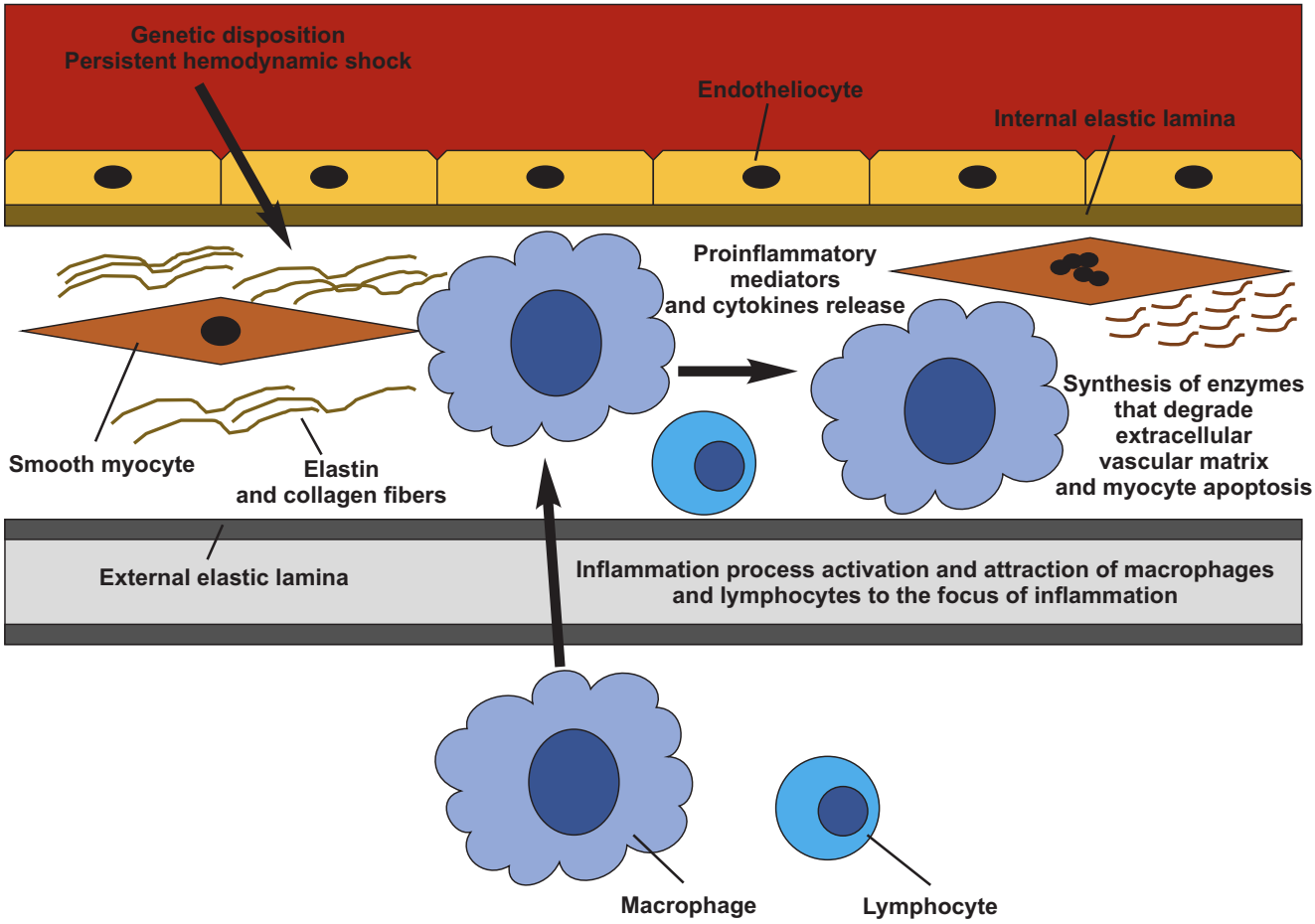

FIG. 1.
Schematic representation of the role of the inflammatory process in CAs growth and rupture

1p34.3-p36.14, 19q13.3, Xp22 and 7q11[20]. The 7q11 locus contains the *COL1A2* gene, the product of which is collagen type I, as well as an adjacent gene responsible for elastin synthesis. In turn, collagen type I and elastin represent the structural basis of the vascular wall [21]. C.B. Theodotou et al. [22] showed in their systematic review that the loci of 9p21/CDKN2 chromosomes are responsible for the process of the vascular wall remodeling and are statistically significantly associated with CA rupture. The study of K. Bilguvar et al. [23], involving more than 2000 patients with CA and 8000 control group respondents, demonstrated that the presence of single-nucleotide polymorphisms in the loci of 2q33.1, 8q11.23 and 9p21.3 chromosomes is statistically significantly associated with cases of sporadic and familial CA. Other potential genetic targets for study on CA formation and growth are MMPs, angiotensin-converting enzyme (ACE), phospholipase C, eNOS and other genes [23]. At the same time, the authors of these studies do not exclude the role of external factors in the formation and rupture of CA.

The International Study of Unruptured Intracranial Aneurysms (ISUIA) analyzed unruptured CAs taking into account patient demographics and the localization of multiple CAs. It was found that more often multiple CAs are localized in the region of the middle cerebral artery (28.6 %) and posterior communicating arteries (13.7 %) [24]. The risk of developing CA is statistically significantly higher in families with a history of CA, especially in Japan and Finland. Globally, about 3 % of the population suffers from CA, but the incidence of aneurysms in Finland is twice as high. Three new loci on 18q11.2 and 10q24.32 chromosomes associated with CA were identified among the Finnish population. Three loci were associated with CA (2q23.3; 5q31.3; 6q24.2) and one with the number of CA (7p22.1). The 7p22.1 locus was more frequent in Finland (4.6 %) than in the Netherlands (0.3 %). Five loci account for 2.1 % of inherited CA in Finland [25]. The previously mentioned *COL1A2* gene has been associated with aneurysms among patients from Japan, China, and South Korea. Nevertheless, this does not fully explain the formation of most CA [25].

Currently, a number of inherited diseases are known to be associated with CA formation, growth and rupture. They include Ehlers-Danlos syndromes (types I and IV), Fabry disease, Osler – Weber – Rendu disease, Pompe disease and autosomal dominant polycystic kidney disease (ADPKD) [1, 25]. Hereditary diseases associated with CA are summarized in Table 1. ADPKD is associated with mutation of *PKD1* and *PKD2* genes [1, 25]. The frequency of occurrence of CA among patients with ADPKD is 10–13 %, and at least 25 % of this number have a positive family history of CA with/without the development of intracranial hemorrhage [25].

TABLE 1

HEREDITARY DISEASES ASSOCIATED WITH CEREBRAL ANEURYSMS [1]

Disease	Type of inheritance	Chromosome locus	Gene
Alkaptonuria	Autosomal recessive	3q2	*AKU*
Thoracic aortic aneurysm	–	9q, 3p8, 1p	–
Autosomal dominant polycystic kidney disease (ADPKD)	Autosomal dominant	16p13.3 4q21	*PKDI* *PKD2*
Achondroplasia	Autosomal dominant	4p16.3	*FGFR3*
Osler – Weber – Rendu disease	Autosomal dominant	9q34.1 12q	*HHT1* *HHT2*
Pompe disease	Autosomal recessive	17q23	GAA
Fabry disease	Autosomal recessive, X-linked	Xq22.1	*GLA*
Osteogenesis imperfecta (type I)	Autosomal dominant	17q22.1 7q22.1	*COL1A1* *COL1A2*
Neurofibromatosis (type I)	Autosomal dominant	17ql1.2	*NF1*
Wermer's syndrome	Autosomal dominant	llql13	*MEN1*
Kahn syndrome	Autosomal recessive	9	0
Cohen syndrome	Autosomal recessive	8q21	*CHSI*
Marfan syndrome	Autosomal dominant	15q21.l	*FBNI*
Noonan syndrome	Autosomal dominant	12q22	9
Rembaud syndrome	Autosomal recessive	9	9
Williams – Beuren syndrome	–	7q11	–
Ehlers – Danlos syndrome (type I)	Autosomal dominant	9q	*COL5A1*
Ehlers – Danlos syndrome (type IV)	Autosomal dominant	2q31	*COL3A1*
Tuberous sclerosis	Autosomal dominant	9q34.1 16ql3.3	*TSC1* *TSC2*
Chronic obstructive pulmonary disease (COPD)	–	19p13.3 14q32	–
Pseudoxanthoma elasticum (PXE)	Autosomal dominant Autosomal recessive	9	9

Analysis of the significance of biochemical markers of connective tissue protein breakdown is a promising area of the study of the pathophysiology of CA formation and growth. Currently, some amino acids and their various forms (oxyproline, hydroxyproline), as well as glycosaminoglycans are the main markers of connective tissue disorder [26–29]. Thus, the study by M.A. Nokhsorova et al. [26] showed that the parameters of the content of some amino acids and their various forms can act as markers of early diagnosis of connective tissue dysplasia (CTD). Similar results were obtained by T.A. Siraeva et al. [27] in their study on pediatric patients with glomerulonephritis. The study by L. Wang et al. [28] and Y. Guo et al. [29] demonstrated the dependence of the level of some amino acids in plasma and urine of patients with aortic dissection and aortic aneurysm. B. Sokół et al. [30] point to a statistically significant correlation between the level of certain amino acids in the cerebrospinal fluid (CSF) and the risk of CA rupture.

BIOPHYSICS OF CEREBRAL ANEURYSMS

Geometric factors

Sizes of cerebral aneurysms

The maximum size of CA is generally considered to be the main risk factor for CA rupture. According to J. Beck et al. [31] and M. Korja et al. [32], in 70–80 % of cases the maximum diameter of unruptured aneurysms is 10 mm. Large (16 to 25 mm) and giant (more than 25 mm) CAs are less common. The clinical and experimental observation of J. Suzuki and H. Ohara [33] showed that the wall of CA, the size of which is not more than 3 mm, is formed by endothelial lining and fibrous tissue, and at the size of CA 4 mm and more, a large number of coarse collagen fibers appear in the wall of the latter. Such morphological changes in the vascular wall significantly reduce its elastic properties with the formation of thinning areas [33]. The changes reduce the degree of resistance of the vascular wall to hemodynamic loads. On the other hand, some authors claim that the difference between the diameters of ruptured and unruptured CA is no more than 1.5 mm and has no statistically significant effect on the risk of CA rupture [34].

A detailed study of the dependence of large CA rupture without taking into account other factors is an extremely difficult problem due to the fact that the analysis of the influence of hemodynamic risk factors gives ambiguous and, in some cases, contradictory results. According to the study of P.B. Canham and G.G. Ferguson [35], CAs with a size of 5 to 9 mm have the highest risk of rupture. However, it is known that the blood flow velocity in the CA is inversely proportional to its dome diameter squared, i. e., as the CA size increases, the blood flow velocity in the cavity of the latter will decrease. A decrease in blood flow velocity will lead to a decrease in the hemodynamic load on the vascular wall. Similar data were obtained by S. Tateshima et al. in their study [36].

Shapes of cerebral aneurysms

It has been proven by numerous studies that the shape of CA has a greater impact on the risk of CA rupture as opposed to its size. Oval, oblong or lobulated CAs have been shown to have a high risk of rupture [36]. According to S. Tateshima et al. [36], in the CA dome region the greatest wall shear stress is noted. The prevalence of ruptured multicameral CAs is statistically significantly higher by 2–7 times [37]. The irregularity of CA contours according to the data of digital subtraction angiography (DFA) is an important risk factor of CA rupture, which confirms thinning of the CA wall, disturbance of its elastic properties and presence of thrombotic masses [38]. C. Sadasivan et al. [39] in their clinical observation noted that CA of the indicated type is statistically significantly associated with rapid growth and a high risk of rupture.

The average CA wall thickness is 16–400 μm [40]. It is known that the CA wall has less pronounced elastic properties in contrast to the arterial wall. At the same time, the stretchability of the CA wall differs in its different parts [40]. The study by J.G. Isaksen et al. [41] clearly showed that in hemodynamic systole the maximum degree of stretching of the CA wall occurs in the region of its *locus minoris resistentia* – the dome wall. The lobular structure of the CA wall confirms the heterogeneity of elasticity of its different sections and correspondingly lower resistance to hemodynamic shocks in comparison with CA of the correct spherical shape [42].

Ratio of cerebral aneurysms

The CA ratio is the ratio of the CA dome height to the width of its neck. Comparison of the values of the ratios of ruptured and unruptured CA indicates a statistically significant predominance of this parameter in the group of ruptured CA (2.4 vs 1.6, respectively) [43]. The clinical case series by H. Ujiie et al. [44], including 129 respondents with ruptured CA and 72 respondents with unruptured CA, showed that the value of the ratio of ruptured CA exceeds 1.6. The unruptured CA ratio, in turn, does not exceed 1.6 [44]. The authors of this study conclude that if the ratio value is less than 1.4, it is safe to say that the risk of CA rupture is low; if the ratio is more than 3, the risk of CA rupture increases significantly [44].

Ratio of CA dome height to the diameter of the parent artery

The ratio of CA dome height to the diameter of the parent artery as a risk factor for its rupture was proposed for the first time by M. Tremmel et al. [45]. The authors statistically significantly found that in 77 % of cases of ruptured CA the ratio of CA dome height to the diameter of the parent artery exceeded 2.05, in contrast to unruptured CA – less than 2.05 [45]. The authors also clearly showed that a 5 mm CA located on the anterior communicating artery with a diameter of 2 mm has a ratio of 2.5, which indicates a very high risk of its rupture, while a CA of similar size but located on the internal carotid artery with a diameter of 4 mm has a ratio of 1.25 and a much lower risk of rupture (about 10 times) [45]. The prospective clinical study by M. Rahman et al. [46] noted that the ratio of CA dome height to the diameter of the parent artery is a sta-

tistically significant risk factor for CA rupture (OR = 2.12; 95% CI: 1.09–4.13).

Mathematical models of cerebral aneurysms

Currently, there are a number of studies on the predictive value of mathematical models of CA growth and rupture risk and its formation. Thus, A.L. Rogozin [47] presented and studied in detail the prognostic value of a mathematical model of the risk of rupture of the internal carotid artery CA. The authors have developed the following formula:

$$P = \frac{1}{1+e^{-z}},$$

where P is the probability of CA rupture, $z = b_1 \times X_1 + b_2 \times X_2 + ... + b_n \times X_n + a$, X – values of independent variables, b – regression ratios, a – constant, e – base of natural logarithm. A more extended mathematical model with several parameters was developed and studied in detail by H. Meng et al. [48]. The researchers presented the final formula as follows:

$$\eta(\lambda,\mu) = \frac{\mu\left(1+\sqrt{1-\mu^2}\right)}{4} \times \frac{\left(1+\lambda^2\right)}{\lambda},$$

where μ is the ratio of CA neck to the parent artery radius, λ is the CA ratio, η is the value of the CA stress factor, which is a function of the ratio of CA neck to the parent artery radius and the CA ratio. In contrast to previous mathematical models, R. Berguer et al. [49] presented a trigonometric model of CA formation, where special attention is paid to the angle between daughter arterial branches forming a bifurcation CA:

$$\cos\theta = \sqrt{\frac{1}{2\beta}},$$

where β is the ratio of the CA neck area to the diameter of the parent artery, θ is the angle between the daughter arterial branches forming the bifurcation CA.

It is clear that the presented mathematical models cannot fully characterize all the processes occurring in the CA cavity and statistically significantly assess the risk of its rupture. Nevertheless, some neurosurgical clinics in the world actively use mathematical models as a rationale for selecting surgical treatment tactics for CA patients in a particular clinical situation.

Hemodynamics of cerebral aneurysms

Blood flows in the CA cavity into simple steady and complex unsteady, or turbulent blood flows [1]. Simple steady blood flow in the CA cavity has a unidirectional constant motion during a single cardiac cycle and may rarely have a single vortex with a constant or changing localization. The turbulent flow is usually unsteady and has a multidirectional motion with many swirls of different localizations in the CA cavity [1, 3].

The type of blood flow in the CA cavity depends largely on the size, shape of the CA and the geometry of the parent artery [1, 16]. In some cases, the blood flow directed into the CA cavity has a high velocity, small width and has a significant hemodynamic effect on certain regions of the CA wall. In other cases, the blood flow is wider and slower, and exerts less hemodynamic shock on the CA wall [1, 16].

Particular attention should be paid to hemodynamic features in bifurcation CAs. Thus, the blood flow velocity in a narrow-neck CA is significantly lower than in the parent artery. The blood flow velocity is higher in a wide-neck CA than in a narrow-neck CA. In a wide-neck CA, blood exchange with the cavity of the parent arterial trunk occurs in greater volume than in a narrow-neck CA [1, 50]. Moreover, the risk of thrombosis is much higher in narrow-neck CA [50].

CAs, which are located at asymmetric arterial bifurcations, have individual hemodynamic characteristics. The part of the CA neck that is adjacent to the larger-diameter daughter artery is subjected to the highest blood pressure, while the part of the neck belonging to the smaller daughter arterial trunk experiences a large degree of stretching under the pulsatile blood flow [51]. The blood volume in the smaller daughter arterial branch comes from the CA cavity, while the larger daughter branch is filled from the parent artery [51].

As for the hemodynamic features of lateral CAs, the filling of the CA cavity with blood is carried out in the distal part of the CA neck. The process of constant change of the blood flow direction occurs in the CA cavity, and the blood exit is verified in the proximal part of the CA neck [52]. The hemodynamic pressure on the distal part of the CA neck is higher than on the proximal part and on the CA dome [53, 54]. According to C.M. Strother et al. [55], lateral CA grows in the direction of blood flow due to the stretching of the CA wall in the distal part of the neck.

The report by D.D. Dolotova et al. [56] showed that a vessel branching from the neck or dome of CA causes their classification as "complex" not only because of the difficulties of surgical intervention, but also because an additional vascular branch and its disconnection from the blood flow can have a significant effect on the change in the parameters of local hemodynamics. The nature of these changes may be determined by such factors as the diameter of the vessel arising from the CA and the location of the CA relative to the parent vessel [56]. The authors of the study also noted that the hemodynamic parameters of bifurcation CAs were much less susceptible to changes: virtual "removal" of the vessel had an insignificant effect on the neck wall and the CA dome located on the flow path from the parent vessel. In lateral CAs, the behavior of the velocity profile and wall shear stress was more diverse, which can be explained by taking into account the totality of local and systemic factors [56].

CONCLUSION

Currently, a great number of studies on the biological and biophysical mechanisms of CA formation, growth, and rupture has been conducted. The role of the inflammatory process, molecular genetics and hemodynamic factors

has been confirmed by numerous experimental and clinical studies. The analysis of risk factors for CA growth and subsequent rupture allows to predict the course of this disease, to choose optimal methods of surgical treatment of this group of patients or to monitor patients with unruptured CA. Undoubtedly, further study of the indicated mechanisms of CA growth and rupture will allow to study in depth the peculiarities of this nosological form from the positions of both fundamental and applied science. This kind of multidisciplinary approach opens up new opportunities in terms of development and introduction of the latest methods of diagnosis and surgical treatment of patients with CA into widespread clinical practice in the near future.

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Conflict of interest
The authors declare the absence of a conflict of interest.

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THE RESULTS OF SURGICAL TREATMENT OF SACRAL SCHWANNOMAS WITH EXTENSION INTO PELVIC CAVITY

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ABSTRACT

The aim. *To analyze the results of surgical treatment of sacral schwannomas with their extension into lower pelvis.*

Materials and methods. *We analyzed the clinical results of surgical treatment of 25 patients with sacral schwannomas, including those with ventral extension and growth into pelvic cavity. All patients were operated at the Clinic of Neurosurgery of the Novosibirsk Research Institute of Traumatology and Orthopedics named after Ya.L. Tsivyan for a 10-year period. The analysis of literature describing prevalence, classification, diagnosis and surgical treatment of sacral tumors and schwannomas in particular was carried out. The features of the course, diagnosis and surgical treatment of sacral schwannomas and accepted surgical methods for their treatment are described.*

Results. *The average follow-up period of the patients in our study was 61.6 ± 2 months. Compared with the initial indicators of patients in the postoperative period (1 year after the surgery), a good clinical result with the regression of symptoms was registered in 17 (68 %) out of 25 patients, and 10 (40 %) of them had a significant regression of neurological disorders. 3 (12 %) operated patients had a deterioration of neurological disorders in the form of aggravation of sensitive disorders; one patient had an aggravation of pelvic organ dysfunction. Radical removal of the tumor was achieved in 24 (96 %) out of 25 cases; subtotal resection was performed in 1 (4 %) patient. The number of relapses and continued growth of tumors in our series was 12 % (3 out of 25 patients). The mortality rate was 0 %. The complications were registered in 3 (12 %) cases and manifested as wound liquorrhea in cases of using posterior approach.*

Conclusion. *The degree of radicality of tumor resection and clinical outcomes is directly related to the selected surgical approach.*

Key words: *sacral schwannomas, retroperitoneal space neurinoma, tumors of the retroperitoneal space of the pelvis and the anterior wall of the sacrum*

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РЕЗУЛЬТАТЫ ХИРУРГИЧЕСКОГО ЛЕЧЕНИЯ ШВАННОМ КРЕСТЦА С РАСПРОСТРАНЕНИЕМ В ПОЛОСТЬ МАЛОГО ТАЗА

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РЕЗЮМЕ

Цель исследования. *Анализ результатов хирургического лечения шванном крестца с распространением в малый таз.*

Материалы и методы. *Проведён анализ клинических результатов хирургического лечения 25 пациентов с шванномами крестцовой локализации, в том числе с вентральным распространением и ростом в полость малого таза, оперированных в клинике нейрохирургии Новосибирского НИИТО им. Я.Л. Цивьяна Минздрава России за 10 лет. Проведён анализ литературных данных, описывающих распространённость, классификации, диагностику и хирургическое лечение опухолей крестца и шванном в частности. Описаны особенности течения, диагностики и хирургического лечения опухолей данной локализации, принятые хирургические подходы.*

Результаты. *Период наблюдения за пациентами в нашем исследовании составил в среднем 61,6 ± 2 мес. По сравнению с исходными показателями больных в послеоперационном периоде (на сроке 1 год) хороший клинический результат с регрессом симптоматики отмечен у 17 (68 %) из 25 пациентов, причём у 10 (40 %) из них имелся значительный регресс неврологических нарушений. У 3 (12 %) оперированных отмечено усугубление неврологических нарушений в виде усугубления чувствительных нарушений, у одного пациента отмечено усугубление нарушений функции тазовых органов. Радикальное удаление новообразования достигнуто в 24 (96 %) из 25 случаев; субтотальная резекция проведена у 1 (4 %) пациента. Число рецидивов и продолженного роста новообразований составило в нашей серии 12 % (3 из 25 пациентов). Летальность составила 0 %. Осложнения, встречаемые в нашей группе, были отмечены в 3 (12 %) случаях и проявлялись в виде раневой ликвореи в случаях задних доступов.*

Заключение. *Степень радикальности резекции опухоли и клинических исходов напрямую связана с выбранным операционным доступом.*

Ключевые слова: *шванномы крестца, невринома забрюшинного пространства, опухоли забрюшинного пространства малого таза и передней стенки крестца*

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INTRODUCTION

Primary spinal cord tumors account for 5 % to 10 % of all spinal neoplasms in adults and 4.5 % of all central nervous system (CNS) tumors [1, 2]. Their incidence is 1.3 cases per 100,000 populations per year [2]. Extramedullary neoplasms occur in 70-80 % of all primary spinal cord tumors and in 53–68.5 % of all spinal cord neoplasms [2–4]. The most common histologic variants of extramedullary tumors are meningiomas (24.4 %), ependymomas (23.7 %), and neurinomas (21.2 %) [5–7]. Tumors of the sacrum account for 1–7 % among spinal tumors [1, 2, 4, 8]. Schwannomas account for about 25–29 % of all primary spinal tumors [8]. Retroperitoneal localization of schwannomas is quite rare and accounts for approximately 1 to 5 % of all retroperitoneal localization masses [1, 2, 4–6, 9]. Investigating the problem of sacral tumor treatment, especially in the case of ventral extension and their retroperitoneal localization, it should be noted the late detection of this pathology and sometimes asymptomatic course [2, 6, 10, 11]. There are many factors contributing to this, mainly the presence of sufficient reserve spaces in this area, atypical symptomatology indicated by patients, and difficulties in interpreting diagnostic results [11].

It is believed that the earliest symptom is local pain in the sacral region, and later, with the progression of tumor growth, radicular symptoms appear, and, as a consequence, sciatic manifestations, pelvic disorders, and radicular pain syndrome appear [4, 12, 13].

Currently, there is no single universally accepted classification of sacral tumors due to their great diversity and genetic origin. The two most common classifications described in the current literature are Enneking classification, based on tumor grade, and Weinstein – Boriani – Biagini (WBB) classification [8]. These classifications help to evaluate treatment tactics, also from the oncologic side [8]. K. Sridhar developed a classification of tumors based on magnetic resonance imaging (MRI) [14]. In 2003, P. Klimo proposed a classification scheme of cauda equina tumors at the sacral level depending on the direction of growth and distinguished three types: first – limited to the sacrum (in this case, the tumor can be excised from the posterior approach); second – tumors adhering to/destroying the posterior and anterior walls of the sacrum (in this case, a combined approach is required for tumor excision – anterior and posterior); third – tumors within the presacral space on the anterior wall of the sacrum (in this case, anterior approach is required for excision) [14].

Due to the available topographic-anatomical features of the small pelvis, in some cases surgical treatment can be performed in one or several stages – removal of the intracanalicular portion first, and then the portion from the pelvic cavity. The main problem of tumors extending from the sacral canal into the pelvic cavity, and in our case schwannomas, is the need to make approach to the pelvis.

Despite the improvement of diagnostic methods, the development of new surgical approaches using microsurgical and endoscopic techniques, and the improvement of anesthesia, it has become possible to perform one-stage operations or surgical approaches – both posterior and anterior – to excise tumors of sacral localization [10]. However, even today, the problem of sparing surgical approach to neoplasms of this type, one-stage excise of the entire tumor and minimizing the number of relapses has not been completely solved [4, 15, 16].

The choice of adequate approach in order to obtain a good clinical effect and a high degree of radicality of tumor excision will depend on proper, careful preoperative planning based on imaging the interaction of the tumor with adjacent organs and great vessels [12, 15]. In the case of schwannomas, it is common to adhere to surgical tactics of treatment with maximum radical excision of the tumor substrate in order to obtain a favorable prognosis due to the fact that these tumors are not sensitive to chemo- and X-ray therapy [2, 3, 12, 16, 17].

THE AIM OF THE STUDY

Retrospective analysis of clinical outcomes of sacral schwannoma surgical treatment, including those with extension into lower pelvis.

MATERIALS AND METHODS

An open (non-blind), uncontrolled, non-randomized, single-center, retrospective study of clinical results of surgical treatment of patients with schwannomas in the sacrum, including those with ventral extension and retroperitoneal localization in the pelvis, operated at the Clinic of Neurosurgery of the Novosibirsk Research Institute of Traumatology and Orthopedics named after Ya.L. Tsivyan for a 10-year period was conducted. Inclusion criteria: the presence of schwannomas localized at the level of the sacrum with a confirmed pathomorphologic diagnosis; surgical intervention in accordance with the standard protocol. Exclusion criterion: severe concomitant somatic pathology in decompensation stage.

During this period, the Clinic of Neurosurgery has gathered the experience of surgical treatment of 69 patients with tumors of sacral localization having different histological structure. Among them 25 (36.2 %) were diagnosed with schwannomas of the sacrum; and according to the histopathological classification adopted by the World Health Organization, the neoplasms of all patients were of the I degree of malignancy. There were 16 (64 %) females and 9 (36 %) males; mean age was 49.6 ± 3 years.

The diagnosis of sacral neoplasms, the degree of schwannoma resection, and confirmation of the presence of relapses or continued tumor growth, adhering to the adopted standards, were performed using MRI studies of the spine and spinal cord on ExcelartVantage MR tomographs (Toshiba, Japan) with intravenous injection of contrast agent. Magnetic field strength was 1.5 T, slice thickness – up to 3 mm in T1, T2, FLAIR, DWI modes

(in three projections). If clinical signs that could be accompanied by structural changes of the spine were detected, the patients underwent additional computed tomography (CT) of the spine on Aquilion 64 multislice spiral computed tomography scanner (Toshiba, Japan); slice thickness was 1 mm. Ultrasound diagnostics was also used when necessary [3, 15].

According to P. Klimo's classification, schwannomas were divided into three groups depending on the localization and direction of growth according to MRI and CT data: 16 (64 %) subjects had tumors limited to the sacrum; 6 (24 %) – intracanalicular, with destruction of the anterior wall of the sacrum and with extension into the pelvic cavity; 3 (12 %) patients had tumors that were localized presacral, directly in the retroperitoneal space in the area of the anterior wall of the sacrum, in the pelvic cavity, without extending into the sacral canal (Table 1).

TABLE 1

DISTRIBUTION OF TUMORS BY THE DIRECTION OF GROWTH

Characteristics of growth direction	Number of patients	
	abs.	%
Tumor limited to the sacrum	16	64
Tumor with extension into the pelvic cavity	6	24
Tumor directly in the retroperitoneal space	3	12
Total	25	100

Intradural tumor growth was noted in 12 (48 %) cases, intra-/extradural – in 8 (32 %), and extradural – in 5 (20 %) cases.

The mean tumor volume was 32.6 ± 0.9 cm^3. In this case, the smallest tumor size was 1.2 cm^3 and the largest was 100.6 cm^3. The follow-up of patients in our study series averaged 61.6 ± 2 months.

The clinical picture of the disease in 21 (84 %) patients consisted of pain syndrome, neurological symptoms and pelvic organ dysfunction. In 4 (16 %) patients, the tumor was diagnosed by MRI as an incidental finding. The distribution of clinical manifestations is summarized in Table 2.

Pain syndrome of varying severity in the sacral region occurred in 16 (64 %) cases. Absence of pain symptoms was noted in 9 (36 %) patients. Mild pain degree according to Visual Analogue Scales (VAS) was noted in 12 (75 %) patients, moderate pain – in 3 (18.7 %) cases, in one case (6.3 %) the pain was severe. Irritation of the pelvic organs, in particular the rectum, expressed in frequent urges to defecate, was noted in 2 (8 %) patients with tumors localized directly in the retroperitoneum of the pelvis. Irritative symptoms along the sacral roots were noted in 20 (80 %) cases. Sensitive disorders were noted in 12 (48 %) cases.

TABLE 2

DISTRIBUTION OF CLINICAL SYMPTOMS

Symptoms	Number of patients	
	abs.	%
Pain syndrome in the sacrum	16	64
Pelvic irritation	2	8
Irritation of the sacral roots	20	80
Sensitive disorders	12	48
POD	3	12
No manifestations	4	16

Note. POD – pelvic organ dysfunction.

Pelvic organ dysfunction (POD) was noted in 3 (12 %) cases, with one patient having dysuric disorders associated with mechanical impact of a large-sized tumor located in the retroperitoneum on the pelvic organs.

All patients were operated using microsurgical instruments, OPMI Vario/NC33 microscope of Carl Zeiss company (Germany). Magnification from 10 to 20 times was used. High-speed drills supplied by Aesculap (USA) and Synthes (USA) were used for approach. A Sonoca 300 ultrasonic dissector/aspirator (Söring, Germany) was used at the stages of excision, allowing, if necessary, to perform internal decompression of the tumor for its further mobilization and minimize the impact on the adjacent neural structures.

Two types of approaches were used in the patient group. The first one was a posterior approach with resection of the posterior wall of the sacrum; performed in 22 patients. Posterior approach allows a fairly clear identification of the nerve structures that are located directly in the sacral canal. In case of tumor localization within the sacral canal, it is limited to trepanation of its posterior wall and skeletonization of the anterior wall of the canal and intervertebral foramen zones. All posterior approaches done were performed

with the patient in the abdominal position on the operating table. In this type of approach, opening and resection of the anterior wall of the sacrum from behind (or enlargement of the already existing enlarged intervertebral foramen) was performed if necessary in order to remove the fragment extending ventrally, which was performed in 6 cases. In 2 of these 6 cases, the posterior and anterior walls of the sacrum were resected and a large ventral component located in the pelvis was removed. Posterior approaches are quite typical, they are widely described in the literature, so there is no point in dwelling on them in more detail.

The second approach was an anterior retroperitoneal approach to excise tumors mainly localized on the anterior wall of the sacrum in the pelvis. It was performed in 3 cases in the supine position of the patient on the operating table. Retroperitoneal approach consists of making an oblique paramedian incision of the anterior abdominal wall. The peritoneum is peeled off the pelvic surface along with the intestine and displaced from the iliac vessels and ureters; the peritoneum is pushed medially. During the tumor approach phases, it is important to assess the risk of injury to the great vessel and ureter. On the lateral side, the iliac neurovascular bundle was mobilized and withdrawn. Bipolar coagulation is used retroperitoneally to enter the pelvis and approach the tumor mass. Subsequently, tumor mobilization with internal decompression using an ultrasonic disintegrator was performed. As the volume of the tumor node decreased after its internal decompression, we separated the schwannoma capsule from the anterior wall of the sacrum and important anatomical formations (peritoneum, vessels, nerves, ureters). After its intracapsular removal and its separation from the pelvic organs, the sacral intervertebral foramen was examined, the site of tumor growth (spinal root) was identified, and after preliminary coagulation it was cut off from the root. The operation ended with hemostasis, layer-by-layer sutures on the wound with drainage in the retroperitoneal space for 1–2 days.

In all 3 cases of purely anteriorly localized tumors excised from anterior approach, the surgical team included a vascular surgeon, but we did not experience any complications or difficulties during excision. The tumors were localized retroperitoneally and were not intimate with the great vessels.

In order to assess the results of surgical treatment and quality of life, we used the McCormick Scale, and to assess the pain syndrome and its dynamics – VAS. It should be noted that microsurgical interventions mostly did not cause a marked worsening of the patients' condition and deepening of the existing focal symptoms in the group of our patients, as well as any significant change in the patients' quality of life index in the postoperative period and transfer of patients from one group to another.

The conducted studies comply with the ethical standards developed on the basis of the Declaration of Helsinki of the World Medical Association (WMA) "Ethical Principles for Medical Research Involving Human Subjects", as amended in 2000, and the Rules for Good Clinical Practice approved by the Order No. 200n dated April 01, 2016 of the Ministry of Health of the Russian Federation. The study was approved by the Biomedical Ethics Committee of Novosibirsk Research Institute of Traumatology and Orthopedics named after Ya.L. Tsivyan of the Russian Ministry of Health. All data were depersonalized.

RESULTS

Compared to the initial parameters of patients in the postoperative period at 1 year, a good clinical result with regression of symptoms was noted in 17 out of 25 patients, which amounted to 68 % ($p < 0.01$) – this corresponded to class I according to the McCormick Scale (1999), – and 10 (5,8 %) of them had significant regression of neurology, expressed in a clear reduction of pain syndrome (from 64 % to 24 %), irritative symptoms on sacral roots, irritation of pelvic organs, restoration of sensitive disorders. In 6 (24 %) individuals the symptoms corresponded to grade II as per the McCormick Scale ($p = 0.291$), in 2 (8 %) cases – to grade III ($p = 0.027$). There were no patients with gross neurological disorders of persistent nature (grade IV) in the group. Positive symptoms noted at 1 year were not significant at later dates.

Regarding POD, we can say that of the three patients with POD in the preoperative period, regression of the disorders was noted in 2 (8 %) patients; 1 (4 %) patient retained POD with gradual incomplete regression during the following 1.5 years. In addition, in one patient who did not have POD preoperatively, this type of disorder appeared in the postoperative period.

Operation radically was achieved in 24 out of 25 cases (96 %), subtotal resection was performed in 1 (4 %) patient.

The number of relapses and continued growth of neoplasms amounted to 12 % in our series (3 out of 25 patients, which was confirmed by clinical and tomographic data). Analysis of neoplasm recurrences revealed that they recurred at 18 and 26 months, after primary surgery and total tumor excision, and in one case continued growth was noted 11 months after subtotal tumor excision. Patients were reoperated without worsening neurologic deficits or relapse during the remaining follow-up period.

Long-term (more than 5 years) treatment results were traced in 10 (40 %) of our operated patients. In 3 (12 %) patients, long-term results could not be followed up due to loss of contact with them. There were no fatal long-term outcomes in the group of patients available for catamnesis collection during the postoperative follow-up period.

Complications observed in our group were noted in 3 (12 %) cases and manifested as wound liquorrhea. All of them are noted in cases of posterior approaches. In all three cases, the liquorrhea was controlled by secondary suturing with placement of lumbar drainage. In cases

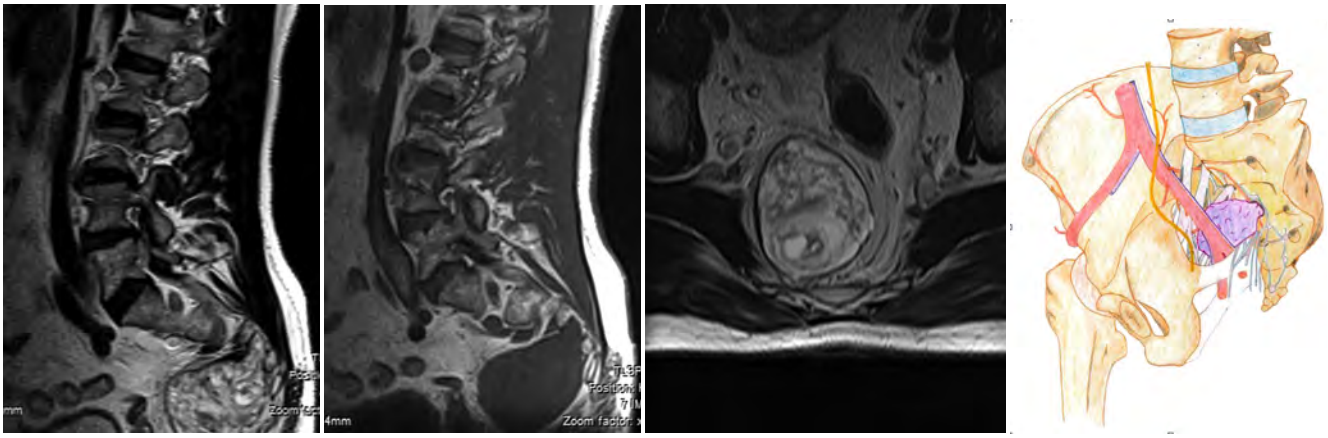
of anterior, retroperitoneal approach, there were no complications. Infections of the surgical area as well as inflammatory changes from the subarachnoid space did not occur among the patients.

CLINICAL CASE STUDY

Patient R., born in 1957, complains of weakness in the left foot and fingers. Intermittent moderate pain in the lumbosacral region, in recent months – fecal urgency. Dx: Giant-sized volumetric mass (schwannoma?) of intraforaminal localization at the level of S3 on the right with ventral extension into the pelvic cavity. The diagnosis was confirmed by contrast-enhanced MRI of the lumbosacral spine, which revealed a giant presacral, contrast-enhanced mass at the S3 level on the right with an intraforaminal component totaling 51 × 52.6 × 65.3 mm, with the intraforaminal component measuring 10 × 11 × 12 mm (Fig. 1).

Surgical treatment was performed routinely: trepanation of the posterior and anterior walls of the sacrum in the projection of tumor growth at the level of S3–S4 segments on the right, microsurgical excision of the tumor.

Among the surgical peculiarities, we would like to emphasize the fact that the tumor was completely excised from the posterior approach without damaging the peritoneum; the resulting postoperative cavity of the posterior wall of the sacrum was filled with autogyre; con-



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

FIG. 1.

Patient R., MRI of the lumbosacral spine in 2 projections with contrast enhancement, before the surgery. Giant presacral tumor of retroperitoneal localization at the S3 level on the right, with intraforaminal component. ***a***, ***b*** *– sagittal sections;* ***c*** *– axial section;* ***d*** *– scheme of topographic and anatomical features of the tumor localization.*

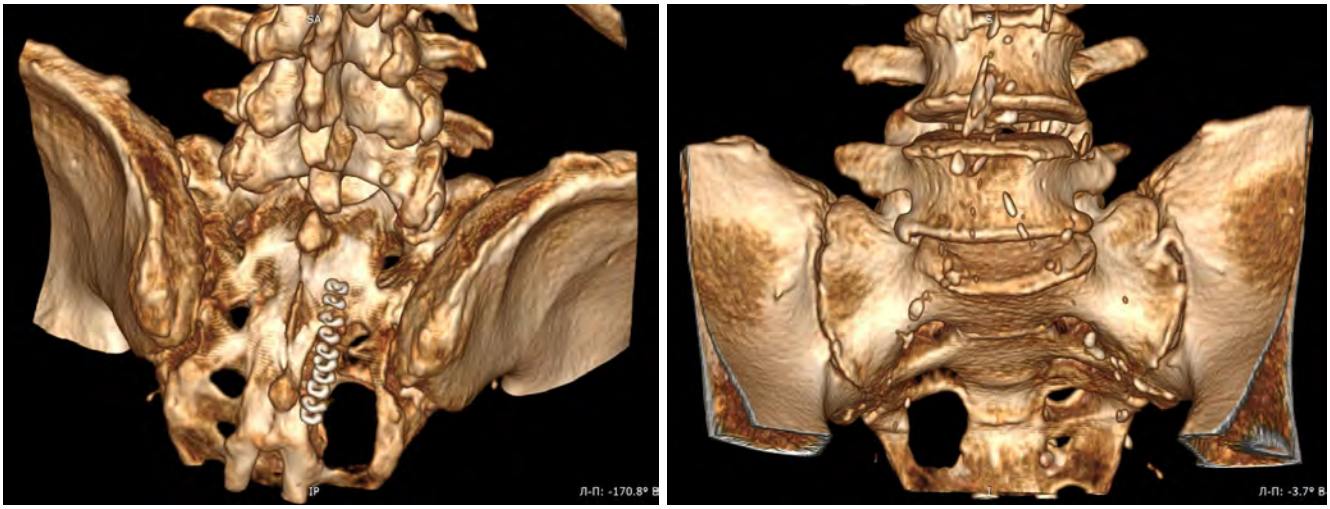


FIG. 2.

Patient R., 3D reconstruction of the MSCT image of the sacrum, after the surgery. Defect of lateral sacral masses in the area of surgical intervention

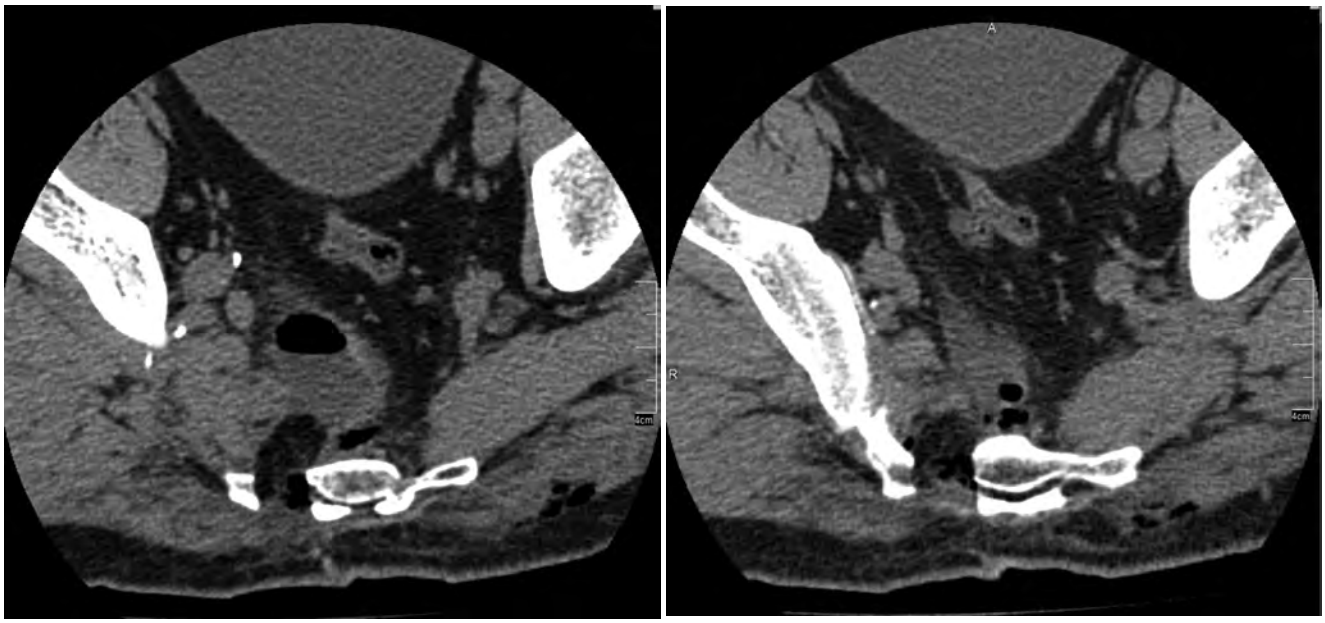
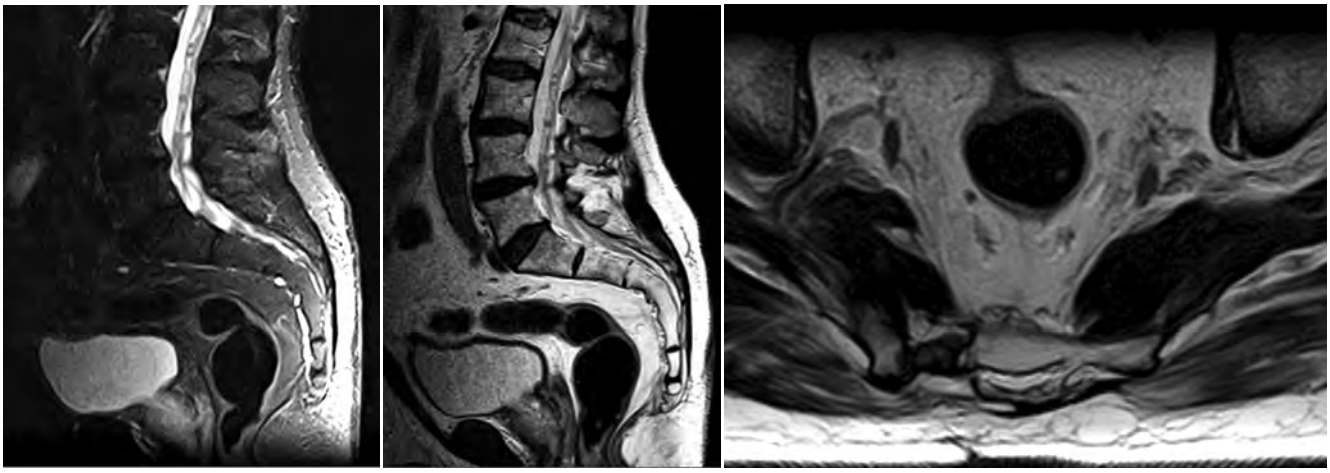


FIG. 3.

Patient R., MSCT images of the sacral spine and the pelvis with contrast enhancement, 1 day after the surgery. No signs of the tumor tissue



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

FIG. 4.

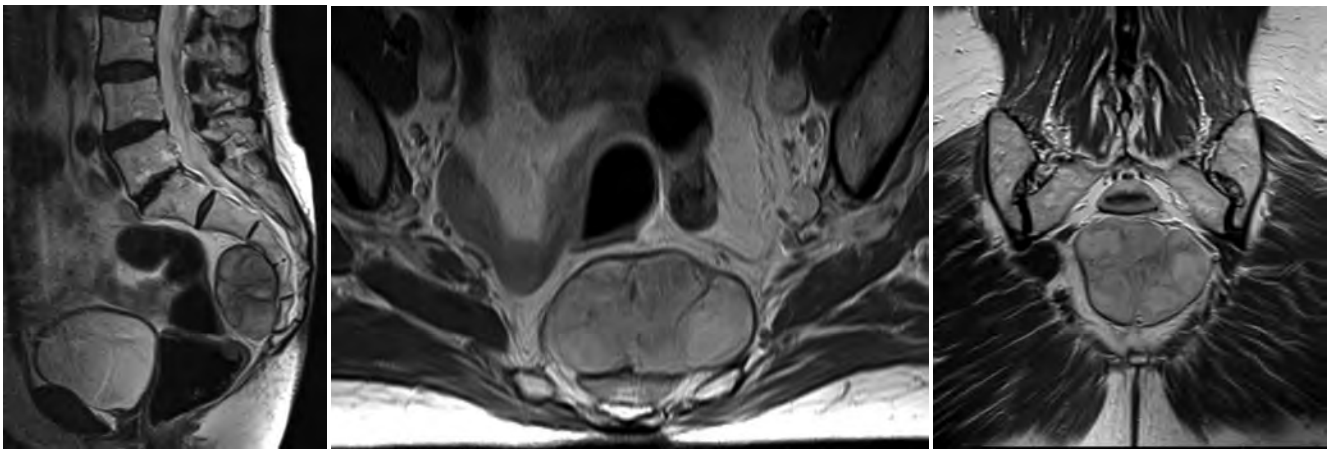
Patient R., MRI of the lumbosacral spine with contrast enhancement, 34 months after the surgery. No signs of recurrence of tumor in the sacrum. ***a***, ***b*** *– sagittal sections;* ***c*** *– axial section.*

sidering adequate hemostasis, no drainage systems were placed in the wound. The resection volume of the sacrum (in the area of the enlarged intervertebral foramen) is represented by a small defect (35.0 × 25.0 mm) that does not compromise the orthopedic integrity of the pelvic ring (Fig. 2).

Intraoperative blood loss amounted to 320 ml. The volume of the excised tumor was 100.6 cm^3. Histologic and immunohistochemical diagnosis: Schwannoma, stage 1 (ICD-O: 9560/0). Based on the results of Multislice Computed Tomography (MSCT) 24 hours after surgery, there were no signs of tumorigenesis (Fig. 3).

The postoperative period was uneventful. Wound healing is primary. In neurologic status – without aggravation of neurologic symptoms, and the urgencies regressed. The patient was discharged from the clinic on the day 12 in stable, satisfactory condition. The patient is on outpatient follow-up at the clinic. The results of control MRI after 34 months showed no signs of recurrence of the mass at the operated level (S3–S4) (Fig. 4)

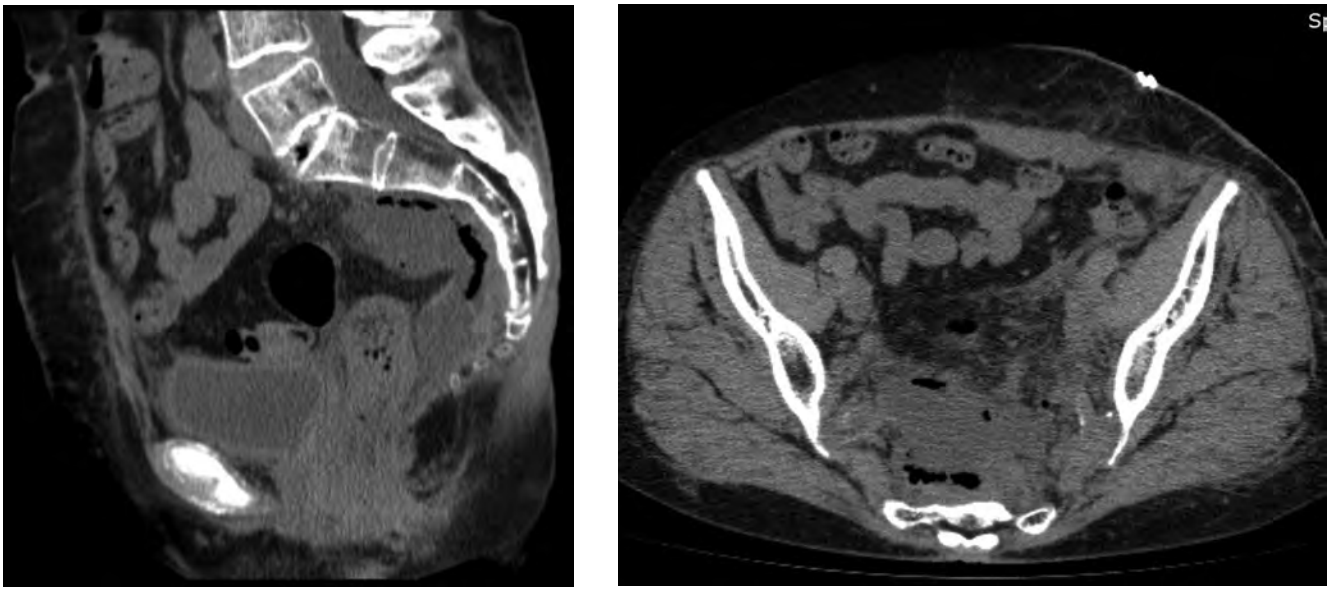
Patient N., born in 1946. Complaints of periodic moderate painful sensations ("feeling of discomfort") in the pelvic area. The results of ultrasound (U/S) re-



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

FIG. 5.

Patient N., MRI of the lumbosacral spine in 2 projections with contrast enhancement, before the surgery: ***a*** *– sagittal section;* ***b***, ***c*** *– axial sections.*



a ***b***

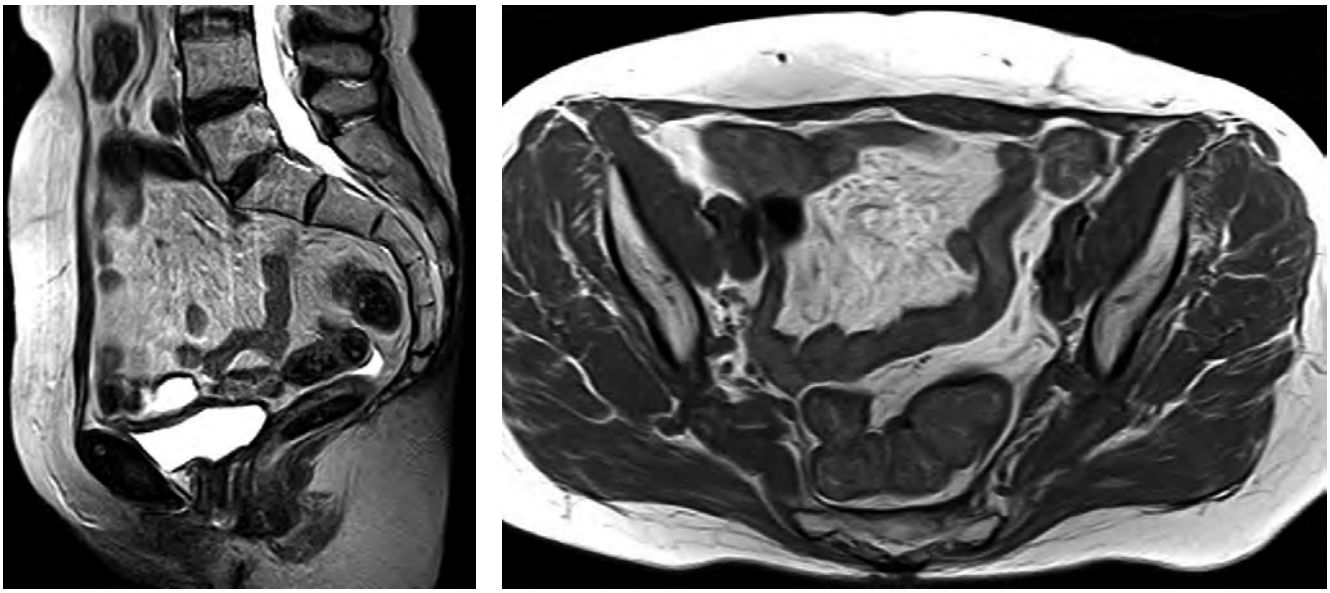
FIG. 6.

Patient N., MSCT images of the lumbosacral spine, spinal cord and the pelvis with contrast enhancement, 1 day after the surgery: no signs of tumor. ***a*** *– sagittal section;* ***b*** *– axial section.*

vealed a mass in the pelvic area, and the patient was referred to an oncologist. In neurologic status at examination: without focal neurologic deficit; no pelvic organ dysfunction. The diagnosis was clarified by contrast-enhanced MRI, which revealed a volumetric contrast-enhanced mass measuring 60.3 × 38.7 × 72 mm in the presacral region, on the ventral surface of the S3–S5 vertebrae (Fig. 5).

The patient underwent a planned surgical intervention: microsurgical removal of a volumetric mass at the level of S3–S5 segments of the sacrum in the pelvic area by left-sided retroperitoneal approach. The tumor was excised total, and a small tumor fragment extending into the intervertebral foramen of the sacrum was also removed. Intraoperative blood loss amounted to 150 ml. The volume of tumor excised was 96.5 cm^3. Histologic and immunohistochemical diagnosis: Schwannoma, stage I (ICD-O: 9560/0). Based on the results of postoperative MSCT control 1 day after surgery, no signs of residual tumor tissue were detected (Fig. 6).

The postoperative period was satisfactory, wound healing was by primary intention. The sutures were removed on the day 10. The patient was activated on the day 2. She was discharged from the clinic in satis-



a *b*

FIG. 7.

Patient N., MRI of the lumbosacral spine in 2 projections with contrast enhancement, 62 months after the surgery. No signs of relapse of tumor. ***a*** *– sagittal section;* ***b*** *– axial section.*

factory condition with improvement on the day 10 after surgery. In neurologic status – without focal symptoms and increasing neurologic deficit. POD in normal state.

Based on the results of the most recent contrast-enhanced MRI of the pelvis and sacrum 62 months after surgery, there was no evidence of tumor relapse (Fig. 7).

DISCUSSION

The necessary set of diagnostic measures for surgical treatment, in addition to clinical and neurological data, should include the results of MRI and MSCT with contrast enhancement, and if necessary, MSCT with angioprogram and ultrasound examination of the pelvic region.

The priority in the treatment of schwannomas of sacral localization should be surgical treatment with complete excision of the tumor process. This ensures minimized and recurrence-free, continued good quality of life for patients, sparing them from reoperations and other treatments to target residual tumor tissue. However, we should not forget that in the pursuit of radical surgery, it is necessary to clearly plan the surgical approach, its features and volume, taking into account the topographic-anatomical features of the area. Despite the fact that the most applicable in neurosurgical practice is the posterior approach, which allows the most radical removal of voluminous masses, mainly intracanalicular localization, in cases of ventral extension, it is also possible to use the posterior approach to remove a ventrally extending tumor node (as presented in the clinical case study), and in this case it is possible to do with minimal, clinically insignificant blood loss, a small resection of bone structures of the sacrum and achieve radicality of removal. Currently, there is no clear algorithm regulating the choice of surgical approach [11]. Some authors are of the opinion that posterior approaches should be chosen when excising giant neurogenic tumors of the sacrum [16, 18]. The anterior retroperitoneal approach we used in several cases also allowed us to excise tumors of ventral localization in the pelvis radically and minimally traumatically.

However, based on our own experience, it is clear that excision of anterior sacral wall tumors at lower sacral levels from anterior retroperitoneal approach will be very difficult due to the formation of a longer operative approach to the tumor, which will reduce the view and angle of surgical attack. Taking this into account, as well as analyzing the peculiarities of the extension of some tumors in our group, the presence of a sufficiently deep surgical approach in the ventral direction at posterior approaches, we would like to note the possibility and expediency of using endovideo-assistance during excision, which, in our opinion, will allow to visualize more fully the removed tumor bed and contact with anatomical formations in the depth of the wound.

We would like to note that in the group of operated patients we did not have to use two-stage (ventral, dorsal) surgical intervention, as well as transperitoneal approach. At the same time it was possible to excise tumors radically enough in almost all cases when correctly chosen posterior or anterior approach was performed. The orthopedic integrity of the spine and pelvic ring was not compromised in all 25 cases and, accordingly, no stabilizing systems

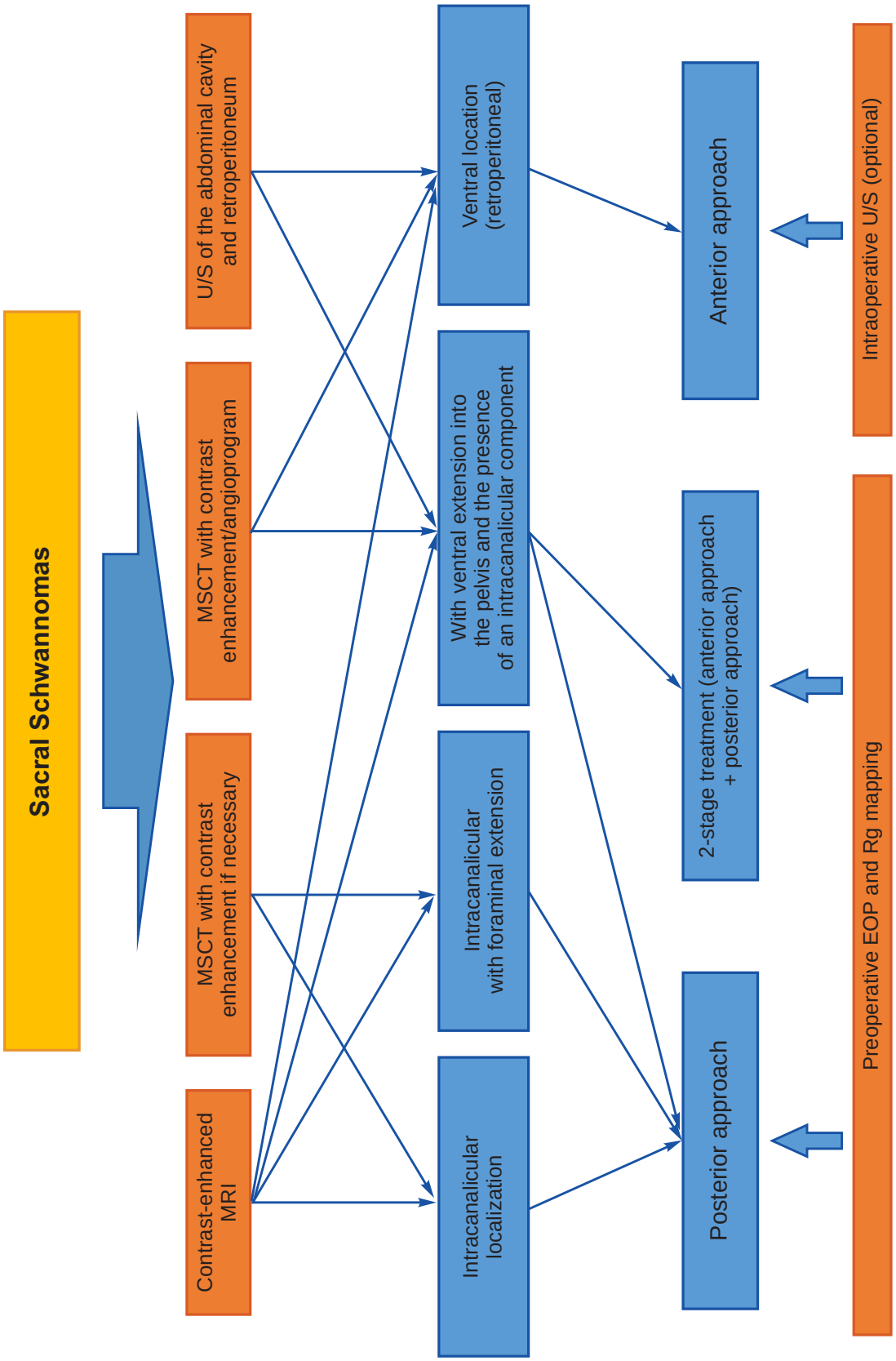


FIG. 8.
Algorithm of surgical and diagnostic approaches in the treatment of sacral schwannomas

were required. Good clinical results with analysis of the dynamics of neurological status, pain syndrome and pelvic organ function confirm the correct choice of surgical approach in each case.

In case of significant ventral extension of the tumor and its large volume, anterior approach or two-stage intervention is preferred. However, our experience and literature data show that sufficiently large sacral schwannomas extending predominantly into the retroperitoneum can be removed from the posterior approach, minimally traumatically and with a good clinical outcome [15, 18]. In this case, it is necessary to carry out an individual approach to the patient, focusing on the localization, extension of the tumor, as well as on the constitutional features of the patient.

The paradigm used in our clinic in treatment and diagnostic approaches in sacral schwannoma surgery is detailed in the flowchart (Fig. 8).

When analyzing complications, taking into account schwannoma localization area at the level of the sacral canal and interaction with the roots of the cauda equina, perhaps the most significant postoperative disorders, significantly worsening the quality of life of patients, are pelvic organ dysfunctions. Therefore, in our opinion, starting from preoperative planning, it is necessary to assess possible risks of postoperative disorders, including POD, together with urologists or neurourologists, and, if such complications are present, to develop and apply a rehabilitation program for them, starting from the early postoperative period. Complications in the form of wound liquorrhea are associated with the particular structure of the terminal portions of the dural sac in the form of thinning of the dura mater, therefore, in order to control this complication, as our experience shows, it is optimal to use biological glue and fat autograft for additional sealing of the dura mater.

CONCLUSIONS

As our experience shows and literature data confirm, the overwhelming number of tumors of sacral localization were schwannomas with gender distribution towards the female sex.

Radical surgical excision of schwannomas, including those with extracanalicular (ventral) extension, is an effective way to treat them, with a favorable prognosis, including using a single approach, even in large-sized schwannomas.

The choice of adequate approach is directly related to the degree of radicality of tumor excision. An individualized approach and planning are required for each case. Furthermore, it is necessary to clearly understand the possibilities of all existing surgical approaches, and the main goal should be, of course, the patient's quality of life.

Conflict of interest

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

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OPHTALMOLOGY

PREOPERATIVE MANAGEMENT OF OPHTHALMIC PATIENTS TAKING ORAL ANTICOAGULANTS

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ABSTRACT

Surgical treatment is often accompanied by such complication as bleeding, and ophthalmic surgery is not an exception. The bleeding risk depends on many factors, the most significant are age, arterial hypertension, hepatic and renal impairment, prior stroke or treatment with oral anticoagulants.

The aim. *To evaluate the structure of patients taking novel oral anticoagulants (NOACs) with an assessment of activated partial thromboplastin time before ophthalmosurgical treatment.*

Materials and methods. *54 patients taking oral anticoagulants were included in the study. A retrospective analysis of medical histories of patients who had surgery for ocular pathology was carried out. The patients were divided into two groups: group 1 – 28 patients whose activated partial thromboplastin time (APTT) did not exceed 45 seconds; group 2 – 26 patients with APTT more than 45 seconds. The criterion for the numerical expression of APTT is the safety of performing regional anesthesia against the background of taking anticoagulant drugs. Statistical processing was performed using the Mann – Whitney test ($p < 0.05$).*

Results. *A comparative analysis of the results showed that the patients of the group 2 had higher rates of APTT. At the same time, they were less likely to have acute cerebrovascular accident (11.5 % compared to 21 % of patients in the group 1) and prior acute myocardial infarction (19 % and 28 %, respectively). Among all the patients, women and slightly older patients prevailed.*

Conclusion. *Patients with atrial fibrillation make up the majority of patients undergoing ophthalmosurgical treatment and taking NOACs. Surgical treatment method was phacoemulsification with intraocular lens implantation. Studying APTT before the surgery allowed us to identify a category of patients with high APTT, to prescribe the withdrawal of the drug before the surgery in order to create optimal conditions for surgical treatment.*

Key words: *vitrectomy, posterior segment eye disease, novel oral anticoagulants, activated partial thromboplastin time, hemorrhagic complications, hypocoagulation effect*

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ОПЫТ ПРЕДОПЕРАЦИОННОГО ВЕДЕНИЯ ОФТАЛЬМОЛОГИЧЕСКИХ БОЛЬНЫХ, ПРИНИМАЮЩИХ ПЕРОРАЛЬНЫЕ АНТИКОАГУЛЯНТЫ

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РЕЗЮМЕ

Хирургическая помощь пациентам зачастую сопровождается таким осложнением, как кровотечение; не является исключением и офтальмологическая хирургия. Вероятность кровотечений зависит от многих факторов, наиболее значимыми из которых являются наличие артериальной гипертонии, нарушение функции печени и почек, перенесённый инсульт, лечение пероральными антикоагулянтами.

Цель работы. *Оценить структуру пациентов, принимающих новые оральные антикоагулянты (НОАК), с оценкой активированного частичного тромбопластинового времени перед офтальмохирургическим лечением.*

Материалы и методы. *В исследование были включены 54 пациента, принимающие пероральные антикоагулянты. Был проведён ретроспективный анализ историй болезни пациентов, прооперированных по поводу глазной патологии. Пациенты были разделены на две группы: 1-я группа – 28 пациентов, у которых активированное частичное тромбопластиновое время (АЧТВ) не превышало 45 с; 2-я группа – 26 пациентов, у которых АЧТВ было больше 45 с, согласно рекомендациям безопасности выполнения регионарной анестезии на фоне приёма антикоагулянтных препаратов. Статистическая обработка выполнялась с использованием критерия Манна – Уитни ($p < 0,05$).*

Результаты. *Сравнительный анализ полученных результатов продемонстрировал, что у пациентов второй группы реже выявлялись острое нарушение мозгового кровообращения (11,5 % по сравнению с 21 % больных первой группы) и острый инфаркт миокарда в анамнезе (19 % и 28 % соответственно). Преобладали лица женского пола и незначительно больший возраст больных.*

Заключение. *Основную долю пациентов, находящихся на офтальмохирургическом лечении и принимающих НОАК, составили больные с фибрилляцией предсердий, в качестве метода хирургического лечения выбрана факоэмульсификация катаракты с имплантацией интраокулярной линзы. Исследование АЧТВ перед операцией позволило выявить категорию пациентов с высоким АЧТВ, назначить отмену препарата перед операцией для создания оптимальных условий для хирургического лечения.*

Ключевые слова: *витрэктомия, заболевания заднего отрезка глаза, новые оральные антикоагулянты, активированное частичное тромбопластиновое время, геморрагические осложнения, гипокоагуляционный эффект*

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RELEVANCE

The majority of patients in ophthalmology clinics who undergo surgery for cataracts, glaucoma, and other eye diseases are elderly and senile. As it is known, with age the specific weight of patients having concomitant cardiovascular pathology increases, which is represented mostly by arterial hypertension (AH) and coronary heart disease (CHD). One of the clinical manifestations of CHD is atrial fibrillation (AF). AF in turn determines a certain risk of cardioembolic complications. This risk is assessed using the CHA_2DS_2-VASc score, which takes into account such indicators as gender, age of the patient, presence of comorbidities (AH, diabetes mellitus (DM), vascular disease, history of acute cerebrovascular accidents (ACVA), signs of heart failure) [1]. Each of the above signs corresponds to a certain score, and the summation of these figures reflects the risk of thromboembolic complications (events). If the total score is 1 or more, the prescription of direct oral anticoagulants (DOACs) should be considered. However, taking anticoagulants is accompanied by the risk of hemorrhagic complications [2]. Bleeding probability depends on many factors, the most significant of which (age, presence of AH, hepatic and renal dysfunction, prior stroke or bleeding) are taken into account in the HAS-BLED score [1]. All these points should be taken into account when providing surgical care to patients, including those with ophthalmologic pathology.

It should be kept in mind that any surgical intervention itself carries the risk of complications, both cardiovascular and localized. Surgical risk assessment (i.e., the incidence rate of cardiovascular complications during and after surgical intervention) depends primarily on the possible volume of the upcoming surgery. According to the National Guidelines [3], ophthalmic surgery is categorized as low-risk surgery, that is, the risk of myocardial infarction (MI) or death from cardiac causes within 30 days after surgery is less than 1 %. That is, large-scale cardiac risk assessment is of no practical use in small-volume surgeries [4]. However, the possibility of non-systemic hemorrhagic complications should not be neglected, especially in patients taking new oral anticoagulants (NOACs), including when performing regional anesthesia [5, 6]. In ophthalmology, this is especially relevant in surgeries requiring regional anesthesia in the form of retrobulbar and pterygopalatine blockades, which can be accompanied by major complications, even though ultrasound navigation levels out technical difficulties and the possibility of damage to anatomical structures [7].

THE AIM OF THE WORK

To evaluate the structure of patients taking novel oral anticoagulants with an assessment of activated partial thromboplastin time before ophthalmosurgical treatment.

MATERIALS AND METHODS

A retrospective analysis of medical histories included data from 54 patients receiving NOACs operated for ocular pathology (Table 1). Patients receiving dual antithrombotic therapy (i. e. NOACs and antiaggregants together), as well as those with severe liver disease, hematologic pathology, and end stage renal disease were excluded from the study.

Based on clinical guidelines on the perioperative management of patients receiving long-term antithrombotic therapy [8, 9], the patients were divided into two groups. Group 1 included patients whose activated partial thromboplastin time (APTT) did not exceed 45 s; group 2 was made up of those who`s APTT was greater than 45 s (Table 2). The criterion for the numerical expression of APTT is the safety of performing regional anesthesia against the background of taking anticoagulant drugs.

Statistical analysis was performed using the Mann – Whitney test. Statistical significance level < 0.05.

TABLE 1

TYPES OF SURGICAL INTERVENTION PERFORMED IN PATIENTS TAKING ORAL ANTICOAGULANTS

Intraocular surgical procedures (*n* = 39)				Laser surgeries (*n* = 15)			
Phaco + IOL	AGO	Vitreoretinal surgeries	Administration of angiogenesis inhibitors	PC discission	Laser iridectomy	Retinal laser photocoagulation	SLP
22	5	7	5	7	4	1	3

Note. Phaco + IOL – phacoemulsification with intraocular lens implantation; AGS – antiglaucomatous surgery; PC discission – posterior capsule discission (performed for secondary cataract); SLP – scatter laser photocoagulation (performed in the proliferative stage of diabetic retinopathy).

RESULTS AND DISCUSSION

The patients included in the study underwent both intraocular surgical interventions and laser surgeries of different types, as presented in Table 1.

Patients were taking oral anticoagulants such as rivaroxaban, apixaban, and dabigatran.

Rivaroxaban was prescribed in 43 % of cases (23 patients), of which 11 patients took the drug at a dose of 20 mg/day, 7 patients at 15 mg/day, 4 patients at 10 mg/day, and one patient at 5 mg/day. Apixaban was given to two patients (4 % of cases) at a dose of 5 mg twice daily. Dabigatran was prescribed in 54 % of cases (29 patients), of which 16 patients received the drug at a dose of 110 mg twice a day, 13 patients – 150 mg twice a day. All patients were analyzed on admission and the APTT was analyzed (laboratory normal value – 25–35 s). Distribution of the patients is summarized in Table 2.

TABLE 2

DISTRIBUTION OF THE PATIENTS DEPENDING ON THE APTT VALUES AND THE ORAL ANTICOAGULANT USED

Parameter, *n* (%)	APTT ≤ 45 s (*n* = 28)	APTT > 45 s (*n* = 26)
Male, *n* (%)	20 (72 %)	11 (42 %)
Female, *n* (%)	8 (28 %)	15 (58 %)
Age, years	74 (56–93)	77 (63–87)
AF, *n* (%)	26 (92 %)	25 (96 %)
AH, *n* (%)	26 (92 %)	26 (100 %)
DM, *n* (%)	7 (25 %)	10 (38 %)
PICS, *n* (%)	8 (28 %)	5 (19 %)
ACVA, *n* (%)	6 (21 %)	3 (11.5 %)
Dabigatran, *n* (%)	12 (43 %)	17 (65 %)
Apixaban, *n* (%)	1 (3 %)	1 (4 %)
Rivaroxaban, *n* (%)	15 (54 %)	8 (31 %)

Note. PICS – postinfarction cardiosclerosis.

Group 1 included 28 patients. Of these, there were 20 (72 %) males and 8 (28 %) females; mean age, 73 ± 0.1 years (56–93 years). Cataract surgery was performed in 39 % of cases (11 patients), vitreoretinal surgery in 21 % of cases (6 patients), antiglaucomatous surgery in 1 patient, and intravitreal injection of drugs in 1 patient. Laser surgical intervention was performed in 29 % of cases (8 patients): laser iridectomy – in 12 %, laser photocoagulation of the retina for its rupture – in 12 %, discission of the posterior capsule (performed for secondary cataract) – in 38 %, laser photocoagulation of the retina in diabetic retinopathy – in 38 %.

Evaluation of somatic status showed that in the majority of cases (93 % – 26 patients) DOACs were prescribed for AF. The remaining patients showed no signs of AF; the indication for NOAC prescription was previously performed prosthesis of the iliac/femoral segments for obliterating atherosclerosis of the lower limb arteries and pulmonary embolism.

8 patients had a history of acute myocardial infarction (AMI), 3 patients underwent coronary artery stenting, 1 patient underwent coronary artery bypass graft surgery, and 4 patients underwent pacemaker implantation. 6 patients had a history of acute cerebrovascular accidents. 1 patient with AF underwent thrombectomy of the left radial artery.

Arterial hypertension was detected in 25 of 28 patients in this group, diabetes mellitus – in 6 patients.

The APTT had a variation from 32 to 46.8 seconds; the mean was 39.6 ± 0.05 seconds. When rivaroxaban was administered, the average APTT was 39.9 ± 0.19 s, when apixaban was administered, it was 41 ± 0 s, and when dabigatran was administered, it was 39.1 ± 0.29 s.

Group 2 included 26 patients, of whom 11 (42 %) were males, 15 (58 %) were females. The age of the patients ranged from 63 to 87 years, with a mean of 76 ± 0.11 years. Intraocular surgery was performed for cataract in 42 % of cases (11 patients), antiglaucomatous surgery – in 12 % of cases (3 patients), intravitreal injection of angiogenesis inhibitors was performed in 15 % of cases (4 patients), vitreoretinal intervention – in 4 % of cases (1 patient). Laser intervention was required in 27 % of cases (7 patients).

Oral anticoagulants were prescribed for AF in the majority of patients (96 % of cases – 25 patients), as well as in the group 1. One patient without signs of AF had a history of deep vein thrombosis of the lower leg, post-thrombotic syndrome.

Arterial hypertension was detected in all patients. Five patients had a history of AMI, 1 patient underwent coronary artery bypass graft (CABG) surgery, 2 patients underwent coronary artery stenting, and 1 patient was implanted with an artificial pacemaker. A history of ACVA was revealed in 7 patients, and 1 patient had recurrent ACVA. Type 2 diabetes mellitus was recorded in 10 patients, of whom 1 patient was receiving insulin.

The range of the APTT values was from 47 to 114 s, with an average of 65.9 ± 0.1 s. When receiving rivaroxa-

TABLE 3

DISTRIBUTION OF APTT VALUES DEPENDING ON THE ORAL ANTICOAGULANT USED

Groups of patients depending on the APTT duration	APTT mean value, s	Rivaroxaban	Apixaban	Dabigatrian
APTT ≤ 45 s	36.9 ± 0.19	39.6 ± 0.19	41	39.1 ± 0.29
APTT > 45 s	65.96 ± 0.1	60 ± 1.07	47	69.7 ± 0.8
p (Mann – Whitney)	0.01	0.001		0.001

ban, the average APTT was 60 ± 1.07 s, apixaban – 47 ± 0 s, and dabigatran – 69.7 ± 0.8 s (Table 3).

Comparative analysis of the obtained results demonstrated that the patients of the group 2 had higher APTT values. At the same time, they had less frequent ACVA (11.5 % compared to 21 % of patients in the group 1) and a history of AMI (19 % and 28 %, respectively). The predominance of female patients and slightly higher age of patients can be noted. It is possible that better hemorheologic control, reflected by the elevated APTT values, contributed to the rarer occurrence of vascular accidents among these patients.

In addition, the medical histories of 20 patients who received oral anticoagulants and who had baseline APTT values greater than 60 s (62.25 ± 0.16 s) were analyzed separately. Patients received dabigatran in 60 % of cases (12 patients), rivaroxaban in 35 % of cases (7 patients), and apixaban was administered in 5 % of cases (1 patient). Taking into account the initial high values of APTT, anticoagulant withdrawal was carried out for a period of 2 to 5 days, on average for 3 days. On reexamination, the APTT values decreased to 37 ± 0.097 s.

DISCUSSION

Current oral anticoagulants include rivaroxaban, apixaban, and dabigatran. Rivaroxaban and apixaban are direct inhibitors of blood clotting factor Xa. Both drugs have a dose-dependent effect on prothrombin time, as well as dose-dependently increase in APTT. Dabigatran is a direct reversible thrombin inhibitor. The most informative parameters reflecting the anticoagulant activity are APTT (for apixaban and rivaroxaban) and thrombin time (for dabigatran).

The use of anticoagulant therapy has increased in patients seeking ophthalmic surgery over the past decade. The decision to discontinue anticoagulants prior to ophthalmic surgery is nuanced and ultimately depends on multiple factors, including the type of surgery, comorbidities, and the patient's risk profile. According to clinical guidelines [3, 9], NOAC therapy should not be interrupted for minor surgeries, which include, for example, cataract treatment. At the same time, at the stage of planning patients for surgery in order to prevent intraoperative hemorrhagic complications, it is necessary to take into account the initial state of the blood coagulation system [10]. To a greater extent, this applies to surgeries where retrobulbar anesthesia and pterygopalatine blockade are performed for the purpose of anesthesia, the most serious complications of which are retrobulbar hematoma and irreversible loss of vision [11]. Most ophthalmologic surgeries can be safely performed when anticoagulant therapy is within the therapeutic range. Certain difficulties may also arise during surgical interventions on the uvea in the treatment of glaucoma, vitreoretinal intervention performance. This is especially true for diabetic patients, as the newly formed vessels have imperfect angioarchitecture and are prone to rupture. The high risk of these complications dictates the necessity of short-term withdrawal of NOACs in case of high values of APTT with subsequent rechecking before surgical treatment.

The greatest hypocoagulant effect, according to our data, was found for dabigatran, which is consistent with the work of L. Ong-Tone et al. [12], where studied the risks of intraocular bleeding in patients taking anticoagulants.

CONCLUSIONS

The main share of patients undergoing ophthalmosurgical treatment and receiving NOACs were patients with atrial fibrillation, and phacoemulsification of cataract with intraocular lens implantation was used as a method

of surgical treatment. Studying APTT before the surgery allowed us to identify a category of patients with high APTT, to prescribe the withdrawal of the drug before the surgery in order to create optimal conditions for surgical treatment.

Conflict of interest

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

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PROSPECTIVE ASSESSMENT OF CYTOKINES AND REGULATORY PROTEINS CONCENTRATION IN THE TEAR FLUID OF POAG PATIENTS WITH VARIOUS HYPOTENSIVE EFFECTS AFTER NON-PENETRATING DEEP SCLERECTOMY

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ABSTRACT

The aim. *To assess the dynamics of cytokine content in tear fluid of primary open-angle glaucoma (POAG) patients at various terms after non-penetrating deep sclerectomy (NPDS) in relation to the functional state of the outflow tracts.*
Material and methods. *We carried out prospective examination of 65 patients with advanced stage of primary open-angle glaucoma after NPDS. Depending on the course of the postoperative period and the conditions for achieving the hypotensive effect of NPDS, all patients were divided into three groups: group 1 – with the optimal hypotensive effect; group 2 – with the conditional hypotensive effect; group 3 – with no hypotensive effect after NPDS. The intraocular pressure and the concentration of TGF-β, MMP-9, IL-6, IL-8, VEGF-A (121 and 165) in the tear fluid were studied using ELISA method in pre- and postoperative period. We studied the outflow tracts using optical coherence tomography and carried out ultrastructural analysis of filtering blebs tissue.*
Results. *In group 1, the minimum initial concentrations of IL-6, IL-8 and TGF-β in the tear fluid and their moderate increase in the postoperative period; high concentrations of MMP-9 at all stages and an increase in VEGF-A by 2 months after NPDS were registered. In group 2, there was a high level of VEGF-A in the tear fluid before and 2 months after NPDS, an increase in TGF-β, IL-6 and IL-8 in the tear fluid in the early period with their suppression in the late period, as well as an increase in MMP-9 in the early postoperative period. Group 3 had maximum concentrations of TGF-β and IL-8 in the tear fluid initially and in the early postoperative period, suppression of MMP-9 in the tear fluid 2 weeks after and of VEGF-A 2 months after NPDS.*
Conclusion. *Initially high concentrations of IL-6, IL-8, and TGF-β in the tear fluid and the suppression of MMP-9 and VEGF-A in the postoperative period contribute to the surgical failure of the NPDS.*

Key words: *non-penetrating deep sclerectomy, transforming growth factor β, TGF-β, matrix metalloproteinase 9, MMP-9, interleukin 6, IL-6, interleukin 8, IL-8, VEGF-A (121 and 165), cytokines in the tear fluid, extracellular matrix*

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ПРОСПЕКТИВНАЯ ОЦЕНКА КОНЦЕНТРАЦИИ ЦИТОКИНОВ И РЕГУЛЯТОРНЫХ БЕЛКОВ В СЛЁЗНОЙ ЖИДКОСТИ ПАЦИЕНТОВ С ОТКРЫТОУГОЛЬНОЙ ГЛАУКОМОЙ С РАЗЛИЧНЫМ ГИПОТЕНЗИВНЫМ ЭФФЕКТОМ ПОСЛЕ НЕПРОНИКАЮЩЕЙ ГЛУБОКОЙ СКЛЕРЭКТОМИИ

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РЕЗЮМЕ

Цель работы. *Оценить динамику содержания цитокинов в слезе у пациентов с первичной открытоугольной глаукомой (ПОУГ) в различные сроки после непроникающей глубокой склерэктомии (НГСЭ) во взаимосвязи с функциональным состоянием путей оттока.*

Материалы и методы. *Проведено проспективное обследование 65 пациентов с развитой стадией ПОУГ после НГСЭ. В зависимости от течения послеоперационного периода и условий достижения гипотензивного эффекта НГСЭ все пациенты разделены на три группы: группа 1 – с оптимальным гипотензивным эффектом НГСЭ; группа 2 – с условным гипотензивным эффектом НГСЭ; группа 3 – с отсутствием гипотензивного эффекта после НГСЭ. Проводилось исследование внутриглазного давления и концентраций TGF-β, MMP-9, ИЛ-6, ИЛ-8, VEGF-A (121 и 165) в слезе методом иммуноферментного анализа в до- и послеоперационном периоде, а также оптическая когерентная томография путей оттока и ультраструктурный анализ ткани фильтрационных подушек.*

Результаты. *В группе 1 определены минимальные исходные концентрации ИЛ-6, ИЛ-8 и TGF-β слезы и их умеренное повышение в послеоперационном периоде; высокие концентрации MMP-9 на всех этапах и нарастание VEGF-A ко 2-му месяцу после НГСЭ. В группе2 отмечен высокий уровень VEGF-A слезы перед и через 2 месяца после НГСЭ; нарастание TGF-β, ИЛ-6 и ИЛ-8 слезы в раннем периоде с подавлением в позднем, а также повышение MMP-9 в раннем послеоперационном периоде. Для группы 3 характерны максимальные концентрации TGF-β и ИЛ-8 слезы исходно и в раннем послеоперационном периоде, подавление MMP-9 слезы через 2 недели и VEGF-A через 2 месяца после НГСЭ.*

Выводы. *Исходно высокие концентрации ИЛ-6, ИЛ-8, TGF-β в слезе и подавление MMP-9 и VEGF-A в послеоперационном периоде способствуют хирургическому неуспеху НГСЭ.*

Ключевые слова: *непроникающая глубокая склерэктомия, трансформирующий фактор роста β, TGF-β, матриксная металлопротеиназа 9, MMP-9, интерлейкин 6, ИЛ-6, интерлейкин 8, ИЛ-8, VEGF-A (121 и 165), цитокины в слёзной жидкости, внеклеточный матрикс*

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RELEVANCE

In the step-by-step algorithm of primary open-angle glaucoma (POAG) treatment, glaucoma surgery performed to normalize intraocular pressure (IOP) is considered as a step following hypotensive drug therapy in case of its ineffectiveness.

At the same time, the main disadvantage of filtering and fistulizing surgeries is excessive scarring in the area of surgical site, which disrupts the outflow of intraocular fluid (IOF) through the newly created pathways. This process is stimulated by pro-fibrogenic and pro-inflammatory regulatory proteins [1]. Abnormalities in the composition of the anterior chamber aqueous humor of glaucoma patients, in particular an increase in active forms of transforming growth factor β (TGF-β), as well as tumor necrosis factor α (TNF-α), interleukins (ILs) 6 and 8, contribute to fibrogenesis [2, 3]. It is known that a decrease in the activity of matrix metalloproteinases (MMPs) leads to impaired degradation of extracellular matrix (ECM) components [4].

In addition to factors in the anterior chamber aqueous humor, wound processes in the glaucoma surgery region are influenced by regulatory proteins (RPs) and cytokines expressed by conjunctival tissue cells in response to surgical trauma [5]. In addition, subclinical conjunctival inflammation, which is often due to long-term use of some antiglaucoma drugs, is also accompanied by monocyte/macrophage tissue infiltration and expression of growth factors and pro-inflammatory cytokines, predisposing to early filtering bleb (FB) scarring [6, 7].

A number of studies, including the data we obtained and published earlier [8], revealed a correlation between effective IOP reduction after trabeculectomy and non-penetrating deep sclerectomy (NPDS) and the state of the conjunctival lymphatic system. It is assumed, given the data presented, that the negative impact on the outcome of antiglaucoma interventions is caused not only by the initial imbalance of regulatory proteins, but also by the use of antimetabolites during surgery and in the postoperative period that inhibit the growth of both connective tissue and conjunctival lymphatic vessels involved in the outflow of intraocular fluid from FB. Thus, the study by R.A. Bouhenni et al. showed that after intraoperative application of mitomycin C in filtering blebs, a decrease in the density of lymphatic and blood vessels was indicated [9].

Thus, it is relevant in the study of the formation of intraocular fluid outflow pathways after glaucoma surgeries to determine the role of cytokines and regulatory proteins in the structural transformation of the extracellular matrix of filtering blebs and the formation of conjunctival lymphatic vessels.

THE AIM OF THE WORK

To assess the dynamics of cytokines and regulatory proteins content in tear fluid of patients with primary open-angle glaucoma after non-penetrating deep sclerectomy at different stages of the postoperative period in correlation with the functional state of the newly created outflow pathways.

MATERIALS AND METHODS

A prospective examination of 65 patients aged 50 to 70 years (mean age 63.6 ± 4.8 years) with advanced stage POAG and decompensated intraocular pressure (IOP) who underwent NPDS and laser descemetopuncture 14–18 days after the surgery. All operations were performed by the same surgeon and were comparable in terms of intervention volume. Depending on the course of the postoperative period and conditions for achieving hypotensive effect of NPDS, all patients 12 months after surgery were divided into three groups: with optimal hypotensive effect of NPDS (group 1), with conditional hypotensive effect of NPDS (group 2), and with no hypotensive effect of NPDS (group 3).

Group 1included 21 POAG patients (age – 65.4 (53.1; 67.3) years) with IOPg ≤ 16 mmHg; according to optical coherence tomography (OCT) the outflow pathways are functional; FBs are diffuse, according to OCT their content is represented by sparse, hyporeflective extracellular matrix [10]. Based on the results of ultrastructural immunohistochemical examination of tissue samples of functional FPs (n = 4), 5 to 7 lymphatic vessels with steady podoplanin expression were indicated [8]. The postoperative period among group 1 patients was areactive. The patients received standard instillation antibacterial and anti-inflammatory therapy (levofloxacin 0.5 %, dexamethasone 0.1 % and nepafenac 0.1 % in a decreasing order).

Group 2 included 23 patients (age – 63.7 (55.2; 66.8) years) with IOPg ≤ 16 mmHg; according to OCT data, in the early postoperative period the substrate of FBs was sparse ECM with loci of rigid matrix, which in some cases was accompanied by transient IOP elevation. Increased inflammatory reaction of the conjunctiva 2 weeks after surgery was an indication for additional anti-inflammatory and antifibrotic therapy, the detailed description of which is presented below. In the late and delayed postoperative periods in this group a qualified hypotensive effect was achieved. Functional postoperative IOF outflow pathways were formed. According to OCT data, filtration blebs were visualized as widespread hyporeflective subconjunctival structures with sparse ECM.

Group 3 included 21 patients (age – 64.3 (52.2; 67.1) years) with IOPg > 16 mmHg; according to OCT data, changes in the newly created outflow tracts already in the early postoperative period were characterized by the presence of predominantly hyperreflective, rigid ECM. The results of ultrastructural immunohistochemical examination of the tissue of non-functional filtering blebs (n = 8) established the absence of fully developed lymphatic vessels among the studied samples [8]. The postoperative period was characterized by a severe inflam-

matory reaction of the conjunctiva and subconjunctival structures. Despite additional treatment, the hypotensive effect of NPDS was not achieved in this group.

Patients of groups 2 and 3 received a comparable amount of additional anti-inflammatory and cytostatic therapy during the postoperative period, which included subconjunctival injections of corticosteroids and antimetabolites (No. 5), as well as microinvasive needling revisions of the filtering bleb (No. 3) within 2 to 6 weeks after NPDS [11–13].

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.

Patients with POAG were examined before surgical treatment and 2 weeks (early postoperative period), 2 months (late postoperative period), and 12 months (delayed postoperative period) after surgery. The IOP level was determined (ORA, ORA Reichert, USA), the degree of ocular inflammatory reaction was assessed by biomicroscopy, and the state of newly created IOF outflow pathways and reflectivity of extracellular matrix of filtration blebs were determined by OCT (Anterior Segment OCT CASIA2, Tomey, Germany). Patients were divided into 3 groups based on these data. The group with optimal hypotensive effect of NPDS included patients with IOPg ≤ 16 mmHg during the delayed postoperative period without local hypotensive therapy, with functional postoperative IOP outflow pathways according to biomicroscopy and OCT/ultrasound biomicroscopy (UBM), as well as with areactive early postoperative period and no indications for additional anti-inflammatory and antifibrotic therapy.

The criteria of conditional hypotensive effect of NPDS were achievement of IOPg ≤ 16 mmHg in 12 months without local hypotensive therapy with functional outflow pathways according to biomicroscopy and OCT/UBM data, but the hypotensive effect of NPDS was obtained after additional anti-inflammatory and antifibrotic therapy during the early postoperative period.

The criteria for the absence of hypotensive effect of NPDS were as follows: IOPg > 16 mmHg; non-functional or partially functional newly created outflow pathways according to biomicroscopy and OCT/UBM data, despite additional anti-inflammatory and antifibrotic therapy.

Moreover, ultrastructural examination of filtering bleb tissue samples that were obtained during repeated surgical interventions was performed in 12 cases 12–18 months after NPDS. In 8 cases samples of non-functional scar-altered blebs were examined: in 4 cases fragments of functional, leaking filtering blebs were excised due to their significant displacement on the cornea and visual discomfort of the patients. Immunohistochemical staining for DNA expression of cell nuclei (DAPI) and podoplanin, a marker of lymphatic vessel endothelium, was performed with ultrastructural study of the obtained drugs using laser confocal microscope LSM 710 (Carl Zeiss AG, Germany).

The concentration of TGF-β, MMP-9, IL-6, IL-8 and vascular endothelial growth factor A (VEGF-A) (121 and 165) was determined in the tear fluid by ELISA using Human TGF-β, Human MMP-9 ELISA, IL-6-ELISA-Best, IL-8-ELISA-Best and VEGF-ELISA-Best kits (Vector-Best, Novosibirsk). Tear fluid from patients with POAG in the amount of 100 μl was collected capillary method from the lower conjunctival arch 4–6 hours before glaucoma surgery, as well as 2 weeks later (before laser descemetopuncture) and 2 months after NPDS.

Patients were included in the study on a voluntary basis, in accordance with the provisions of the World Medical Association Declaration of Helsinki (1964, rev. 2013). The study was approved by the decision of the Biomedical Ethics Committee of the Scientific Centre for Family Health and Human Reproduction Problems.

Statistical processing of the results of the clinical study was carried out using non-parametric statistics methods with calculation of the Mann-Whitney test. The obtained indicators were considered statistically significant with a significance level of $p < 0.05$. The median (Me) and interquartile range (25th–75th percentiles) were calculated to characterize the scattering in the sample. Taking into account significant deviation in the concentration of the investigated regulatory proteins and cytokines, line graphs of several variables were constructed in Statistica (StatSoft Inc., USA), which allowed us to visualize the dynamics of their content in the tear fluid in the pre- and postoperative period.

RESULTS

Group 1 patients were characterized by minimal (among the comparison groups) initial concentrations of TGF-β in tears and moderate values of this factor 2 weeks and 2 months (in 62 % of cases) after NPDS. However, in 38 % of the cases after 2 months there was a complete suppression of TGF-β tear, probably due to the resolution of postoperative inflammation (Table 1, Fig. 1). Moreover, high concentrations of MMP-9, increase of VEGF-A by the 2nd month after HPDS, as well as minimal (among the comparison groups) concentrations of proinflammatory IL-6 and IL-8 in the early postoperative period and their suppression in the late postoperative period were revealed in group 1 at all stages of the pre- and postoperative period.

Group 2 patients were characterized by extremely high baseline tear VEGF-A level and its peak increase by the 2nd month of the postoperative period. The initial concentrations of TGF-β, IL-6 and IL-8 in tears exceeded the values of group 1, and in the early postoperative period they increased by 1.5–2 times; this was accompanied by an increase in the inflammatory reaction of the conjunctiva 2 weeks after NPDS and was an indication for additional anti-inflammatory and antifibrotic therapy. After 2 months, there was suppression of these factors. Also, patients with conditional hypotensive effect of NPDS were characterized by a signifi-

TABLE 1

REGULATORY PROTEINS CONCENTRATION IN THE TEAR FLUID OF POAG PATIENTS AT VARIOUS STAGES OF THE PERIOPERATIVE PERIOD

Indicators	Group 1, Me (IQR)	Group 1, Me (IQR)	Group 1, Me (IQR)	*p* (Mann – Whitney test)
MMP-9 (baseline), ng/ml	16.1 (6.3–36.3)	15.2 (0.4–28.2)	14.4 (3.18–25.8)	$p_{1-2} > 0.05$ $p_{1-3} > 0.05$ $p_{2-3} > 0.05$
MMP-9 (2 weeks), ng/ ml	112.0 (96.5–142.1)	63.0 (18.5–102.4)	1.7 (0.16–2.2)	$p_{1-2} = 0.001$ $p_{1-3} = 0.001$ $p_{2-3} = 0.001$
MMP-9 (2 months), ng/ ml	25.1 (11.1–57.4)	4.2 (2.04–6.1)	3.6 (0.9–5.9)	$p_{1-2} = 0.001$ $p_{1-3} = 0.001$ $p_{2-3} > 0.05$
TGF-β (baseline), pg/ml	9.1 (0.0–22.0)	36.7 (0.0–96.0)	107.7 (15.7–142.4)	$p_{1-2} > 0.05$ $p_{1-3} = 0.003$ $p_{2-3} = 0.02$
TGF-β (2 weeks), pg/ml	294.6 (189–324.8)	150.4 (104.6–177.6)	590.6 (311.2–669.0)	$p_{1-2} = 0.001$ $p_{1-3} = 0.004$ $p_{2-3} = 0.001$
TGF-β (2 months), pg/ml	153 (0–256.0)	34.4 (0–130.2)	11.7 (0–47.7)	$p_{1-2} > 0.05$ $p_{1-3} = 0.03$ $p_{2-3} > 0.05$
VEGF-A (baseline), pg/ml	504.0 (452.0–572.0)	1018.0 (856.0–1409.0)	617.0 (503.0–709.0)	$p_{1-2} = 0.001$ $p_{1-3} > 0.05$ $p_{2-3} = 0.001$
VEGF-A (2 weeks), pg/ml	703.0 (370.0–847.0)	604.0 (452.0–705.0)	780.0 (624.0–815.0)	$p_{1-2} > 0.05$ $p_{1-3} > 0.05$ $p_{2-3} = 0.001$
VEGF-A (2 months), pg/ml	709.0 (622.0–1180.0)	1015.0 (826.0–1137.0)	439.0 (390.0–437.0)	$p_{1-2} = 0.04$ $p_{1-3} = 0.001$ $p_{2-3} = 0.001$
IL-6 (baseline), pg/ml	23.10 (21.3–25.0)	32.7 (29.3–34.9)	32.3 (30.0–34.4)	$p_{1-2} = 0.001$ $p_{1-3} = 0.001$ $p_{2-3} > 0.05$
IL-6 (2 weeks), pg/ml	55.4 (50.4–72.3)	41.6 (39.6–44.2)	36.8 (22.1–40.1)	$p_{1-2} = 0.001$ $p_{1-3} = 0.001$ $p_{2-3} = 0.003$
IL-6 (2 months), pg/ml	16.2 (11.0–20.6)	17.8 (15.9–19.0)	25.2 (19.3–64.4)	$p_{1-2} > 0.05$ $p_{1-3} = 0.001$ $p_{2-3} = 0.001$
IL-8 (baseline), pg/ml	182.0 (158.3–225.1)	211.6 (181.4–253.4)	461.0 (337.2–649.2)	$p_{1-2} > 0.05$ $p_{1-3} = 0.001$ $p_{2-3} = 0.001$
IL-8 (2 weeks), pg/ml	607.2 (537.0–690.0)	710.4 (607.0–822.6)	919.2 (747.8–1131.0)	$p_{1-2} > 0.05$ $p_{1-3} = 0.001$ $p_{2-3} = 0.006$
IL-8 (2 months), pg/ml	112.0 (82.0–171.0)	84.5 (49.5–118.3)	164.2 (110.9–224.6)	$p_{1-2} = 0.02$ $p_{1-3} = 0.04$ $p_{2-3} = 0.001$

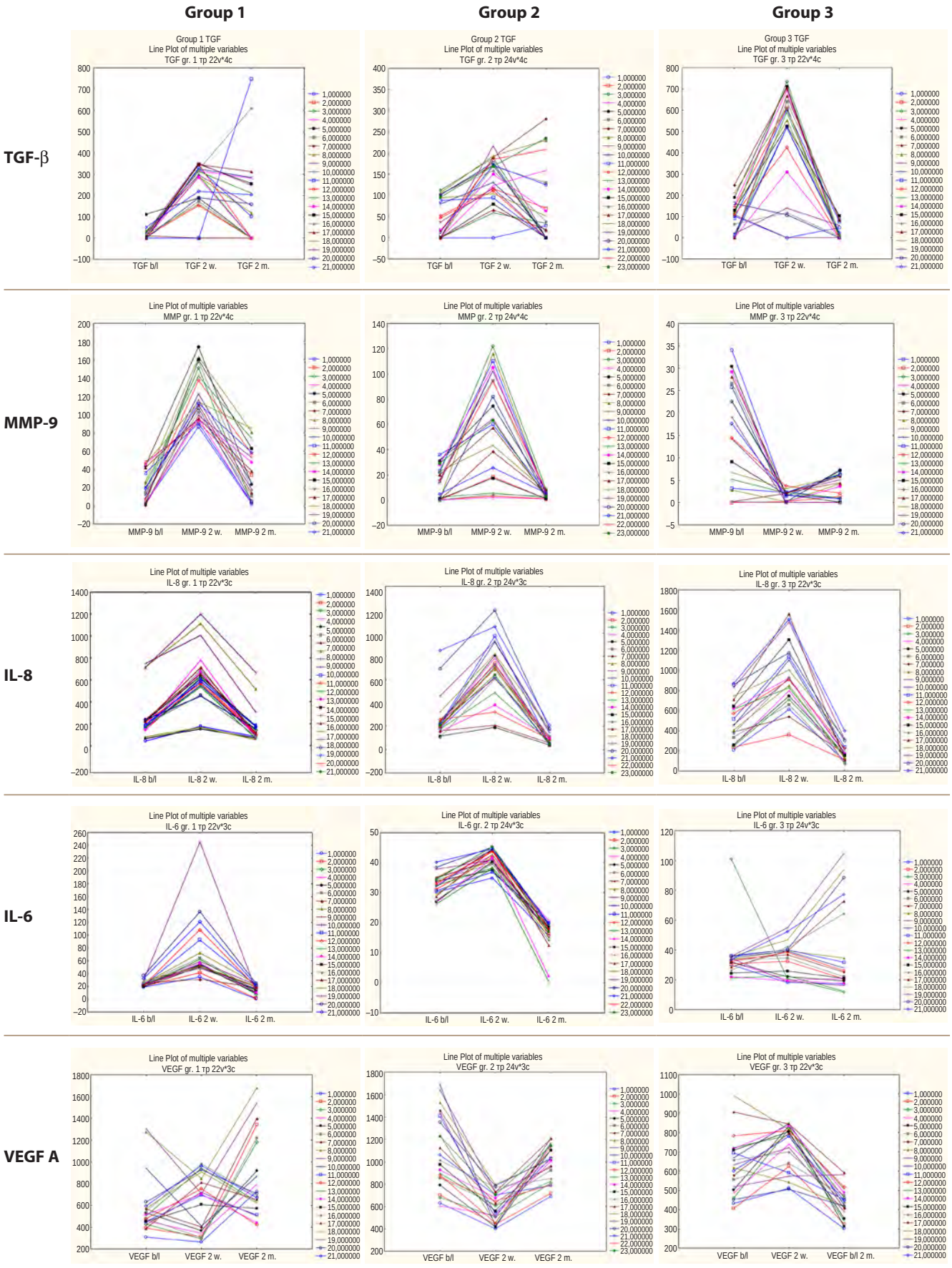

FIG. 1.

Linear graphs of changes in the cytokines and regulatory proteins concentration in the tear fluid at different follow-up periods in POAG patients of three clinical groups

cant increase in tear MMP-9 concentration in the early postoperative period (see Table 1, Fig. 1).

In group 3, tear TGF-β and IL-8 concentrations were maximal both before surgical treatment and in the early postoperative period. The principal difference between groups 1 and 2 was the decrease in tear MMP-9 2 weeks after NPDS. In addition, these patients maintained high IL-6 concentrations and had significant suppression of VEGF-A tears during the late postoperative period.

DISCUSSION

Wound healing is a complex dynamic process that is under constant biochemical control of regulatory molecules that provide specific interactions between cells and components of the extracellular matrix, leading to the restoration of the structural integrity of the tissue. As it was shown earlier [10, 14], functional FB after NPDS is a hypocellular subconjunctival structure in the form of a sparse ECM with fully developed lymphatic vessels, which allows effective outflow of intraocular fluid through the postoperative outflow pathways and determines the persistent hypotensive effect of glaucoma surgery.

The factors predisposing to the formation of optimal hypotensive effect of NPDS are: low initial concentrations of proinflammatory cytokines and regulatory proteins, in particular IL-6, IL-8, TGF-β, which determines minimal inflammatory reaction of the conjunctiva in the early postoperative period; high levels of MMP-9, responsible for timely degradation of the components of the temporary ECM at all stages of the postoperative period, as well as an increase in VEGF-A by the 2nd month after NPDS, which ensures conjunctival lymphangiogenesis.

The significant increase of MMP-9 during the early postoperative period and the peak increase of tear VEGF-A level in the late postoperative period in group 2 may have also contributed to the formation of functional pathways of intraocular fluid outflow, despite the significant inflammatory reaction from the conjunctiva 2 weeks after NPDS. Moreover, the obtained data allowed to establish biological markers of the effectiveness of additional therapeutic measures aimed at controlling the inflammatory reaction and excessive scarring processes in the area of surgical intervention. These are IL-6 and IL-8, as well as TGF-β, suppression of which by the 2nd month of the postoperative period determined the formation of a sufficient (conditional) hypotensive effect of NPDS.

The failure of surgery in group 3 was due to the highest initial level of factors with proinflammatory and profibrogenic activity, increased expression of IL-6, IL-8 and TGF-β in tears in the early postoperative period against the background of a significant decrease in MMP-9, as well as the persistence of high concentrations of IL-6 and suppression of VEGF-A 2 months after NPDS. This determined a severe ocular inflammatory reaction to surgical trauma and its chronization during the delayed postoperative period, active fibrogenesis, impaired degradation of components of the temporary ECM and absence of conjunctival lymphangiogenesis.

CONCLUSIONS

Thus, in the course of this study it was proved that the important conditions for the formation of functional pathways of IOF outflow after NPDS are the preservation of the balance between cytokines and regulatory proteins with proinflammatory and profibrogenic properties, and the factors that ensure timely degradation of temporary ECM components and activation of conjunctival lymphangiogenesis.

Initially high concentrations of IL-6, IL-8 and TGF-β in tear, suppression of MMP-9 and VEGF-A as a result of active and most likely excessive anti-inflammatory and cytostatic therapy in some cases contribute to the surgical failure of glaucoma surgery, as they lead to impaired structural reorganization of the temporary ECM and conjunctival lymphangiogenesis designed to ensure steady intraocular fluid outflow from the filtering blebs.

Conflict of interest

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

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PEDIATRICS

A CLINICAL CASE OF THROMBOSIS IN A TEENAGER IN THE POST COVID-19 PERIOD

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ABSTRACT

The article presents a case of a fatal outcome of a 15-year-old teenager with cerebral vascular thrombosis, which developed in the post COVID-19 period. The young man came in with complaints of headache, vomiting, photophobia, hyperacusis. It was known that at an early age he had been operated on for the Arnold – Chiari anomaly, had a subdural-peritoneal shunt. Two weeks before hospitalization, he suffered a new coronavirus infection of mild severity, which was confirmed by a positive analysis of a smear from the oropharynx by polymerase chain reaction.

Cephalgia was acute, stopped for a short time after the use of analgesics. During the examination, the patient laid with his eyes closed, asked for silence and blackout in the ward. Any movement of the head was accompanied by dizziness, headache, vomiting. During an objective examination, no pathological changes were found on the part of the internal organs.

The neurological status was determined by photophobia, double vision, asymmetric face, asymmetry of the eye slits S > D, drooping of the left corner of the mouth, nystagmus, hyperacusis. There was no rigidity of the occipital muscles. Meningeal signs were negative. There were no pelvic disorders.

According to the laboratory examination, lymphocytopenia, thrombocytosis, acceleration of ESR, moderate increase in ferritin, D-dimers were detected. Conducted neuroimaging methods (MSCT of the brain with intravenous contrast, brain MRI, ultrasound diagnostics of cerebral vessels) did not find any blood clots in intracranial and extracranial vessels. Low-positive values of IgM antibodies to cardiolipin were revealed. According to the results of polymerase chain reaction, polymorphism G20210A was detected in the prothrombin gene. The patient received high-dose glucocorticoids and heparin for treatment.

Despite the therapy, a month and a half after hospitalization, the teenager died from thrombosis of the central venous sinuses (cavernous sinus on the left, transverse sinuses, jugular veins), which were confirmed on autopsy.

This clinical case of venous sinus thrombosis in a teenager in the post COVID-19 period presented diagnostic difficulties, since accurate imaging methods did not detect the presence of a blood clot in the cerebral vessels.

Key words: *post COVID-19 syndrome, thrombosis, children, prothrombin gene mutation, antiphospholipid antibodies*

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КЛИНИЧЕСКИЙ СЛУЧАЙ ТРОМБОЗА У ПОДРОСТКА В ПОСТКОВИДНЫЙ ПЕРИОД

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РЕЗЮМЕ

В статье представлен случай летального исхода подростка 15 лет с тромбозом церебральных сосудов, развившимся в постковидном периоде. Юноша поступил с жалобами на головную боль, рвоту, светобоязнь, гиперакузию. Известно, что в раннем возрасте он был прооперирован по поводу аномалии Арнольда – Киари, имел субдурально-перитонеальный шунт. За две недели до госпитализации перенёс новую коронавирусную инфекцию в лёгкой степени тяжести, которая была подтверждена положительным анализом мазка из ротоглотки методом полимеразной цепной реакции.
Цефалгии носили острый характер, купировались кратковременно после применения анальгетиков. При осмотре лежал с закрытыми глазами, просил тишины и затемнения в палате. Любое движение головой сопровождалась головокружением, головной болью, рвотой. При объективном осмотре патологических изменений со стороны внутренних органов не обнаружено. В неврологическом статусе определялись светобоязнь, двоение в глазах, асимметричное лицо, асимметрия глазных щелей S > D, опущение левого угла рта, нистагм, гиперакузия. Ригидности затылочных мышц нет. Менингеальные знаки отрицательные. Тазовых нарушений нет.
По данным лабораторного обследования обнаружены лимфоцитопения, тромбоцитоз, ускорение скорости оседания эритроцитов, умеренное повышение ферритина, D-димеров. Проведённые методы нейровизуализации (мультиспиральная компьютерная томография головного мозга с внутривенным контрастированием, магнитно-резонансная томография головного мозга, ультразвуковая диагностика сосудов головного мозга) тромбов в интракраниальных и экстракраниальных сосудах не обнаружили. Выявлены низкопозитивные значения антител IgM к кардиолипину. По результатам полимеразной цепной реакции обнаружен полиморфизм G20210A в гене протромбина. В качестве лечения получал высокодозные глюкокортикоиды, гепарин.
Несмотря на проводимую терапию через полтора месяца от момента госпитализации подросток умер от тромбоза центральных венозных синусов (кавернозного синуса слева, поперечных синусов, яремных вен), которые подтверждены на аутопсии.
Данный клинический случай тромбоза венозного синуса у подростка в постковидном периоде представлял диагностические трудности, так как методы точной визуализации не обнаружили наличие тромба в церебральных сосудах.

Ключевые слова: *постковидный синдром, тромбоз, дети, мутация гена протромбина, антифосфолипидные антитела*

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RELEVANCE

For the past two years, the entire world has been focused on the infection caused by SARS-CoV-2. To date, the pathogenetic mechanisms that determine the severity of the course of this infection have been studied. Understanding of the main pathogenesis links led to the revision of therapeutic treatment, which is aimed at suppressing the synthesis of proinflammatory cytokines that cause the hyperinflammation syndrome, to stop hypercoagulability, which also affects the severity of the course of SARS-CoV-2 infection.

To a lesser extent, the mechanisms of the development of the so-called post COVID-19 syndrome have been studied. Post COVID-19 syndrome is characterized by various clinical manifestations that occur after SARS-CoV-2 infection and can last up to 6 months. Hyperproduction of cytokines, fibrosis, autoantibody production, and direct tissue damage by the virus are considered as the main pathogenetic links of this pathological condition. No less frequent manifestation of post COVID-19 syndrome is thrombosis in vessels of various caliber and localizations, which are due to vasculopathy and impaired hemostasis.

This article presents a death case of a teenager with venous sinus thrombosis that developed in the post COVID-19 period.

INTRODUCTION

Thrombosis in pediatric practice is quite rare. There are recognized risk factors for thrombosis: surgical treatment; presence of a central venous catheter; nephrotic syndrome; oncopathology; carrying mutations and polymorphisms of genes associated with a high risk of thrombosis; antibodies to phospholipids; and infections. The pathogenesis of SARS-CoV-2 infection includes hemostasis disorders that affect all its links: activation of vascular hemostasis, suppression of fibrinolysis, increased thrombin formation, which determines the risk of thrombosis [1–3]. To date, there are insufficient data on the incidence of thrombosis in children with SARS-CoV-2 infection, and there are no recommendations for the prevention of thrombosis in the post COVID-19 period. Nevertheless, hemostasis studies in children with SARS-CoV-2 infection show the presence of hypercoagulability [4]. In a systematic review of the literature [5], the authors presented data on 19 children with clinical thrombosis. The most frequent localization of thrombosis was pulmonary vessels (21 %); thromboses of various localizations (cerebral, intestinal, renal, and deep veins of the extremities) were also described. A more recent publication on the results of a multicenter retrospective study [6] determined that thrombosis is more likely to occur in children older than 12 years of age, and risk factors include the presence of multisystem inflammatory syndrome and a central venous catheter. These findings are supported by a literature review of 16 publications on the high incidence of thrombosis in children with multisystem inflammatory disease [7]. The authors showed that the incidence of thromboembolism in multisystem inflammatory syndrome is 1.4–6.5 %. One third of thrombosis cases are localized in cerebral vessels, which is accompanied by a high mortality rate. So of the three children with cerebral infarctions, all of them died. All publications on the presence of thrombosis in children refer to the acute period of SARS-CoV infection.

A search for publications in the scientific electronic libraries PubMed, e-Library yielded no data on the development of thrombosis in children in the post COVID-19 period.

THE AIM OF THE PUBLICATION

To present a case of venous sinus thrombosis in a post COVID-19 teenager.

CASE HISTORY

A 15-year-old boy was hospitalized at the Children's Republican Clinical Hospital in Ulan-Ude with complaints of headache, vomiting, photophobia, hyperacusis. From the patient's life history it is known that he was born from the first normal pregnancy. The delivery was physiological, on time, birth weight 3,076 g, height 52 cm. In physical and neuro-psychological development did not lag behind. No chronic illnesses. At the age of 11 months of life, he was operated for Arnold – Chiari malformation and had a subdural-peritoneal shunt. Monitored by a neurologist and neurosurgeon on an irregular basis.

Two weeks prior to hospitalization, he had a new mild form of coronavirus infection after family contact. In clinic there was subfebrile temperature for 2 days, weakness, sore throat, runny nose. SARS-CoV-2 infection was confirmed by positive analysis of an oropharyngeal swab by polymerase chain reaction.

As treatment he received Grippferon, throat gargle with antiseptics, decongestants. Recovery was recorded one week after the onset of the disease by a negative nasopharyngeal swab by polymerase chain reaction.

From the history of the disease it is known that 2 weeks after recovery from SARS-CoV-2 infection he began to complain of headaches with predominant localization in the occipital region. Cephalgia was acute, stopped for a short time after the use of analgesics. When seeking medical help at the place of residence, no focal neurological symptoms were detected. Continued symptomatic treatment was recommended. Subsequently, the headaches became continuous, accompanied by vomiting. Started noting that bright lights and loud noises increased the intensity of the cephalgias. In 5 days from the onset of the disease he was hospitalized at the Children's Republican Clinical Hospital in Ulan-Ude. On admission, the patient's condition was considered moderately severe. On examination: forced position due to intense cephalgias. The patient tried to lie still, as any movement of the head caused dizziness, headache, vomiting. Preferred to lie with his eyes closed, asked for silence and blackout in the room. On physical examination:

the skin is clean, pink, no pathologic changes in the pharynx. Lungs are vesicular. Heart tones are rhythmic, audible. The abdomen is soft, painless, liver and spleen are not enlarged.

Neurological status on admission

Cranial nerves: 1st pair – olfaction is not affected; 2nd pair – follows objects for a short time, photophobia; 3rd pair, 4th pair, 6th pair – pupils D = S, photoreaction is alive, eye movements in full volume, convergence, accommodation are normal, the patient noted double vision in extreme leads; 5th pair – sensitivity on the face is preserved, pain at palpation of the 1st branch on both sides; 7th pair – face asymmetrical, eye slits S > D, left corner of the mouth was lowered; 8th pair – nystagmus at the extremes of gaze, hearing preserved, hyperacusis; 9th pair, 10th pair, 12th pair – no bulbar disorders, tongue along the midline; 11th pair – movements in the cervical spine preserved, not limited. There are no sensory disturbances, active and passive movements in the limbs are not limited. Muscle tone in all muscle groups is satisfactory. Symmetrical and brisk tendon reflexes from the arms and legs. Doesn't walk, doesn't sit up, rolls over in bed. There was no rigidity of the occipital muscles. Meningeal signs were negative. There were no pelvic disorders.

Additional health examination results

A complete blood count (CBC) on admission: hemoglobin – 166 g/L, erythrocytes – 5.97 million/L**,** leukocytes – 16.6 thousand/μL, neutrophils – 80 %, lymphocytes – 7 %, monocytes – 12 %, platelets – 482 thousand/μL, an erythrocyte sedimentation rate (ESR) – 23 mm/h. There are no pathologic changes in the biochemical blood analysis. Coagulogram results: decreased thrombin time – 15.5 sec, a high d-dimer test – 2 mg/mL (norm – 0.5 mg/mL). Highly positive IgG values to SARS-CoV-2 were detected with a KP of up to 10.3. According to electroencephalogram: diffuse changes in electrobiological activity. No pathologic changes were found in the cerebrospinal fluid (CSF). Doppler ultrasound revealed decreased blood flow in the left vertebral artery, poor visualization of the left internal carotid artery. According to the data of duplex scanning of extracranial sections of brachiocephalic arteries, the conclusion about the increased level of peripheral resistance in the left internal carotid artery was given. Blood flow through the vertebral arteries without signs of extravascular compression, reduced velocity indices at the level of V1-, V2-segments.

Multispiral computed tomography of the brain was performed, and data were obtained on the presence of signs of the condition after subdural-peritoneal shunt, excision of a cyst of the left frontal and temporal region, Arnold – Chiari malformation, borderline ventriculomegaly, and arachnoid cyst of the left temporal region. Contrast-enhanced magnetic resonance imaging of the brain showed signs of Arnold – Chiari malformation, tonsillar herniation, asymmetric ventriculomegaly, deformation of the lateral ventricles, periventricular leukoareosis, and temporal lobe pole cyst on the left side. No pathologic selective accumulation of contrast agent was noted. No abnormalities in the cerebral vascular bed were found according to the results of neuroimaging methods.

Differential diagnostics was carried out with liquorodynamic disorders on the background of Arnold **–** Chiari malformation, hemorrhagic stroke, brain neoplasms.

The severity of the patient's condition was presumed to be due to post COVID-19 encephalitis. Pulse therapy with methylprednisolone at a dose of 10 mg/kg/injection, for a total dose of 2,500 mg followed by oral prednisolone 35 mg/day for 2 weeks was performed. During the whole period of hospitalization, the patient received anticoagulants – enoxiparin 40 mg/day, then heparin 20 units/kg/hour.

The patient's condition progressively deteriorated. The cerebral syndrome in the form of dizziness, headache persisted, and focal symptoms – complete ophthalmoplegia on the left, flaccid tetraparesis, facial nerve paresis on the left, bulbar paresis – were added. According to the data of blood tests, thrombocytosis remained within 400 thousand/μL, acceleration of ESR – 30–50 mm/h. A moderate increase in ferritin – 268.8 μg/L (reference values – 140 μg/L), IL-6 level – 4.1 pg/mL, which is not out of the norm, progressive increase in D-dimers up to 9 mg/L were revealed. The results of repeated magnetic resonance imaging of the brain with visualization of arterial vessels and venogram showed the absence of focal and diffuse changes in the brain substance, asymmetry of blood flow along the P1 segments of the posterior cerebral artery (D > S). Minimal asymmetry of blood flow along the intracranial section of vertebral arteries (D > S).

The search for prothrombogenic risk factors continued. There was a decrease in antithrombin III – 79.46 % (norm – 96–126 %), low level of homocysteine – 7.61 μm/l. To exclude antiphospholipid syndrome, antibodies to phospholipids were investigated; positive values of IgM antibodies to cardiolipin – 27 units/mL and negative IgG values were determined. No antibodies to β-2 glycoprotein-1, lupus anticoagulant were detected. A genetic study was performed to determine polymorphisms of genes responsible for hereditary thrombophilias. Polymerase chain reaction results revealed a G20210A mutation in the prothrombin gene.

Despite anticoagulant therapy, a month and a half after hospitalization, the teenager died of thrombosis of intracranial central venous sinuses (cavernous sinus on the left, transverse sinuses, jugular veins), which was confirmed on autopsy.

CONCLUSION

Venous sinus thrombosis in children is rare, with an average of 0.5 per 100,000 pediatric population [8]. There is a multifactorial nature of thrombogenic risks in its etiology, and 32 % of cases are hereditary thrombophilias [9]. This case once again confirms that thrombosis in pediatric practice occurs against the background of combined prothrombotic factors. Thus, the following risks of increased thrombosis were found in the teenager: the presence of subdural-peritoneal shunt, G20210A mutation in the prothrombin gene, hypohomocysteinemia, low level of antithrombin III. Low-positive antibodies to cardiolipin class IgM could not be an independent cause of thrombosis. They were more likely to be

produced by SARS-CoV-2 infection, which is also an independent risk for increased thrombosis.

The presented clinical case demonstrates the difficulty in the diagnostic search for cerebral venous thrombosis in a post COVID-19 teenager. The examinations performed with the inclusion of highly sensitive neuroimaging methods did not reveal thrombi in intracranial and extracranial vessels. But the presence of signs of hypercoagulability in a patient with neurologic focal symptoms allowed us to think about the possibility of cerebral vascular thrombosis. And despite the results of magnetic resonance imaging and multispiral computed tomography of the brain, the search for prothrombogenic factors continued.

Thus, we want to draw attention to the possibility of the development of hemostasis disorders in children not only during the acute course of SARS-CoV-2 infection, but also in the post COVID-19 period. Diagnosis of the thrombosis causes should include investigation of inherited thrombophilias as the most significant risk factor for increased thrombosis.

Conflict of interest

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

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COMPONENT COMPOSITION OF THE BODY IN CHILDREN WITH CHRONIC KIDNEY DISEASE ACCORDING TO THE RESULTS OF BIOIMPEDANSOMETRY

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ABSTRACT

Body composition reflects the dynamic processes in a child's development. The recommended restrictive diets for patients with advanced chronic kidney disease (CKD) contribute to a high risk of sarcopenic muscle wasting as diagnosed by bioimpedancemetry.

The aim of the study. *To assess BMI and body composition in children with CKD, to identify features of body composition in patients with different BMI Z-score values. Materials and methods. The physical development of 110 children with CKD of different stages was assessed. Patients were divided into two clusters: Group 1 (92 children) with BMI from 10.95 to 21.5 kg/m^2, BMI Z-score did not exceed +2.0 (without obesity); Group 2 (18 children) – BMI from 24.11 to 37.2 kg/m^2, Z-score BMI – more than +2.0 (obese). All underwent bioimpedancemetry, the proportion of fat and active cell mass was assessed. The comparison was carried out by nonparametric statistics methods.*

Results. *Changes in body composition were revealed: children without obesity had severe protein-energy deficiency in 7 cases (7.6 %). The difference in the proportion of fat mass in children of different groups, Me [Q1; Q3]: Group 1 – 18.00 % [14.00; 22.00], Group 2 – 35.00 % [21.98; 41.00], (Mann – Whitney U-test: U = 279.5, p = 0.00001). In Group 1, the active cell mass was 53.50 % [51.00; 56.00], in Group 2 – 41.50 % [39.00; 47.00] (U = 174.5, p = 0.000001), there were no significant differences in other parameters of bioimpedancemetry.*

Conclusions. *The proportion of active cell mass is lower in overweight children, with a significant predominance of the proportion of fat mass, which indicates the depletion of protein reserves due to their redistribution and possible insufficient alimentary intake in advanced stages of CKD.*

Key words: *children, chronic kidney disease, nutritional status, physical development, bioimpedance measurement, sarcopenia*

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КОМПОНЕНТНЫЙ СОСТАВ ТЕЛА ДЕТЕЙ С ХРОНИЧЕСКОЙ БОЛЕЗНЬЮ ПОЧЕК ПО РЕЗУЛЬТАТАМ БИОИМПЕДАНСОМЕТРИИ

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РЕЗЮМЕ

Актуальность. *Компонентный состав тела отражает динамические процессы в развитии ребёнка. Рекомендованные ограничительные диеты для пациентов с продвинутыми стадиями хронической болезни почек (ХБП) способствуют высокому риску саркопенического истощения мышц, что диагностируется биоимпедансометрией.*

Цель исследования*. Оценить ИМТ и компонентный состав тела детей с ХБП, выявить особенности состава тела у пациентов с разными значениями Z-score ИМТ.*

Материалы и методы. *Оценено физическое развитие 110 детей с ХБП разных стадий. Пациенты разделены на два кластера: группа 1 (92 ребёнка) – с ИМТ от 10,95 до 21,5 кг/м^2, Z-score ИМТ не превышал +2,0 (без ожирения); группа 2 (18 детей) – ИМТ от 24,11 до 37,2 кг/м^2, Z-score ИМТ – более +2,0 (с ожирением). Всем проведена биоимпедансометрия, оценивалась доля жировой и активной клеточной массы. Сравнение проводилось методами непараметрической статистики.*

Результаты. *Выявлены изменения компонентного состава тела: у детей без ожирения имелась тяжёлая белково-энергетическая недостаточность в 7 случаях (7,6 %). Доказано различие содержания доли жировой массы у детей разных групп (Me [Q1; Q3]): в группе 1 – 18,00 [14,00; 22,00] %, в группе 2 – 35,00 [21,98; 41,00] %, (U-критерий Манна – Уитни: U = 279,5; p = 0,00001). В группе 1 активная клеточная масса составила 53,50 [51,00; 56,00] %, в группе 2 – 41,50 [39,00; 47,00] % (U = 174,5; p = 0,000001), по остальным показателям биоимпедансометрии статистически значимых различий не получено.*

Заключение*. Доля активной клеточной массы ниже у детей с избыточной массой тела, при значительном преобладании доли жировой массы, что свидетельствует об истощении белковых запасов за счёт их перераспределения и возможного недостаточного алиментарного поступления при продвинутых стадиях ХБП.*

Ключевые слова*: дети, хроническая болезнь почек, нутритивный статус, физическое развитие, биоимпедансометрия, саркопения*

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INTRODUCTION

Pathology of the urinary system in the pediatric population ranks second in prevalence after respiratory diseases. Chronic kidney disease (CKD), a concept accepted in internal medicine, has entered pediatric nephrology as well. CKD in pediatrics has some peculiarities. Criteria for diagnosing and staging CKD in children adapted in the 2012 KDIGO (Kidney Disease Improving Global Outcomes). Congenital kidney disease is the main cause of chronic kidney disease in children. Congenital anomalies of the kidney and urinary tract (CAKUT) are the most common causes, accounting for 60 % of cases of CKD in children [1]. Genetically determined pathologies (cystinosis, oxalosis, hereditary nephritis, interstitial nephritis) account for 20–30 %. Glomerular lesions (mainly focal segmental glomerulosclerosis and lupus nephritis) account for 10 to 20 %. Diabetic nephropathy and hypertension are rare causes of chronic kidney disease in children compared with adults [2]. Obesity-related nephropathy is a condition recognised in both therapeutic and pediatric nephrology. The risk of accelerated development of chronic kidney disease is associated with low birth weight, often accompanied by a low number of nephrons in the kidneys [3].

Chronic kidney disease in children is accompanied by changes in metabolic processes, intoxication and comorbid diseases of the gastrointestinal, cardiovascular and endocrine systems [4–6]. Irreversible changes entail disorders of physical development and nutritional status, requiring thorough diagnosis and proper tactics for their correction [7, 8]. Nutritional status is a complex of clinical, anthropometric and laboratory indicators that characterise the quantitative ratio of muscle and fat mass of the patient's body. Nutritional status disorders are associated with protein-energy malnutrition, developmental delay, and mineral and bone disorders [9]. Anaemia is a frequent complication in children with chronic kidney disease, the prevalence of which increases as the disease progresses [1]. There are standards for the diagnosis and correction of protein-energy malnutrition, but there is no acceptable definition of this condition for children with chronic kidney disease and therefore no accurate diagnostic criteria [10]. Assessment of nutritional status in children is also complicated by the absence of a gold standard, specific abnormalities in body composition and a slowly progressive disease [11]. The causes of protein-energy malnutrition in children with progression of chronic kidney disease are diverse and are associated with impaired intake and assimilation of essential nutrients [9]. This is caused by poor appetite; dysgeusia may occur against the background of taking a large number of drugs and intoxication. Increasing chronic kidney disease is often accompanied by digestive disorders: vomiting associated with gastroesophageal reflux, delayed bowel voiding due to motility disorder. Numerous studies have proven the close association between nephropathy and impaired gut microbiota already in the onset of CKD. Impared microbiome and toxic endothelial damage interfere with the hydrolysis and nutrient absorption [12]. Any inflammation, infectious or immunopathological, causes metabolic acidosis and oxidative stress, increasing as kidney function declines. This entails aggravation of endothelial dysfunction, impaired permeability of cell membranes, and also changes the ratio of components in the intra- and extracellular space. Assessment of nutritional status and its correction at early stages of chronic kidney disease is the most important task of the pediatric nephrologist. Anthropometry (measurement of height, body mass and calculation of BMI) is the main method of detecting such abnormalities. However, body composition of children with chronic kidney disease, at least from stage 3 onwards, is peculiar; anthropometry is unable to identify the real degree of protein-energy malnutrition. Patients with chronic kidney disease are at high risk of sarcopenic muscle wasting and thus at increased risk of mortality [13, 14]. Most of the existing studies evaluating the correlation between chronic kidney disease and sarcopenia have been conducted in adult dialysis patients [15–19]. There are few current studies that suggest the use of bioimpedanceometry as a component of diagnosis of impaired physical development and nutritional status. Bioimpedanceometry in children with chronic kidney disease is of particular interest and in the future may become an indispensable component of diagnostics of nutritional status of a child [20, 21].

THE AIM OF THE STUDY

To assess the deviation of BMI Z-score and biocomponent body composition of children with chronic kidney disease, to identify the features of body composition in patients with different values of BMI Z-score.

MATERIALS AND METHODS

A continuous prospective single-center study describing a series of hospital cases (patients with chronic kidney disease) was conducted in the pediatric urology and nephrology departments of St. Petersburg State Pediatric Medical University of the Ministry of Health of Russia.

Study period: January 2016 – December 2021, with no unplanned shifts in the time intervals of the study. There were no additional specific factors whose effect during the study period could have influenced the conclusions. The main indicator of the study: distribution of patients according to body fat mass, clarification of the percentage of children with active cell mass deficiency in groups of children with normal and increased body mass index.

Inclusion criteria: 2 to 18 years of age and a confirmed diagnosis of chronic kidney disease. Exclusion criteria: chronic kidney disease before the age of 2 years; patients who failed to perform bioimpedance study due to psycho-emotional agitation and children old-

er than 2 years with height less than 95 cm were not included in the study.

The diagnostic inclusion criteria for chronic kidney disease, were reduced results of glomerular filtration rate, which was determined by the Rehberg test or the Schwartz equation. Chronic kidney disease was divided into 5 stages. Stage 1 CKD is characterised by the glomerular filtration rate more than 90 ml per minute, the absence of manifestations of nephropathy. At Stage 2 CKD, the glomerular filtration rate was 60–89 ml per minute, and there were initial signs of nephropathy. Stages 3A and 3B CKD are characterised by a reduced glomerular filtration rate of 59 to 30 ml per minute and signs of severe nephropathy, shriveling and scarring of renal tissue. Stage 4, severe, of CKD was identified when the glomerular filtration rate ranged from 29 to 15 ml per minute. Stage 5 CKD, kidney failure, is diagnosed when the glomerular filtration rate is less than 15 ml per minute.

Each patient underwent anthropometry (height, body weight and BMI assessment) and bioimpedanceometry using the DIAMANT-AIST apparatus. Physical development data were assessed using WHO Anthro and WHO AnthroPlus, free-access programmes from the official website of the World Health Organisation (WHO). The Z-score of body mass index (BMI), nondimensional statistic indicator used to compare values of different dimensionality, was evaluated. We studied the distribution of patients by body fat percentage, specifying the percentage of children with active cell mass deficiency in the groups of children with normal and increased BMI. We also studied possible differences in the percentage of fat, active cell mass, BMI and Z-score of BMI in different stages of chronic kidney disease, BMI and Z-score of BMI in children of different sexes. When the amount of extracellular/intracellular fluid was assessed, it was noted that none of the patients has oedema syndrome.

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The study was conducted in compliance with the World Medical Association Declaration of Helsinki. Parents (guardians) or the patient himself or herself over the age of 15 years signed a written consent to allow diagnostic and anthropometric procedures to be carried out for the study.

Methods of statistical processing

The sample size was not pre-calculated. The data were described and statistically analysed using Statistica v. 10.0 statistical software package (StatSoft Inc., USA). The data distribution was assessed by calculating Pearson's χ^2 test (Pearson's chi-squared test). Descriptive statistics (median (Me), 25 % and 75 % percentiles [Q1; Q3]) were used for data without normal distribution.

The Mann – Whitney U-criterion (*U*) was used to identify and evaluate the differences of quantitative parameters in two independent samples without normal distribution, and the Kruskal – Wallis test was used in three or more independent samples. The fairness of the tested hypothesis was assessed by the "*p* value", $p < 0.05$ was taken as the critical value.

RESULTS

A group of 130 children was evaluated: 20 cases had criteria for non-inclusion (less than 95 cm, younger than 2 years, psychoemotional reactions in bioimpedanceometry). Thus, the group was represented by 110 patients (65 boys and 45 girls). The sample size was limited to the number of patients staying in the specialised departments of the clinic during the period of anthropometric and bioimpedance studies.

The children included in the study had the following causes of chronic kidney disease: surgical disorders of urodynamics (high-grade vesicoureteral reflux, primary obstructive megaureter, hydronephrosis, multiple malformations of the urinary system, spinal disorders of urination), or manifestations of nephrotic syndrome, glomerulonephritis, systemic lupus erythematosus, systemic vasculitis. All patients suffered from chronic kidney disease, and children with urodynamic disorders were treated in the urology department, where they underwent surgical interventions aimed at correcting the disorders; nephrological patients were treated for the underlying disease in a specialized nephrology department.

Stage 1 CKD was found in 50 (45.45 %) children. Stage 1 CKD was diagnosed in 13 (11.81 %) patients. Stage 3B CKD was detected in 20 (18.18 %) patients, Stage 4 CKD, severe, was found in 13 (11.81 %) patients. This group had residual kidney function. Children had significant somatic and biochemical abnormalities and were registered in a dialysis centre. Stage 5 CKD, kidney failure, was present in 14 (12.72 %) children, in some cases already receiving renal replacement therapy (RRT) or preparing to switch to hemodialysis, with significant somatic, biochemical and anthropometric deviations from age norms.

The data set of 110 patients who were included in the study was clustered and two clusters were formed according to BMI. Group 1 (1st cluster) included 92 patients with BMI between 10.95 and 21.5 kg/m^2, BMI Z-score not exceeding +1.0. 50 patients had BMI Z-score less than –1. Group 2 (2nd cluster) included 18 patients with excessive BMI by Z-score and a BMI score between 24.11 and 37.2 kg/m^2. BMI Z-score was more than +2.0. This gave reason, according to WHO criteria, for the diagnosis of obesity in 18 patients (16.36 % of the total number of children) [22].

Further comparison of the two groups was carried out by such bioimpedancemetry indicators as the percentage of fat mass and the percentage of active cell mass, which allows to assess the reserves and saturation of muscle tissue and tissues of internal organs with protein, and to diagnose fat protein replacement in some cases.

TABLE 1

DISTRIBUTION OF CHILDREN OF THE FIRST CLUSTER ACCORDING TO Z-SCORE OF BODY MASS INDEX

Age group	Malnutrition		Standard	Body mass excess (BME)		Total
	Z-score < –3	Z-score from –3 to –2	Z-score from –2 to –1	Z-score from –1 to +1	Z-score from +1 to +2	
Early childhood*	2	4	10	17	3	36
Middle childhood**	2	7	8	11	2	30
Adolescence***	3	4	9	7	3	26
Total	7	15	27	35	7	92

Note. * – children 3–7 years old of both sexes; ** – children 7–11 years old (girls) and 7–12 years old (boys); *** – children 11–18 years old (girls) and 12–18 years old (boys).

52 children out of 92 in the first cluster had stage 1–2 CKD and 40 had stage 3–5 CKD. Overweight was found in 4 children with stage 1–2 CKD. Among patients with stage 3–5 CKD, overweight was found in 9 patients. There were no children with obesity among them (Table 1).

92 (83.63 %) had normal or low BMI (Group 1) and 18 (16.36 %) had obesity according to WHO criteria (Group 2). There was a statistically significant difference between the groups: the age of Group 1 was 8.63 [2.64; 17.69] years and the age of Group 2 was 15.27 [7.27; 18.97] years ($U = 377.5$; $p = 0.0002$). Children with low or normal index values showed half the median age at clustering (Fig. 1).

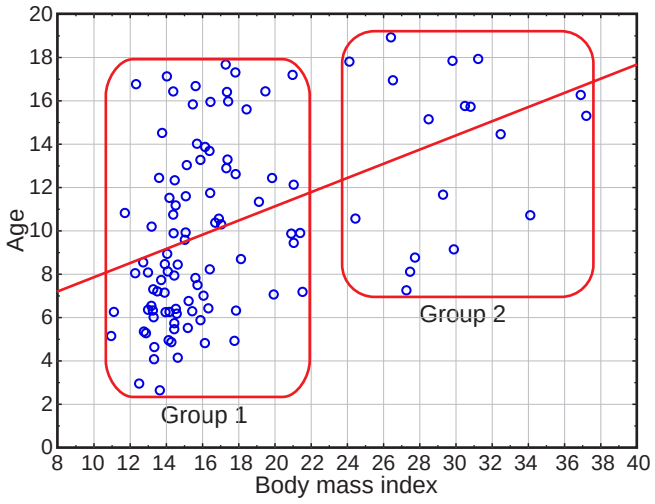

FIG. 1.

Distribution of body mass index in Groups 1 and 2 depending on age, with the formation of two clusters

According to bioimpedanceometry data, the percentage of fat mass in Group 1 was 18.00 [14.00; 22.00] %, which corresponded to 9.6 % deficiency, 85.1 % normal and 5.3 % excess fat mass; in Group 2 the percentage of fat mass was 35.00 [21.98; 41.00] %, which corresponded to 25 % normal and 75 % excess. Thus, the increase in fat mass deviation was more significant in Group 2 ($U = 279.5$; $p = 0.00001$) (Fig. 2).

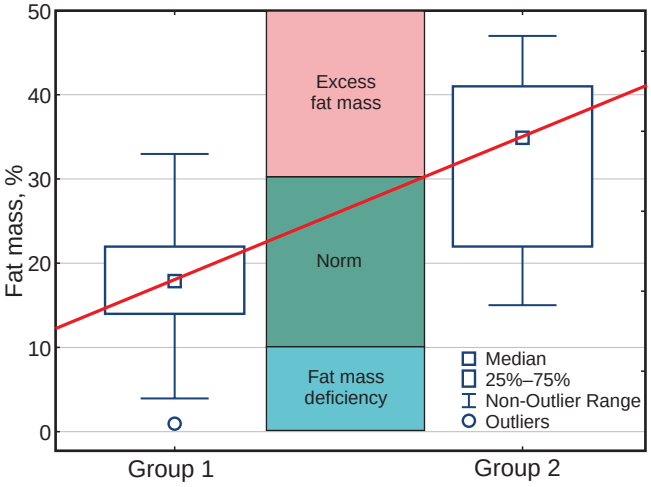

FIG. 2.

Comparison of deviations in the content of fat mass in Groups 1 and 2 (%)

In Group 1, active cell mass was 53.50 [51.00; 56.00] %, deficiency of active cell mass was noted in 19.1 %, excess in 7.4 %. In Group 2, active cell mass was 41.50 [39.00; 47.00] %, deficiency of active cell mass was noted in 81.0 %, excess was not found in any case. When analysed, there

was a statistically significant difference in the proportion of active cell mass in patients of Groups 1 and 2 ($U = 174.5$; $p = 0.000001$), with the development of a significant deficiency of active cell mass in Group 2 (Fig. 3). These patients have a significantly reduced amount of deposited muscle protein, due to redistribution and possibly insufficient alimentary intake, due to protein restriction in the diet of children with advanced chronic kidney disease.

When assessing the proportion of fat and active cell mass in subgroups of children with different stages of chronic kidney disease (stage 1–5), no statistically significant differences were obtained, according to the Kruskal – Wallis test (Table 2).

When anthropometric indicators were assessed in subgroups of children with different stages of chronic kidney disease by BMI Z-score, no statistically significant differences were obtained (Kruskal – Wallis test: $H = 2.123676$; $p = 0.7130$). Also, subgroup differences in BMI ($H = 2.776229$; $p = 0.5959$) were not proven (Table 3).

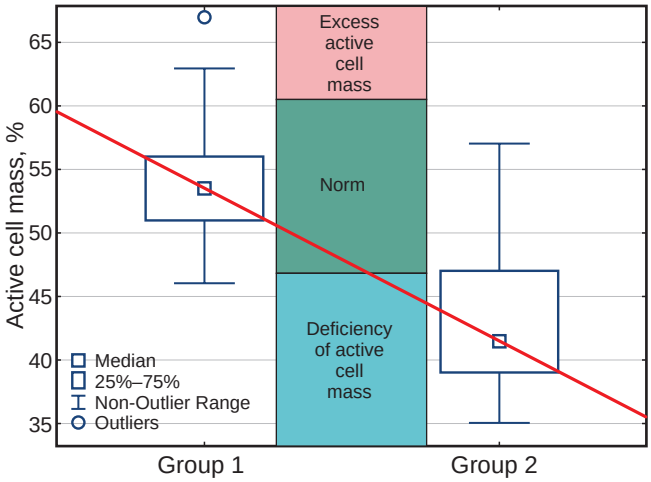

FIG. 3.
Comparison of the actual content of active cell mass in Groups 1 and 2 (%)

TABLE 2

THE PROPORTION OF ACTIVE CELL AND FAT MASS IN CHILDREN, DEPENDING ON THE STAGE OF KIDNEY DISEASE, Me [Q1; Q3]

Stage (*n*)	Age	Fat mass percentage, %	Active cell mass percentage, % ($p = 0.36$)
Stage 1 ($n = 50$)	8.51 [6.39; 11.68]	18.00 [14.00; 24.00]	53.50 [49.00; 56.00]
Stage 2 ($n = 13$)	10.74 [6.26; 15.99]	26.00 [18.00; 30.00]	49.00 [46.00; 53.00]
Stage 3 ($n = 20$)	9.92 [7.12; 15.88]	18.50 [16.00; 22.50]	52.50 [49.00; 55.00]
Stage 4 ($n = 13$)	11.18 [10.30; 13.90]	19.00 [18.00; 23.00]	54.00 [52.00; 55.00]
Stage 5 ($n = 14$)	12.96 [6.35; 14.53]	20.00 [15.00; 24.00]	52.00 [50.00; 54.00]

TABLE 3

MEDIAN DATA OF BODY MASS INDEX AND Z-SCORE OF BODY MASS INDEX IN CHILDREN DEPENDING ON THE STAGE OF KIDNEY DISEASE, Me [Q1; Q3]

Stage (*n*)	Body mass index ($p = 0.59$)	Z-score of body mass index ($p = 0.71$)
Stage 1 ($n = 50$)	15.26 [13.70; 18.11]	–0.97 [–1.94; 1.16]
Stage 2 ($n = 13$)	14.40 [13.58; 21.37]	–0.74 [–2.83; 0.82]
Stage 3 ($n = 20$)	15.90 [14.43; 23.20]	–0.83 [–1.46; 1.97]
Stage 4 ($n = 13$)	16.71 [15.23; 17.36]	–0.42 [–1.47; 0.15]
Stage 5 ($n = 14$)	15.65 [13.79; 17.84]	–1.14 [–2.08; –0.38]

TABLE 4

INDICATORS OF BODY MASS INDEX AND Z-SCORE OF BODY MASS INDEX IN CHILDREN DEPENDING ON GENDER, ME [Q1; Q3]

Stage	Sex	*n*	Body mass index ($p = 0.63$)	Z-score of body mass index ($p = 0.39$)
Stage 1	boys	$n = 30$	14.75 [13.70; 16.90]	–1.28 [–2.44; 0.32]
	girls	$n = 20$	16.15 [13.70; 20.37]	–0.56 [–1.47; 2.05]
Stage 2	boys	$n = 4$	17.35 [12.20; 27.74]	–0.16 [–5.74; 1.41]
	girls	$n = 9$	14.40 [14.20; 19.10]	–0.74 [–2.83; 0.63]
Stage 3	boys	$n = 12$	15.81 [14.44; 22.16]	–0.89 [–1.46; 0.41]
	girls	$n = 8$	15.90 [14.43; 24.18]	1.00 [–1.67; 2.14]
Stage 4	boys	$n = 10$	16.85 [16.17; 17.36]	–0.27 [–1.47; 0.23]
	girls	$n = 3$	15.23 [14.48; 19.50]	–0.49 [–1.62; –0.09]
Stage 5	boys	$n = 9$	14.40 [13.65; 18.42]	–0.79 [–2.08; –0.38]
	girls	$n = 5$	15.70 [15.61; 17.83]	–1.50 [–1.87; –0.61]

No subgroup differences by sex were found in BMI ($U = 1383.5$; $p = 0.6331$) and Z-score BMI ($U = 1323.0$; $p = 0.3980$) (Table 4).

DISCUSSION

The determination of active cell mass, the content of which characterises the percentage of metabolically active cells, is of great practical importance. Active cell mass includes mass of skeletal muscle, internal organs and nervous tissue. The percentage of active cell mass reflects muscle functional activity and indirectly allows us to estimate the physical strength reserve of an individual [20, 23]. There was a decrease in active cell mass percentage in Group 2 by more than a quarter compared to Group 1: 53.50 [51.00; 56.00] and 41.50 [39.00; 47.00] %. In Group 1, deficiency of active cell mass was detected in 19.1 % of cases, in Group 2 – in 81 % of cases. Percentage of fat mass in Group 1 was almost half that of Group 2 children (18.00 [14.00; 22.00] and 35.00 [21.98; 41.00] %). Excess fat mass in Group 1 was diagnosed in 5.3 % of children, in Group 2 excess fat mass was found in 75 % of cases. Adolescent obese patients are characterised by excess fat mass and its prevalence over active cell mass in the body component composition [24, 25]. According to a number of authors, excess fat mass and its prevalence over active cell mass progresses with age and becomes one of the predictors of early development of sarcopenia among adults [20].

The study of nutritional status of patients with CKD often reveals anthropometric abnormalities [26, 27], associated with the peculiarity of nutrition of children in this group (significant limitation of alimentary protein intake) [28], existing fluid and electrolyte disorders that progress with increasing course of CKD and a glomerular filtration rate decline. The percentage of active cell mass of a child with a normal or low BMI of 73.5 % corresponds to the norm, while in Group 2, there is a drop in the percentage of active cell mass and its replacement by fat cells due to muscle protein resorption, which leads to an increase in the percentage of fat

mass. According to bioimpedanceometry data, children with a high body mass index (Group 2) suffering from chronic kidney disease have in most cases active cell mass deficiency (mass of muscles and internal organs), which against the background of the body's load of fat replacement tissue significantly reduces physical abilities to move and vital activity. Children with chronic kidney disease may have both average anthropometric indicators and body mass deficiency or obesity [26]. Patients with chronic kidney disease face serious challenges in maintaining adequate nutrition and growth [28]. Our study showed a higher content of adipose tissue in children with excessive body weight on the background of chronic kidney disease, due to the replacement of active cell mass, which can be interpreted, in fact, as steatosis (adipose degeneration) of the macroorganism. The study allowed for the first time in Russia to assess the state of fat and active cellular components of patients with renal pathology, with the formation of evidence-based conclusions about significant differences in the groups of children with low/normal and excessive body weight, which contributes to the understanding of the formation of nutritional status of these patients. The results obtained in the prospective study have a high level of statistical significance and allowed us to make valid conclusions [29]. To develop precise recommendations for daily protein supplementation for children with deficiency of active cell mass on the background of chronic kidney disease and steatosis (adipose degeneration), further studies are needed to assess the influence of factors of alimentary and renal metabolism, the state of the macroorganism, family and social factors.

Study limitations

There was no objective possibility to fully take into account the peculiarities and quality of nutrition of patients, social status, duration of the disease and effectiveness of the treatment for the study. Thus, it is possible that the actual values of fat and active cell mass in subgroups of children with chronic kidney disease and different social status may slightly differ. A larger sample size could allow for in-depth exploratory analyses in subgroups, assessing possible correlations and other influencing factors.

CONCLUSION

Our study revealed that all children with chronic kidney disease can be divided into two groups based on obesity, according to BMI values and WHO criteria. The biocomponent composition of the patients' body was proved to differ in the percentages of fat and active cell mass. Almost 85 % of children in Group 1 have normal fat mass, while in Group 2 the percentage of such patients is almost 4.5 times lower, and most children have excess body fat mass. In the group of children without obesity, there was severe protein-energy deficiency in 7 cases (7.6 %). Children with pathologically high values of BMI Z-score (more than +2) have a significant deficiency of active cell mass against the background of excess adipose tissue due to resorptive-replacement processes and insufficient nutritional intake, which corresponds to the criteria of sarcopenia [21]. The results of the study demonstrate the necessity of using bioimpedanceometry for complex diagnostics of nutritional status disorders for each particular child with chronic kidney disease, regardless of the stage of the disease.

The work was carried out as a part of the research work (state registration number of NIOKTR (Research, Development and Technological Work) AAA-A18-118113090077-0 dated 30.11.18) "Screening of nutritional status in children with somatic, surgical and neurological pathology, correction possibilities". The study was not sponsored.

Conflict of interest

The authors declare the absence of a conflict of interest.

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PSYCHOLOGY AND PSYCHIATRY

HIGH SLEEP REACTIVITY: CLINICAL, PSYCHOLOGICAL AND POLYSOMNOGRAPHIC FEATURES

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ABSTRACT

Background. *The model of sleep reactivity to stress considers sleep reactivity to stress as a link in the pathogenesis of insomnia disorder – the degree to which stress disturbs sleep, which manifests as difficulty in initiating and maintaining sleep.*
The aim. *To study clinical and psychological features as well as subjective and objective sleep indexes of subjects with high level of sleep reactivity to stress.*
Materials and methods. *The psychological status, subjective indexes of sleep and sleep reactivity to stress according to Ford Insomnia Response to Stress Test were studied among 18–75 year-old subjects without significant sleep disturbances and patients with chronic insomnia. Polysomnography was performed for objective evaluation of sleep parameters.*
Results. *It was found that individuals with high levels of sleep reactivity to stress were characterized by high levels of anxiety, restlessness, and neuroticism. According to results of Pittsburg questionnaire, a lower quality of sleep was revealed. These findings were correlated with objective indexes of sleep according to polysomnographic studies: less deep sleep and its lower efficiency due to sleep disturbances.*
Conclusions. *Individuals with high sleep reactivity to stress are characterized by greater anxiety combined with subjective and objective sleep disturbance like insomnia type.*

Key words: *insomnia, sleep reactivity, anxiety, polysomnography, sleep efficiency*

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КЛИНИКО-ПСИХОЛОГИЧЕСКИЕ И ПОЛИСОМНОГРАФИЧЕСКИЕ ОСОБЕННОСТИ ЛИЦ С ВЫСОКОЙ РЕАКТИВНОСТЬЮ СНА К СТРЕССУ

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РЕЗЮМЕ

Обоснование. *Модель реактивности сна к стрессу рассматривает в качестве звена патогенеза инсомнического расстройства реактивность сна к стрессу – степень, в которой стресс нарушает сон, что проявляется в виде трудностей инициации и поддержания сна.*
Цель работы*. Изучить клинико-психологические особенности, а также субъективные и объективные показатели сна испытуемых с высоким уровнем реактивности сна к стрессу.*
Методы. *Среди респондентов 18–75 лет без значимых жалоб на нарушения сна и среди пациентов с хронической инсомнией оценён психологический статус, субъективные показатели сна и реактивность сна к стрессу по опроснику Форда по влиянию стресса на сон (Ford Insomnia Response to Stress Test), а также проведена полисомнография для объективной оценки показателей сна.*
Результаты. *Установлено, что для лиц с высоким уровнем реактивности сна к стрессу характерны высокие уровни тревожности, тревоги, невротизации. По результатам Питтсбургского опросника выявлено более низкое качество сна. Эти данные согласуются с объективными показателями сна по результатам полисомнографического исследования: менее глубоким сном и его меньшей эффективностью за счёт нарушения поддержания сна.*
Заключение. *Лица с высокой реактивностью сна к стрессу характеризуются большей тревожностью в сочетании с субъективным и объективным нарушением сна по типу инсомнии.*

Ключевые слова*: инсомния, реактивность сна к стрессу, тревога, полисомнография, эффективность сна*

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OBJECTIVES

Insomnia is a condition that characterized by subjectively unsatisfactory quality or duration of sleep associated with difficulties of falling asleep, sleep maintenance disorders, and/or early (unintentional) awakenings 3 times a week with impaired daytime functioning or more when there are opportunities for comfortable sleep [1]. Along with the most commonly used models of the aetiopathogenesis of insomnia, the "three P's" model and the hyperactivation model, a new concept – sleep reactivity to stress – is currently being used [2]. Sleep reactivity to stress is regarded as a complex feature determined both genetically and by environmental influences, which manifests itself in a propensity to produce sleep disturbances in response to exposure to various stressors [2, 3]. Research is needed in order to determine whether the sleep reactivity to stress factor may be a risk marker for the development of insomnia, as reliable premorbid predictors for the development of this variant of insomnia have not been determined to date.

THE AIM OF THE STUDY

To evaluate the clinical-psychological and polysomnographic features of individuals with high sleep reactivity to stress.

METHODS

Study design. Inclusion in the study was conducted among patients 18–75 years old who applied to the consulting and diagnostic department of the Almazov National Medical Research Centre of the Ministry of Health of Russia with complaints of sleep disorders, in whom the Insomnia Severity Index [4] exceeded 15 points. In addition, volunteers without relevant complaints were included in the study as a comparison group. All study participants completed a questionnaire including:

- The Ford Insomnia Response to Stress Test (FIRST) was used to assess sleep reactivity to stress [2];
- subjective assessment of the main sleep parameters over the last month, which was performed using the Pittsburg questionnaire [5] with a total score;
- assessment of insomnia severity using the Insomnia Severity Index [4];
- assessment of clinical and psychological features using the Integrative Anxiety Test [6]. The questionnaire consists of 30 questions with answers on the incidence rate of emotional states recently ("never", "rarely", "often", "almost all the time") with calculation of the sum of scores and division of the questionnaire into subscales of state and trait anxiety. The obtained "raw" scores are converted into standard ten (sten) (from 1 to 9): a score on the general anxiety scale below 4 stens corresponds to a low level of anxiety, 4–6 – to norm, 7 stens and above – to a high level of anxiety;
- assessment of neurotization according to the Scale for psychological express diagnostics of the neuroticism level (NL) [7].

Further, objective sleep assessment by polysomnography (PSG) was performed on the Embla N7000 device (Natus, USA) without medical supervision during one night with assessment of the main sleep characteristics as per the AASM 2.5 rules [8]. Patients with significant acute and chronic comorbidities, including those taking medications that could significantly affect the estimated sleep parameters, were not included in the study. The exclusion criterion was concomitant sleep disorders detected by PSG results (sleep apnea-hypopnea index > 15/h, periodic limb movement index (PLMI) > 15/h). Based on the results of Ford's questionnaire, the subjects were divided into groups with low (< 18 points) and high reactivity (≥ 18 points) [2].

The study was performed in the Almazov National Medical Research Centre of the Ministry of Health of Russia (St. Petersburg) from February 2020 to May 2022. The research report was approved at the meeting of the local ethical committee of the Almazov National Medical Research Centre of the Ministry of Health of Russia No. 02–20 dated February 17, 2020. All subjects signed informed consent to participate in the study prior to the report procedures.

Statistical analysis

The following software was used to analyze statistical data: Statistica v. 8 (StatSoft Inc., USA). The following statistical procedures for analyzing empirical data were used: descriptive statistics (auxiliary indicators for describing the results of other procedures – mean and median), Shapiro – Wilk test (for assessing the normality of distribution and choosing the methodology of intergroup comparison), Student's t-test (applied to parameters having normal distribution and presented in a metric or interval scale) and Mann – Whitney U test (for cases when normal distribution was not observed or the scale was ranked) for comparing quantitative variables, Fisher's exact test for qualitative parameters. The statistical significance level was taken as $p < 0.05$.

STUDY RESULTS

A total of 34 subjects were examined. According to Ford's questionnaire, a high level of sleep reactivity to stress was found in 27 people (76.5 %), of which 8 (23.5 %) were men, with all men having a high level of reactivity. The mean age (Table 1) (35.1 ± 15.5 and 34.9 ± 15.6 years) and other sociodemographic parameters did not differ between the studied groups. The median reactivity level was 24 points, with 26 (10–33) points among those with insomnia, and 22 (13–29) points among those without significant complaints ($p = 0.009$).

When assessing psychological status (Table 2), higher levels of trait anxiety ($p = 0.001$) and state anxiety ($p = 0.002$) were found in the highly reactive group of respondents. In addition, the results of the ITT scales were analyzed. Subjects in the high reactivity group demonstrated higher levels on the following subcomponents of trait anxiety: "emotion-

TABLE 1
SOCIAL AND DEMOGRAPHIC CHARACTERISTICS OF SURVEY GROUPS

Parameters	Total	Low reactivity group (*n* = 7)	High reactivity group (*n* = 27)	*p*
Age	34.96 ± 15.34	35.14 ± 15.53	34.92 ± 15.62	0.739
Gender:				
male	8 (23.5 %)	0 (0 %)	8 (29.6 %)	0.160
female	26 (76.5)	7 (100 %)	19 (70.4 %)	
Education:				
higher	17 (50 %)	2 (28.6 %)	15 (55.6 %)	0.157
secondary	11 (32.35 %)	2 (28.6 %)	9 (33.3 %)	
vocational secondary	6 (17.65 %)	3 (42.8 %)	3 (11.1 %)	
Job:				
employed	24 (70.6 %)	5 (71.4 %)	19 (70.4 %)	1.000
unemployed	10 (29.4 %)	2 (28.6 %)	8 (29.6 %)	
Smoking:				
smokers	8 (23.5 %)	2 (28.6 %)	6 (22.2 %)	1.000
non-smokers	26 (76.5 %)	5 (71.4 %)	21 (77.8 %)	
Comorbidities:				
hypertension	1 (3 %)		1 (4 %)	0.405
diabetes mellitus	1 (3 %)	1 (14 %)		
others	16 (47 %)	2 (28 %)	14 (52 %)	
Alcohol:				
no	9 (26.5 %)	3 (43 %)	6 (22 %)	0.634
1–2 times a month	17 (50 %)	3 (43 %)	14 (52 %)	
on a regular basis	8 (23.5 %)	1 (14 %)	7 (26 %)	
Physical activity:				
No	2 (6 %)	1 (14 %)	1 (4 %)	0.292
occasionally	11 (32 %)	3 (43 %)	8 (30 %)	
on a regular basis	21 (62 %)	3 (43 %)	18 (66 %)	
BMI	24.79 ± 9.85	23.16 ± 5.64	25.21 ± 10.72	0.496
Insomnia severity index > 5 points	15 (44.1 %)	1 (14.3 %)	14 (51.8 %)	0.104
Insomnia severity index, points	12 (2–25)	7 (2–17)	16 (3–25)	0.127

al discomfort" ($p = 0.047$), "asthenic component of anxiety" ($p = 0.009$), "phobic component" ($p = 0.033$), and "anxious evaluation of perspective" ($p = 0.002$). Levels of state anxiety were also higher in the high reactivity group according to its individual components: "emotional discomfort" ($p = 0.029$), "asthenic component" ($p = 0.049$).

When assessing neuroticism level (NL), a predominance of higher values was found in subjects with high reactivity

TABLE 2

PSYCHOLOGICAL INDICATORS IN THE LOW AND HIGH REACTIVITY OF SLEEP TO STRESS GROUPS

Parameters	Low reactivity group, Me (Q1; Q3)	High reactivity group, Me (Q1; Q3)	*p*
ITT_T_st, sten	5 (5; 6)	8 (7; 9)	0.001
ITT_T_ED_st, sten	6 (5; 8)	7 (7; 9)	0.047
ITT_T_AST_st, sten	6 (4; 8)	8 (6; 9)	0.009
ITT_T_PHOB_st, sten	5 (3; 6)	7 (5.75; 8)	0.033
ITT_T_EP_st, sten	5 (4; 6)	7.5 (6; 9)	0.002
ITT_T_SP_st, sten	4 (1; 7)	5 (2.75; 7.25)	0.252
ITT_S_st, sten	1 (1; 2)	5 (2.5; 6)	0.002
ITT_S_ED_st, sten	1 (1; 1)	3 (1; 6)	0.029
ITT_S_AST_st, sten	5 (1; 6)	7 (6; 8.5)	0.049
ITT_S_PHOB_st, sten	1 (1; 3)	4 (1; 6)	0.110
ITT_S_EP_st, sten	1 (1; 5)	4 (2.5; 5.5)	0.121
ITT_S_SP_st, sten	1 (1; 5)	4 (1; 5)	0.425
Insomnia severity index, points	7 (4; 7)	16 (8; 18)	0.058
PSQI_quality of sleep, points	1 (1; 2)	2 (1; 3)	0.048
PSQI_total score, points	6 (4; 8)	10 (9; 15)	0.008

Note. Structure components of trait anxiety: ITT_T_st – trait anxiety; ITT_T_ED_st – emotional discomfort; ITT_T_AST_st – asthenic; ITT_T_PHOB_st – phobic; ITT_T_EP_st – anxious evaluation of perspective; ITT_T_SP_st – social protection. Structure components of state anxiety: ITT_S_st – state anxiety; ITT_S_ED_st – emotional discomfort; ITT_S_AST_st – asthenic; ITT_S_PHOB_st – phobic; ITT_S_EP_st – anxious evaluation of perspective; ITT_S_SP_st – social protection. PSQI – Pittsburgh Sleep Quality Index.

TABLE 3

SLEEP PARAMETERS BY PSG IN THE GROUPS OF HIGH AND LOW REACTIVITY OF SLEEP TO STRESS

Parameters	Low reactivity, Me (Q1; Q3)	High reactivity, Me (Q1; Q3)	*p*
Duration of sleep, min	450.5 (441.6; 474)	383.5 (344; 453)	0.086
Sleep efficacy, %	93 (78.5; 94)	77.9 (65; 85.4)	0.004
Wake time after sleep onset, min	22 (13.2; 24)	85.8 (34.8; 159.8)	0.013
Sleep latency, min	13.9 (6; 43)	28.3 (9.8; 65)	0.273
NREM-sleep stage 1 representation, %	3.5 (2.8; 8)	5 (4.4; 14)	0.036
NREM-sleep stage 2 representation, %	53.5 (49.8; 56)	46.7 (36.5; 53.8)	0.141
NREM-sleep stage 3 representation, %	23.5 (15; 28.4)	16.6 (13; 21)	0.026
REM-sleep representation, %	15.8 (8; 23)	14.7 (10.4; 20.5)	0.961

Note. NREM – non-rapid eye movement; REM – rapid eye movement.

(47.86 ± 24.96; 8.11 ± 38.16; $p = 0.014$). Individuals with low sleep reactivity to stress had a very low NL, indicating a low probability of neuroticism (ranging from 6 % in men to 13 % in women), whereas the group with high sleep reactivity to stress had an indeterminate NL, with a 49–50 % probability of neuroticism. Both subjective sleep quality score ($p = 0.048$) and total score ($p = 0.008$) as per the Pittsburgh Sleep Quality Index were higher in the low sleep reactivity to stress group, but no statistically significant differences were found on the Insomnia Severity Index. The results obtained in the questionnaire are consistent with objective sleep indicators in this group (Table 3): lower sleep efficien-

cy (p = 0.004) mainly due to impaired sleep maintenance (longer wakefulness after sleep onset, p = 0.013) and less deep sleep (1.5 % greater representation of the 1st stage of non-rapid eye movement (p = 0.036) and less representation (as a percentage) of the 3rd stage of non-rapid eye movement (p = 0.026)).

DISCUSSION

In this work, we evaluated the clinical and psychological characteristics of 34 volunteers and patients with complaints of sleep disorders, dividing them into groups with low and high sleep reactivity to stress, which was found in 4/5 of the respondents. Screening techniques for assessing anxiety and neuroticism were chosen to assess psychological status, since individuals with high anxiety are more likely to suffer from sleep disorders, and anxiety is one of the factors in the structure of the Ford scale, with the likelihood of developing sleep disorders before an important event (questions 1, 8, 9) [2]. The findings of high levels of trait anxiety as per the ITT assessing perspective taking, hyperactivation, and phobias in highly reactive individuals are consistent with the notion of predisposing factors for the development of insomnia [9]. And questions assessing the asthenic component of trait anxiety describe typical complaints of persons with insomnia. In contrast to trait anxiety, there were no differences for the "anxious evaluation of perspective" and "phobic component" when assessing state anxiety. The "social protection" component is optional in the anxiety assessment and was not significant for either trait or state anxiety. The level of state anxiety was below normal in the low reactivity group, and average in the high reactivity group, with statistically significant differences on the components of emotional discomfort and asthenia. Thus, the results obtained at this stage of the study are consistent with the data of works describing the association of a high level of sleep reactivity to stress and the severity of anxiety [10, 11], as well as symptoms of insomnia [12, 13]. Current standards for the diagnosis of insomnia do not require instrumental confirmation of sleep disturbance by PSG; it is used to exclude comorbid sleep disturbances [9]. Our data from a previous analysis of objective sleep characteristics of individuals with insomnia symptoms showed no statistically significant differences in PSG scores [14] when compared to healthy volunteers. The changes in PSG detected in our study may be a response to polysomnographic examination, as it is known about the «first night effect», when some people sleep worse on the first night of PSG and better on the second and subsequent nights [15]. At the same time, impaired sleep quality in highly reactive individuals may indicate a more severe sleep disturbance than insomnia. Drake C. data from a prospective 1-year follow-up cohort of individuals without symptoms of insomnia or depression suggest a 3-fold increased risk of developing insomnia among individuals with high Ford Insomnia Response to Stress Test, even after adjusting for stress exposure and sociodemographic factors. Increased sleep latency was also found among those who developed insomnia [16]. A limitation of the study is the small sample size and lack of prospective follow-up. At the same time, a comprehensive assessment of anxiety components and objective sleep assessment by PSG allows us to evaluate the characteristics of individuals with high sleep reactivity to stress.

CONCLUSION

Within the framework of the conducted work the following features of the subjects of the group of high sleep reactivity to stress were revealed: higher levels of anxiety as an individual-typological property, anxiety as a state, neuroticism, as well as worse subjective and objective sleep indices. Assessment of sleep reactivity to stress using the Ford Insomnia Response to Stress Test may be a practical tool for predicting objective sleep disturbances characteristic of insomnia. Prospective follow-up is required to assess the prognostic value of the development of insomnia in individuals with different sleep reactivity to stress.

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Conflict of interest

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

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TRAUMATOLOGY

OUR FIRST EXPERIENCE WITH THE USE OF HYDROXYAPATITE PASTE TO IMPROVE THE INTEGRATION OF THE GLENOID COMPONENT OF A REVERSE PROSTHESIS WITH A BONE DEFECT OF THE SCAPULA (CASE REPORT)

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ABSTRACT

The problem of reverse shoulder arthroplasty with various deformities of the glenoid is relevant for modern traumatology and orthopedics. In addition to various defects, the methods of solving which can be eccentric reaming of the glenoid by milling cutters, bone autoplasty, augmentation, the use of individual implants, orthopedic traumatologists have to deal with a decrease in the mineral density of the bone tissue of the scapula.

***The aim of this study** is to demonstrate the possibility of using hydroxyapatite paste together with bone autoplasty in revision shoulder arthroplasty in conditions of a massive defect and reduced glenoid bone density.*

***Discussion.** The article presents a case of surgical treatment of a patient with the consequences of a fracture of the proximal metaepiphysis of the humerus and local osteoporosis of the glenoid by the method of reverse shoulder arthroplasty in combination with the use of hydroxyapatite paste. A detailed description of the operation technique is given.*

***Conclusion.** The described clinical case demonstrates the effectiveness of the technique of using hydroxyapatite preparations for shoulder joint replacement.*

***Key words:** shoulder joint, omarthrosis, reverse arthroplasty, glenoid, hydroxyapatite,* osteoporosis

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НАШ ПЕРВЫЙ ОПЫТ ИСПОЛЬЗОВАНИЯ ГИДРОКСИАПАТИТНОЙ ПАСТЫ ДЛЯ УЛУЧШЕНИЯ ИНТЕГРАЦИИ ГЛЕНОИДАЛЬНОГО КОМПОНЕНТА РЕВЕРСИВНОГО ПРОТЕЗА ПРИ КОСТНОМ ДЕФЕКТЕ ЛОПАТКИ (СЛУЧАЙ ИЗ ПРАКТИКИ)

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РЕЗЮМЕ

Проблема реверсивного эндопротезирования плечевого сустава при различных деформациях гленоида является актуальной для современной травматологии и ортопедии. Кроме различных дефектов, методами решения которых могут быть эксцентричная разработка гленоида фрезами, костная аутопластика, аугментация, применение индивидуальных конструкций, травматологам-ортопедам приходится сталкиваться со снижением минеральной плотности костной ткани лопатки.

***Целью** настоящего исследования является демонстрация возможности применения пасты с гидроксиапатитом совместно с костной аутопластикой при ревизионном эндопротезировании плечевого сустава в условиях массивного дефекта и сниженной плотности костной ткани гленоида.*

***Обсуждение.** В статье представлен случай оперативного лечения пациентки с последствиями перелома проксимального метаэпифиза плечевой кости и локальным остеопорозом гленоида методом реверсивного эндопротезирования в сочетании с применением гидроксиапатитной пасты. Дано подробное описание техники операции.*

***Заключение.** Описываемый клинический случай демонстрирует эффективность методики применения препаратов гидроксиапатита при эндопротезировании плечевого сустава.*

***Ключевые слова:** плечевой сустав, омартроз, реверсивное эндопротезирование, гленоид, гидроксиапатит, остеопороз*

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The problem of reverse shoulder arthroplasty with various deformities of the glenoid is relevant for modern traumatology and orthopedics [1–7]. In addition to various defects, the methods of solving which can be eccentric reaming of the glenoid by milling cutters, bone autoplasty, augmentation, the use of individual implants, orthopedic traumatologists have to deal with a decrease in the mineral density of the bone tissue of the scapula [8–10]. Local osteoporosis of the glenoid surface of the scapula is at least a risk of aseptic instability of the implanted metaglene; also in case of a pronounced decrease in mineral density of the bone tissue of the scapula, stable metaglene placement is not possible at all. In addition to systemic therapy of osteoporosis, the surgical solution of the problem is the use of grafts, special components for more stable fixation (primary-revision metaglene with revision stem, extended screws, etc.), preparations with osteostimulating effect in the form of granules, plates, pastes based on hydroxyapatite [1–7, 11–13].

Synthetic hydroxyapatite with the formula $Ca_{10}(RO_4)_6(OH)_2$ is identical in its chemical properties to the mineral composition of bone tissue – biological hydroxyapatite. Modern science has revealed that blood and intercellular matrix proteins (fibronectin, vitronectin, fibrinogen, osteocalcin, bone sialoproteins, immunoglobulins, albumin, etc.) are absorbed on the surface of bioactive material immediately after its implantation into the tissue environment [14]. In turn, the surface of any implant almost never comes into direct contact with body tissues [14]. The layer of proteins absorbed on the surface of the biomaterial initiates cell adhesion and also provides information transport to cells through cell adhesion receptors – integrins [14]. Fibronectin and vitronectin, which belong to the family of integrins, are involved in the adhesion processes of osteoblasts and their progenitor cells to the surface of calcium-phosphate biomaterials [14]. The morphology, amount and distribution of absorbed substances depend on the physicochemical properties of the biomaterial surface such as electrical charge, chemical composition, roughness, etc. [14]. Hydroxyapatite preparations serve as a gradually resorbable matrix with osteoconductive and osteoinductive properties to which osteoblast precursors attach with subsequent bone growth and formation [14]. Literature data show positive experience of using cement and pastes with hydroxyapatite together with orthopedic implants in order to increase their stability, especially in case of unsatisfactory bone quality [15, 16].

THE AIM OF THE STUDY

To demonstrate the possibility of using hydroxyapatite paste together with bone autoplasty in revision shoulder arthroplasty in conditions of a massive defect and reduced glenoid bone density.

CLINICAL CASE STUDY

Patient K., born in 1949, starting from January 31, 2022 was treated in the adult orthopedics department of the National Medical Research Center for Traumatology and Orthopedics named after N.N. Priorov of the Ministry of Health of Russia with pain and restricted movement of the right shoulder joint. According to the patient and documentation, the injury occurred on December 19, 2017 as a result of a fall on her right shoulder at home. On emergency indications on December 19, 2017 osteosynthesis of a fracture of the neck of the humerus with a plate was performed, in dynamics – lack of fracture union. Anamnestically, pain syndrome was up to 6 points as per the visual analogue scale (VAS); limb function was assessed with a score of 61.7 as per the DASH (Disability of the Arm, Shoulder and Hand) questionnaire. Due to impaired limb function and pain syndrome, the plate was removed 8 months after surgery (Fig. 1).

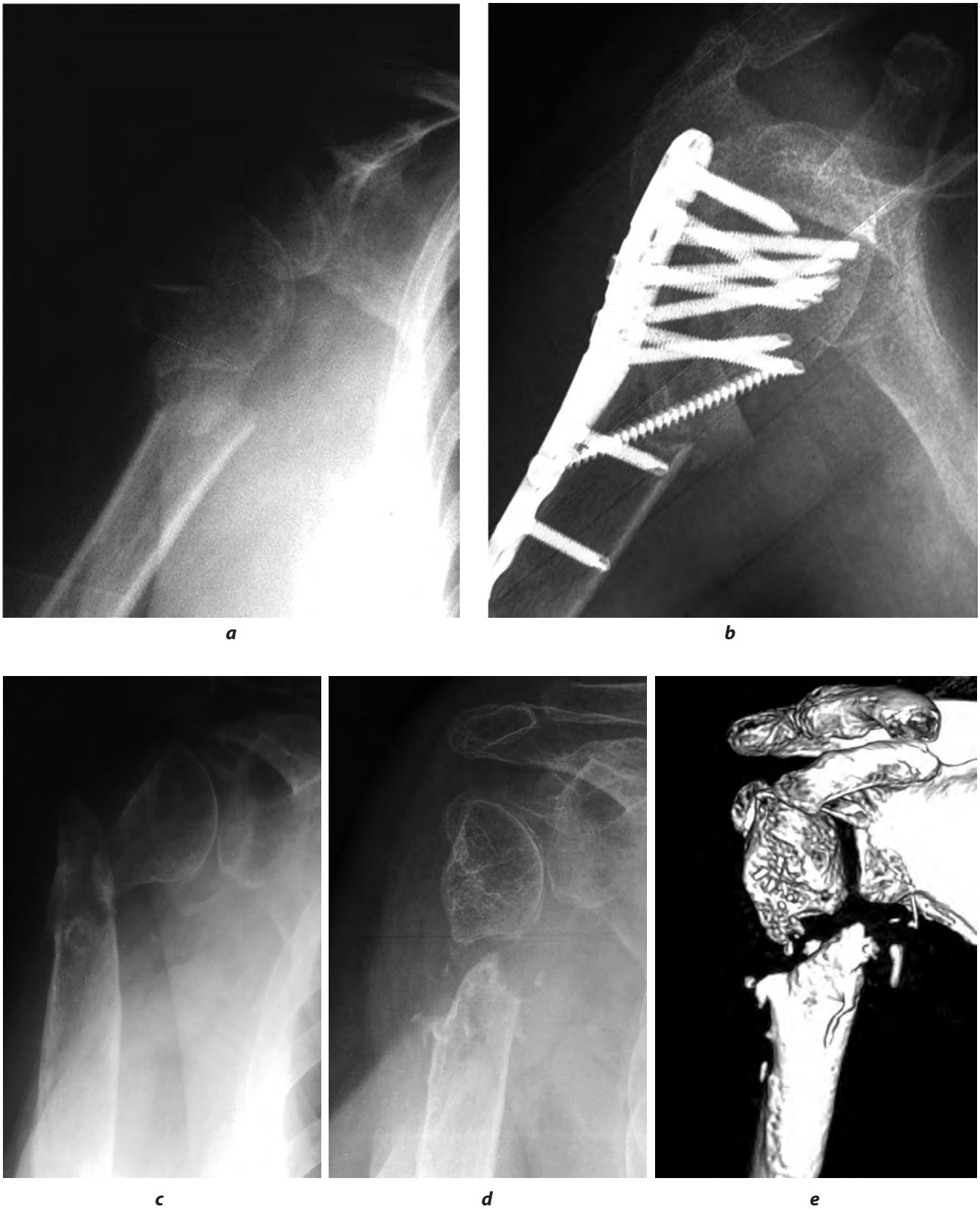
Due to the formation of pseudoarthrosis defect of the proximal humerus and pronounced impairment of the upper limb function, shoulder hemiarthroplasty was performed on September 23, 2019 (Fig. 2).

Taking into account the initial hypotrophy of the deltoid muscle from inactivity, tendon failure of the supraspinous muscle, the joint was not functional after arthroplasty of the humeral head (impaired function of the upper limb – 53.3 DASH points). In this regard, repeated shoulder arthroplasty with a reverse shoulder system was performed on June 10, 2021 (Fig. 3).

The early postoperative period proceeded without complications, the patient underwent rehabilitation courses and noted improvement of joint function. In the dynamics, the patient noted a feeling of instability in the joint, worsening of the limb function (change in the DASH questionnaire scores from 35.8 to 65.8 points), consulted a doctor and on January 31, 2022 was hospitalized in the National Medical Research Center for Traumatology and Orthopedics named after N.N. Priorov of the Ministry of Health of Russia (Fig. 4).

The patient was further examined at the National Medical Research Center for Traumatology and Orthopedics named after N.N. Priorov of the Ministry of Health of Russia: radiological and computed tomography (CT) data revealed aseptic instability, migration of the entire glenoid component of the endoprosthesis (metaglene with glenosphere), glenoid bone resorption with the formation of a pronounced medializing bone defect. Glenoid bone density was also markedly reduced (with areas with a mean value of about 50 HU), despite the fact that the patient had been previously treated for osteoporosis with antiresorptive therapy (Figure 5).

With regard to the migration of the glenoid component, the volume and type of the medializing defect, and unsatisfactory bone quality parameters in the implantation zone, a decision was made to perform revision reverse arthroplasty using revision metaglene, bone autoplasty, and bioactive paste based on hydroxyapatite to improve osseointegration of metaglene and screws. In order to reduce surgical aggression and prevent an increase in the number of surgical interventions, the surgery for removal of the migrated component and revision arthroplasty were performed in a single stage.



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

FIG. 1.

Patient K., X-ray picture of the resulting fracture of the humerus, postoperative radiographs of the patient: ***a*** *– X-ray of the fracture of the humerus;* ***b*** *– X-ray of the patient after osteosynthesis;* ***c***–***e*** *– X-ray picture after removal of the plate: the formation of a defect-pseudoarthrosis of the proximal humerus*

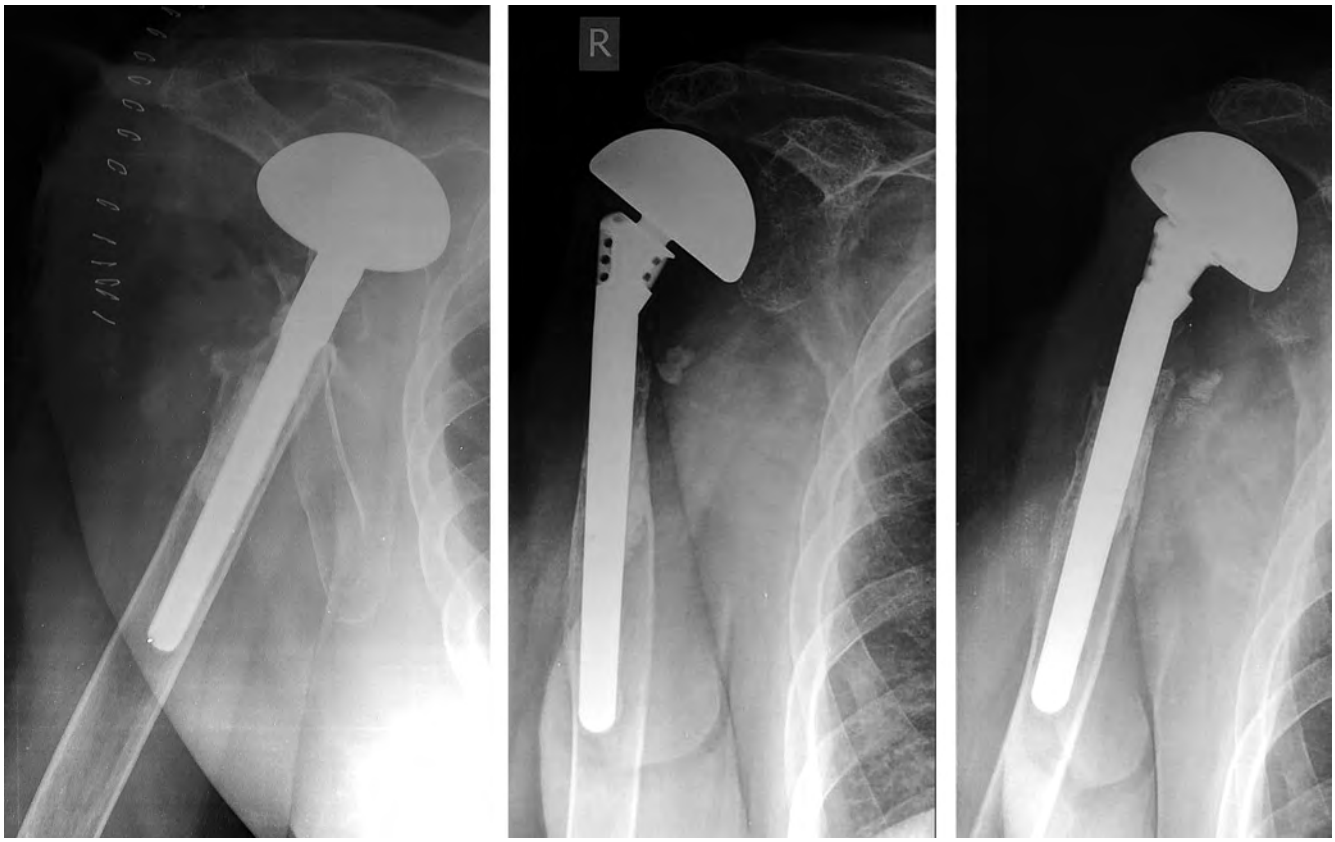
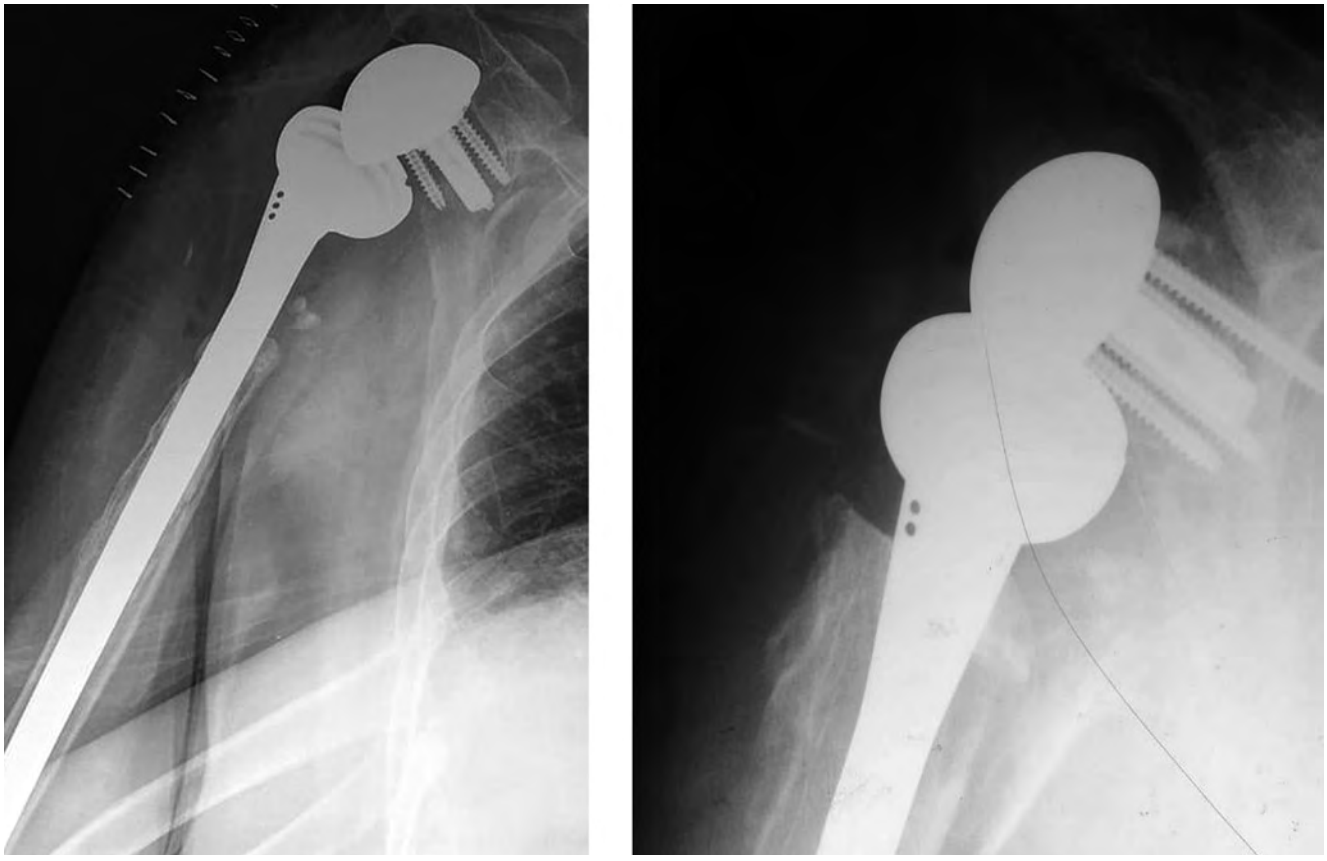


FIG. 2.
Patient K., X-ray after shoulder hemiarthroplasty

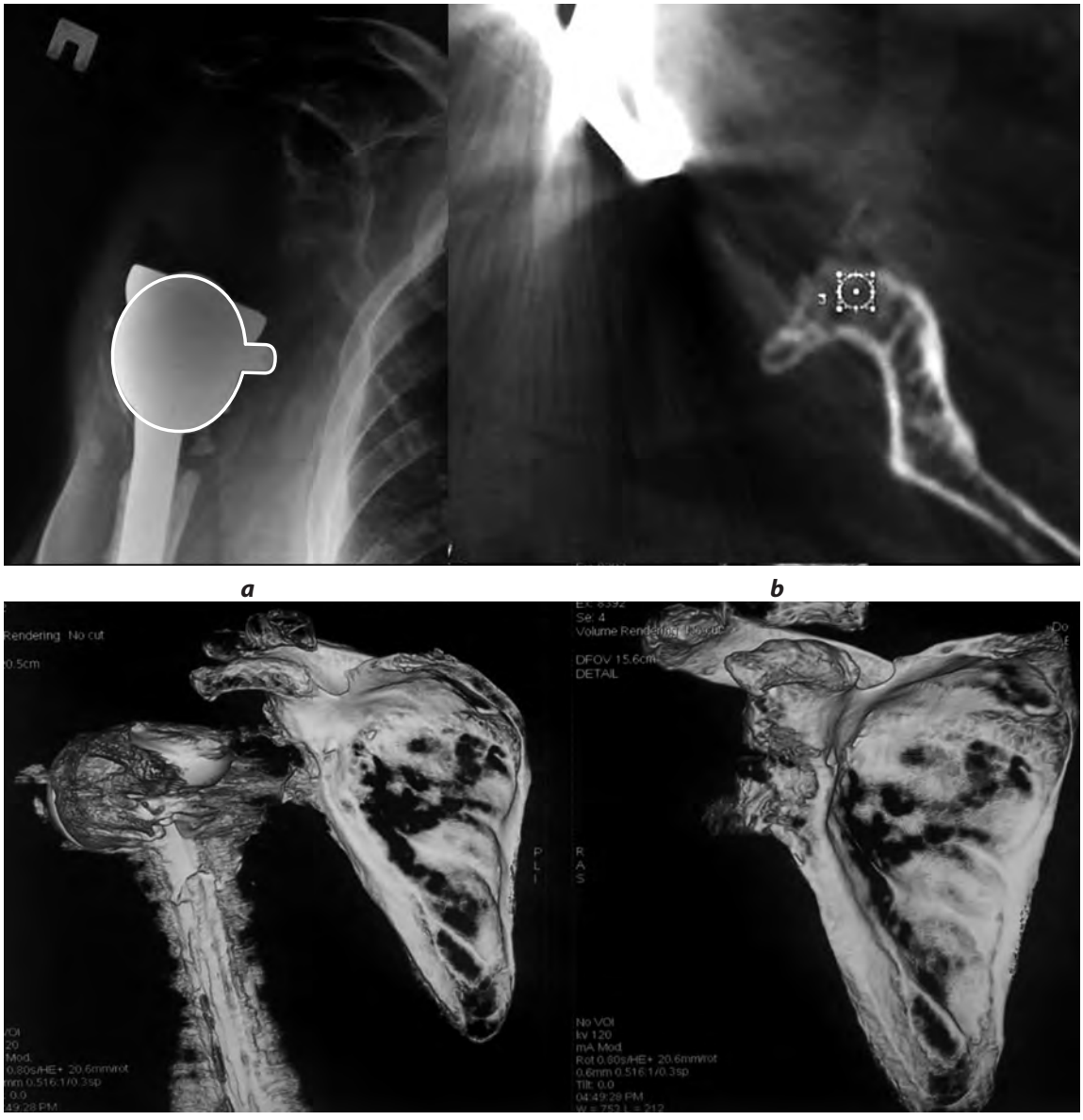


a ***b***

FIG. 3.
*Patient K., X-ray picture straight after (**a**) and 3 months after (**b**) revision reverse shoulder arthroplasty*

FIG. 4.

Patient K., clinical picture at the time of contacting the National Medical Research Center for Traumatology and Orthopedics named after N.N. Priorov



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

FIG. 5.

Patient K., X-ray picture of instability, migration of the scapular component of the endoprosthesis, destruction of the glenoid: ***a*** *– X-ray of the shoulder joint, the outline of the scapular component is circled in white;* ***b*** *– CT is an axial section of the glenoid, a bone defect is visualized;* ***c*** *– three-dimensional modeling of the scapula with visualization of the glenoid according to CT*

Surgery technique

The first stage of the operation after surgical approach was the removal of the glenoid complex of the endoprosthesis components, and the polyethylene insert with signs of wear was removed (Fig. 6a). Intraoperatively, material was collected for microbiologic examination. Removal of scar-altered tissues and glenoid skeletonization was performed. E3 glenoid deformation according to the Gupta, Thussbas, Koch, Seebauer classification was characterized by a significant loss of bone volume and medialization of the glenoid articular pad (Fig. 6b, Fig. 7).

a ***b***

FIG. 6.

*Patient K., intraoperative photographs: **a** – wear mainly on the upper edge of the endoprosthesis liner; **b** – type E3 predominantly on the medial surface of glenoid according to the classification of Gupta, Thussbas, Koch, Seebauer*

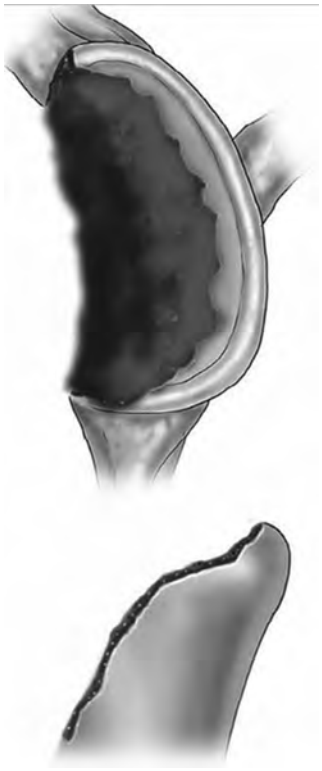


FIG. 7.

Scheme showing E3 glenoid deformity (30–60 % of the articular surface of the scapula) [5]

The next step, taking into account the unsatisfactory bone quality, was the eccentric processing of the glenosphere with a milling cutter until the site for graft and metaglene placement was formed. A surgical approach to the iliac crest was performed, an osteotome was used to take an autograft of the necessary shape and size for adequate lateralization of the glenosphere. The lateralizing graft was placed on the prepared glenoid site and fixed with a wire (Fig. 8).

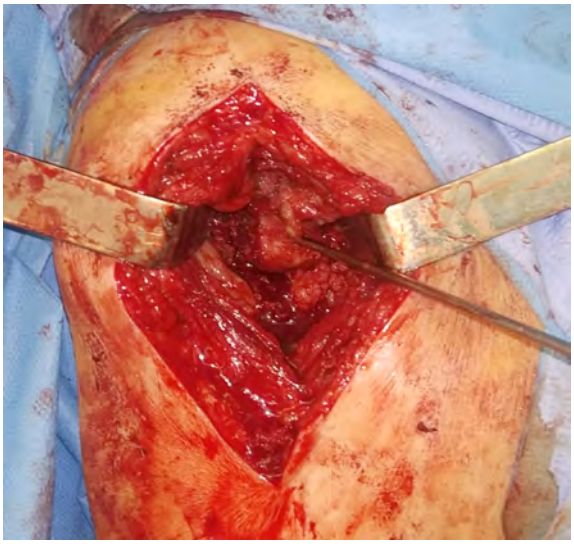


FIG. 8.

Patient K., bone autoplasty of the glenoid: the bone graft was placed along the guide wire

The canal of the extended metaglene stem was reamed through the graft. To improve osseointegration of metaglenes and osteoreparation, syringe injection of hydroxyapatite paste into the formed canal was performed; metaglenes with an extended stem were implanted through the graft.

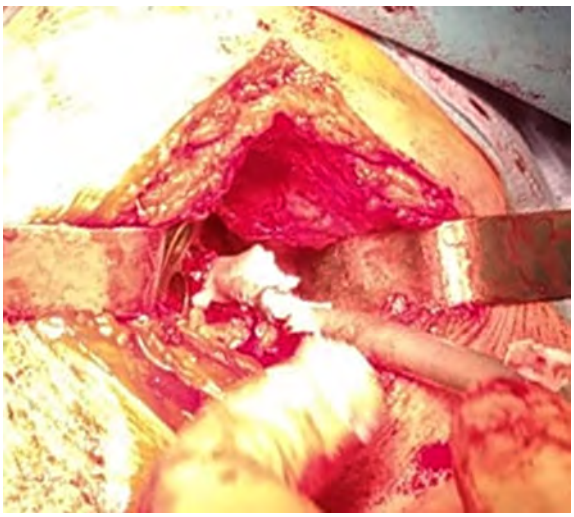


FIG. 9.

Patient K., application of osteoplastic material to the screws to be installed

Based on the preoperative planning, the screw channels were reamed through the areas with the maximum compact content of dense bone tissue, hydroxyapatite paste was injected into the channels with a syringe according to the method described above, and the preparation was additionally applied to the screws themselves (Fig. 9). The screws, glenosphere and liner were inserted, the endoprosthesis was repositioned, and the wounds were sutured.

Postoperative period

Postoperative X-ray control was performed. X-rays performed in two projections visualize a correctly, stably placed reverse shoulder arthroplasty with the offset enlarged at the expense of the bone graft (Fig. 10).

The postoperative period proceeded without complications. The patient received antibacterial prophylaxis for purulent inflammatory complications, anticoagulant prophylaxis, symptomatic and gastroprotective therapy. According to the microbiological study of intraoperative material, no microflora growth was detected. Electrical stimulation of the deltoid muscle and mechanotherapy was started in the early postoperative period in the hospital. After discharge, the patient continued rehabilitation actions, physiotherapy treatment under the supervision of an orthopedist and rehabilitologist. The patient was consulted by osteoporosis treatment specialists at the National Medical Research Center for Traumatology and Orthopedics named after N.N. Priorov of the Ministry of Health of Russia, received osteotropic drug therapy. There were no purulent inflammatory complications.

Computed tomography was performed 3 months after the intervention to evaluate metaglene osseointegration and graft remodeling. Tomographic sections showed rearrangement and autograft union, stable placement of the prosthesis components without signs of peri-implant bone tissue reaction (Fig. 11, 12, 13).

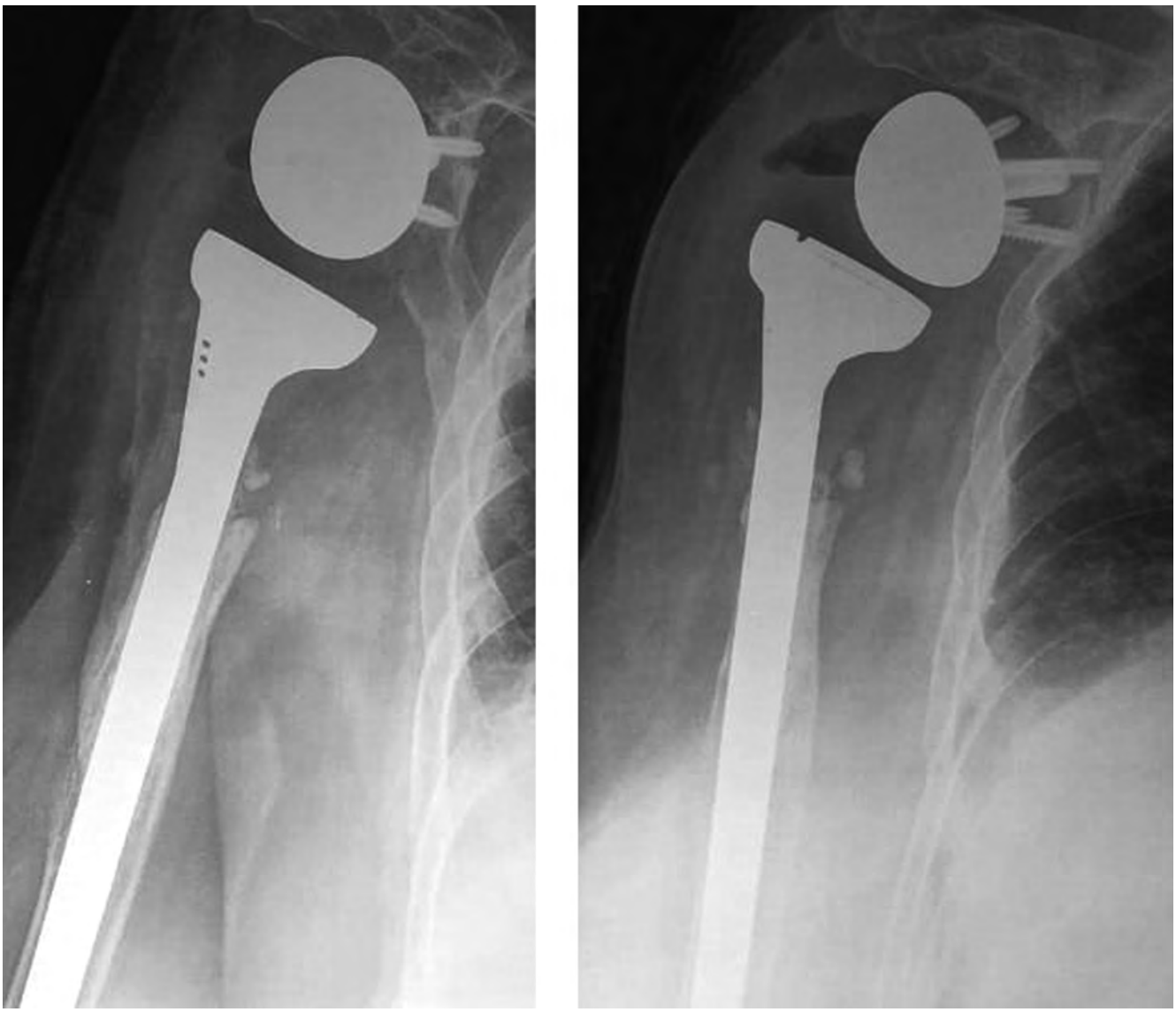


FIG. 10.
Patient K., postoperative X-ray

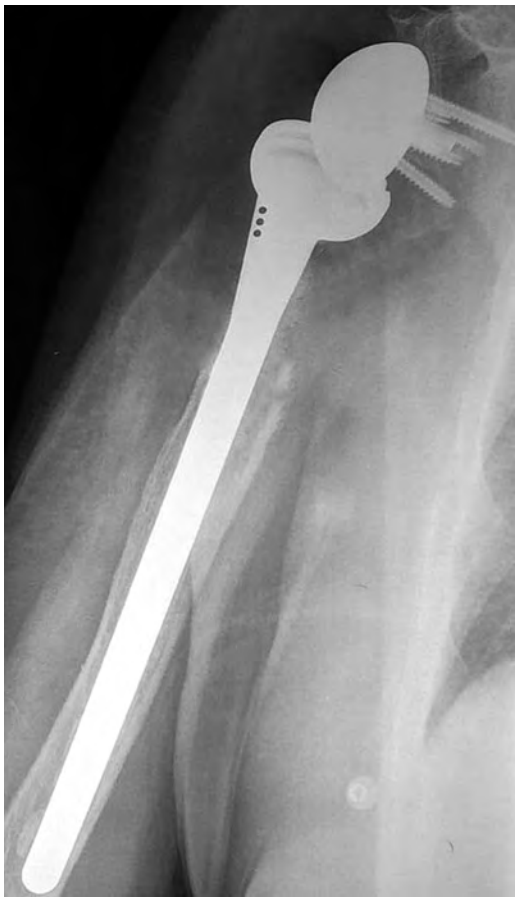


FIG. 11.
Patient K., X-ray control in the long term after surgery

The medium-term clinical results were evaluated after 5 months; considering the complexity of the case, improvement in movement amplitude was achieved, the patient was free of pain, and limb function was evaluated with a DASH score of 25.8 (Fig. 14).

DISCUSSION

National Medical Research Center for Traumatology and Orthopedics named after N.N. Priorov of the Ministry of Health of Russia has accumulated extensive experience in the use of bioactive materials based on hydroxyapatite and collagen for the replacement of bone defects, plasty of osteochondral injuries, stimulation of reparative osteo- and chondrogenesis, treatment of osteomyelitis, and use in onco-orthopedics. Positive osteoinductive and osteoconductive properties of these materials were revealed. The paste used clinically is a non-hardening hydroxyapatite mass that completely fills the bone defect. The material attracts biomolecules necessary for the regenerative process and, along with a localized increase in ion levels, contributes to its osteostimulatory effect. Osteoblast colonization and vascularization occur throughout the paste implant. Cell-mediated resorption of the material occurs over several months concurrently with the formation of mature bone.

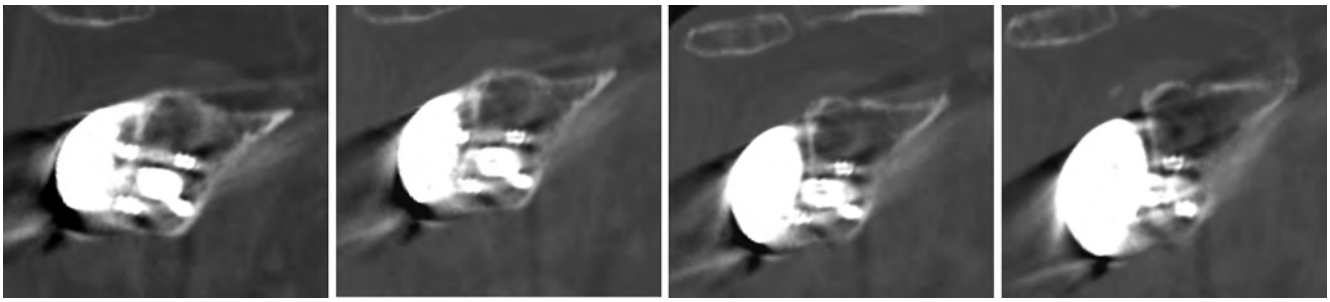


FIG. 12.
Patient K., CT control in the long term after surgery

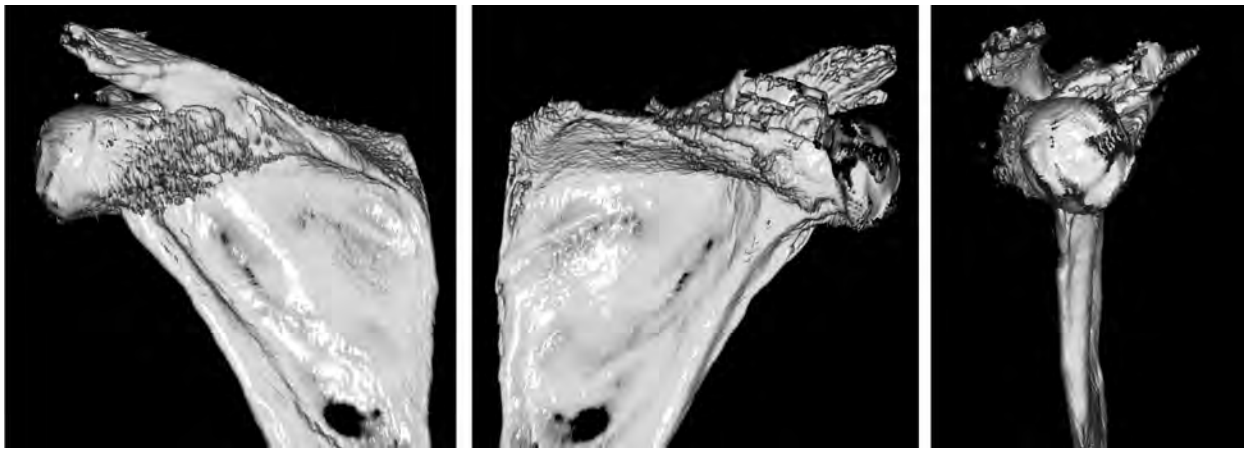


FIG. 13.
Patient K., CT control in the long term after surgery: 3D model of the scapula with the installed glenosphere

FIG. 14.
Patient K., medium-term functional outcome

CONCLUSION

This clinical case illustrates the possibilities of effective application of bioengineering achievements in the form of hydroxyapatite osteoactive material in the form of a paste in complex revision shoulder arthroplasty. This technique makes it possible to level out the undesirable consequences of reduced glenoid bone mineral density, which is aseptic instability of the glenoid component of the endoprosthesis. The use of cement with hydroxyapatite and the combined use of hydroxyapatite in the form of gels, granules, pastes to improve screw fixation has already proven effective in the practice of vertebroplasty and neurosurgery. The positive experience of using this assistive technique in spinal surgery and the present clinical case may justify the need for further development of this problem and introduction of the technology into the practice of shoulder arthroplasty.

Conflict of interest

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

Standards of ethics

Compliance with the bioethical norms of the study was confirmed by the results of the meeting No. 3 of March 17, 2022 of the local ethics committee of the National Medical Research Center for Traumatology and Orthopedics named after N.N. Priorov of the Ministry of Health of Russia; the study was approved for publication.

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SURGERY

DIAGNOSIS AND COMPARATIVE ANALYSIS OF SURGICAL TREATMENT OF PATIENTS WITH LIVER ALVEOCOCCOSIS

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ABSTRACT

Rationale. *Alveococcosis is a rare disease, its diagnosis and treatment depend on surgical techniques, equipment and clinical experience.*
The aim. *To develop a diagnostic algorithm and compare the results of surgical treatment of patients with liver alveococcosis in different periods of time.*
Materials and methods. *At the first stage, we carried out a retrospective analysis (1995–2007) of 33 patients with alveococcosis (a comparison group). At the second stage, a prospective clinical study (2008–2021) was performed on 39 patients (the main group). The number of patients was determined in accordance with the inclusion and exclusion criteria, and the study groups were comparable in age, sex, parasite localization ($p > 0.05$). For the names of operations, the WHO classification of alveococcosis was used.*
Results. *In the main group, there is an increase in the applicability of: enzyme immunoassay; ultrasound and computed tomography; biopsy. Complications decreased by 2.7 times from 54.6 % in the comparison group to 20.6 % in the main group ($\chi^2 = 8.97$; $df = 1$; $p = 0.003$). The average duration of operations, as well as the average volume of blood loss in the comparison group and the main group were, respectively: with atypical resection – 220.4 and 180.2 min ($p = 0.003$), 640.1 and 480.0 ml ($p = 0.005$); with anatomical resection – 296.2 and 247.2 min ($p = 0.002$), 1450.2 and 1150.3 ml ($p = 0.018$); with cytoreductive resection – 230.2 and 200.1 min ($p = 0.004$), 860.3 and 670.4 ml ($p = 0.001$). There were 13 (39 %) cytoreductive resections in the comparison group, and 3 (8 %) in the main group ($\chi^2 = 4.74$; $df = 1$; $p = 0.029$).*
Conclusion. *Timely diagnosis of alveococcosis leads to an increase in the number of radical resections, and modern surgical technologies and equipment can reduce the time of surgery, blood loss and the number of complications.*

Key words: *liver alveococcosis, algorithm for diagnosing liver alveococcosis, minimally invasive surgical interventions, radical liver resections, multi-stage approach to surgical treatment*

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ДИАГНОСТИКА И СРАВНИТЕЛЬНЫЙ АНАЛИЗ ХИРУРГИЧЕСКОГО ЛЕЧЕНИЯ БОЛЬНЫХ АЛЬВЕОКОККОЗОМ ПЕЧЕНИ

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РЕЗЮМЕ

Обоснование. *Альвеококкоз является редким заболеванием, диагностика и лечение которого зависят от хирургических технологий, оборудования и клинического опыта.*

Цель исследования. *Разработать алгоритм диагностики и сравнить результаты хирургического лечения пациентов альвеококкозом в различные периоды времени.*

Материалы и методы. *На первом этапе проведён ретроспективный анализ (1995–2007 гг.) 33 пациентов с альвеококкозом – группа сравнения. На втором этапе выполнено проспективное клиническое исследование (2008–2021 гг.) 39 пациентов – основная группа. Количество пациентов определялось в соответствии с критериями включения и исключения, а исследуемые группы были сопоставимы по возрасту, полу, локализации паразита ($p > 0,05$). Для названий операций использовалась классификация альвеококкоза Всемирной организации здравоохранения.*

Результаты исследования. *В основной группе отмечается увеличение применяемости иммуноферментного анализа, ультразвукового исследования и компьютерной томографии, биопсии. В 2,7 раза снизилось количество осложнений – с 54,6 % в группе сравнения до 20,6 % в основной группе ($\chi^2 = 8,97$; $df = 1$; $p = 0,003$). Средняя длительность операций, а также средний объём кровопотери в группе сравнения и основной группе составили соответственно: при атипичной резекции – 220,4 и 180,2 мин ($p = 0,003$), 640,1 и 480,0 мл ($p = 0,005$); при анатомической резекции – 296,2 и 247,2 мин ($p = 0,002$), 1450,2 и 1150,3 мл ($p = 0,018$); при циторедуктивной резекции – 230,2 и 200,1 мин ($p = 0,004$), 860,3 и 670,4 мл ($p = 0,001$). В группе сравнения было проведено 13 (39 %) циторедуктивных резекций, а в основной группе – 3 (8 %) ($\chi^2 = 4,74$; $df = 1$; $p = 0,029$).*

Заключение. *Своевременная диагностика альвеококкоза ведёт к увеличению количества радикальных резекций, а современные хирургические технологии и оборудование позволяют сократить время операции, кровопотерю и количество осложнений.*

Ключевые слова: *альвеококкоз печени, диагностика альвеококкоза печени и его осложнений, малоинвазивные хирургические вмешательства, радикальные резекции печени, многоэтапный подход к оперативному лечению*

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OBJECTIVES

Alveococcosis is characterized by a parasitic lesion of the liver with slow growth, possible spreading and metastasis to other organs and tissues, making it similar to malignant tumor [1–9]. Late detection of the disease reduces the chances of performing radical or conditionally radical liver resections, as well as organ transplantation and patient recovery [10–13]. Moreover, long-term ongoing disease leads to various complications that significantly worsen the patient's condition and reduce the percentage of positive outcome of surgical intervention [14, 15]. The development of both laboratory and imaging diagnostics, as well as surgical technologies, including minimally invasive ones, makes it possible to detect the disease at early stages and increase the possibility of performing radical surgical interventions [16–18]. Minimally invasive surgeries in such cases become very significant, as they allow to manage various complications of parasitic lesions at the early stage(s) with subsequent safe radical surgical interventions, and when this is not possible, they remain the final surgical aids, allowing to alleviate the patient's condition and improve the quality of life. Modern equipment and various hemostatic agents (e. g. coverings) used for liver resection allow to perform surgical intervention faster, with less blood loss, reliable bile stasis and hemostasis, which leads to a decrease in postoperative complications [19–21].

MATERIALS AND METHODS

An open-label prospective retrospective non-randomized controlled single-center study was performed in two stages. At the first stage, a retrospective analysis of 33 medical records of patients with liver alveococcosis for 1995–2007 was performed, who further made up the comparison group. Methods of operative treatment of alveococcosis were developed and improved based on the obtained data. At the second stage of the study, a prospective clinical study involving 39 patients for 2008–2021 included in the main group was performed to evaluate the effectiveness of the proposed methods. The number of patients was determined in accordance with the inclusion and exclusion criteria, and the study groups were comparable in age, sex, parasite localization ($p > 0.05$). The data on patients in both groups are presented in Table 1. The diagnostic methods used in both groups are summarised in Table 2. When performing resection operations in the main group, carbon dioxide laser and spray coagulator were used, as well as hemostatic agents such as wound resorbable coverings. In case of complications and large spread of alveococcosis in the main group a multi-stage approach of surgical treatment was used, aimed at: biliary drainage using X-ray in case of obstructive jaundice; balloon dilatation and bile duct stenting; puncture and necrotic cavity

TABLE 1

DISTRIBUTION OF PATIENTS BY GROUPS

Parameters	Main group	Comparison group	*p* value	Total
Observation period (years)	2008–2021	1995–2007	–	1995–2021
Number of patients, abs. (%)	39 (54.2 %)	33 (45.8 %)	–	72 (100.0 %)
Average age (years), Me [25th; 75th percentiles]	46.1 [38.8; 53.6]	44.3 [37; 51.1]	$p = 0.692$	45.5 [37.8; 53.2]
Sex, abs. (%)				
male	21 (53.8 %)	19 (57.6 %)	$\chi^2 = 0.10$; df = 1; $p = 0.751$	40 (55.6 %)
female	18 (46.3 %)	14 (42.4 %)		32 (44.4 %)
Parasite localization in the liver, abs. (%)				
right lobe	22 (56.4 %)	20 (60.6 %)		42 (58.3 %)
left lobe	10 (25.6 %)	8 (24.2 %)	$\chi^2 = 0.15$; df = 2; $p = 0.927$	18 (25 %)
bilobed	7 (18.0 %)	5 (15.2 %)		12 (16.7 %)

Note. *p* – level of statistical significance.

TABLE 2
DIAGNOSTIC METHODS IN THE COMPARED GROUPS

Diagnostic method	Main group (n = 39)		Comparison group (n = 33)	
	abs.	%	abs.	%
Enzyme immunoassay	31	79.5	10	30.3
Ultrasound investigation	31	79.5	27	81.8
Native computer tomography	29	74.4	8	24.2
Contrast-enhanced computer tomography	25	64.1	4	12.1
Positron-emission tomography	4	10.3	0	0.0
Duplex ultrasound scanning of liver vessels	9	23.1	2	6.1
Needle biopsy	12	30.8	3	9.1
Diagnostic laparoscopy	1	2.6	3	9.1

drainage with ultrasound guidance; portal vein embolization in order to increase the volume of liver parenchyma. We used the 1996 WHO classification of alveococcosis ($P_{x-4}N_{x-1}M_{x-1}$) to name the surgical options, where: P – primary lesion; N – extrahepatic involvement of adjacent organs or tissues; M – distant metastases, as well as the resectability criterion R_{0-2} ($_0$ – radical; $_1$ – conditionally radical; $_2$ – cytoreductive) [22].

The obtained data was processed using statistical software packages Microsoft Excel (Microsoft Corp., USA) and Statistica 12 (StatSoft Inc., USA). Qualitative variables were described by absolute numbers and relative frequencies (%). Conformity to normal distribution of quantitative data was assessed using the Shapiro – Wilk test. Group variables were presented as median and interquartile range, Me [25%; 75%]. The Mann – Whitney U test was used for intergroup comparison. The χ^2 test was determined to compare categorical variables; the Yates correction was used in cases where tables contained small frequencies ($n < 5$). The Fisher angular transformation (φ-transformation) was used to compare the percentages. Differences were considered statistically significant at $p < 0.05$. The conjugate table method was used to express the results and efficacy of the intervention.

The study was based on the principles established by the International Committee of Medical Journal Editors (ICMJE) and the Universal Declaration on Bioethics and Human Rights.

RESULTS AND DISCUSSION

Table 2 shows that there is a higher number of procedures performed in the main group, except for laparoscopy, which is due to the progressive development and introduction of diagnostic equipment in different periods of time. In addition to quantitative differences, we also noted a qualitative difference associated with more sensitive expert-class diagnostic equipment. Based on all studies, we developed and implemented an algorithm for differential diagnosis of liver alveococcosis, presented in Figure 1.

As a result of analyzing the quantitative ratio of the liver surgeries performed, we did not obtain a statistically significant difference in the compared groups when performing atypical and anatomical resections, although significant differences in absolute numbers are visible: 9 (main group) versus 4 (comparison group) – right lobe, 4 versus 1 respectively – left lobe. Examples of atypical and anatomical resections are shown in Figures 2–5. Extended resections, as shown in Figures 6–8, were performed only in the main group, and therefore the comparison was not possible. However, we obtained a statistically significant difference by evaluating the number of cytoreductive resections performed, of which there were significantly more in the comparison group: 3 (main group) versus 10 (comparison group) – right lobe, 0 versus 3 respectively – left lobe ($\chi^2 = 4.74$; df = 1; $p = 0.029$*). The surgical interventions are summarized in Table 3.

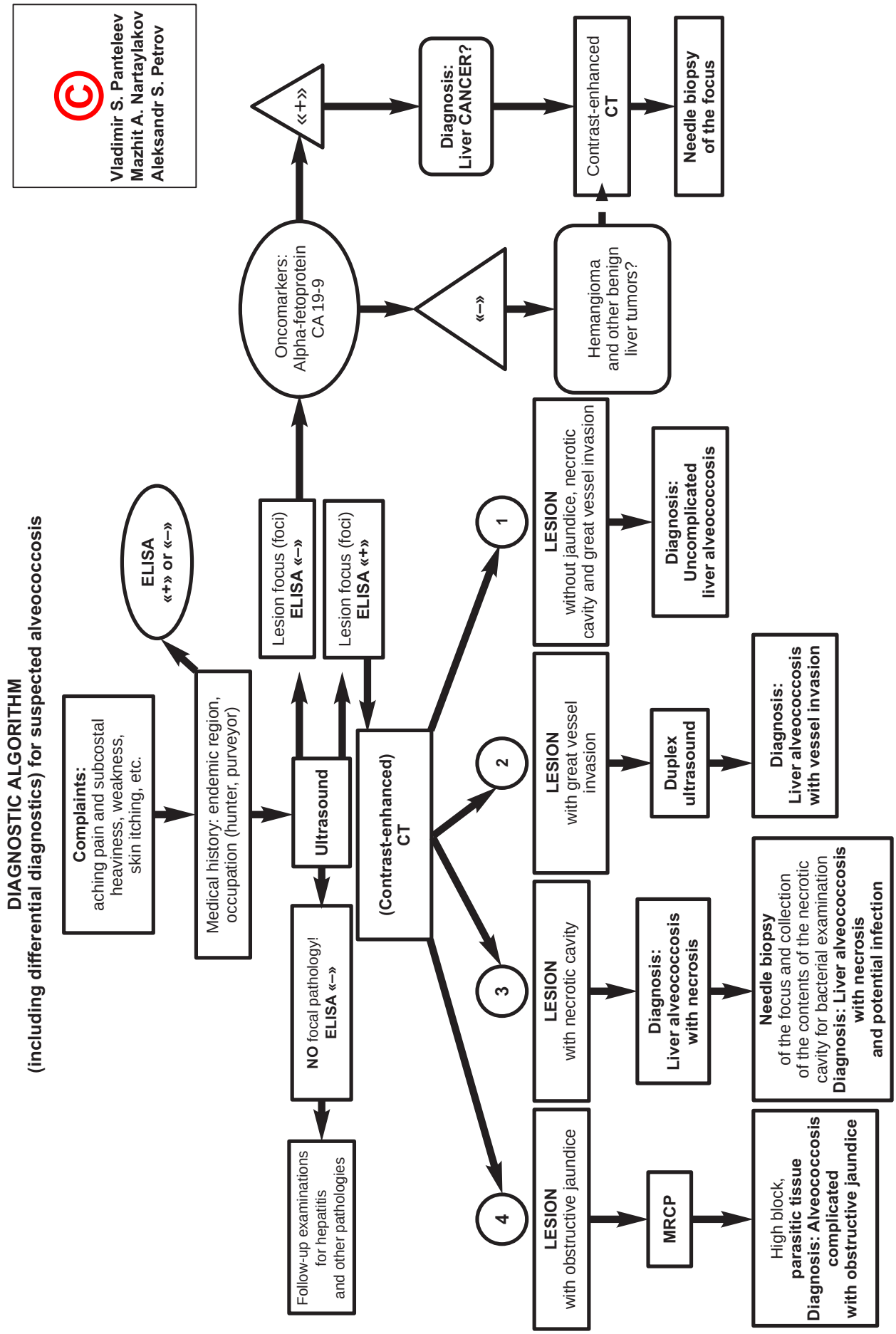

FIG. 1.
Diagnostic algorithm (including differential diagnostics) for suspected alveococcosis

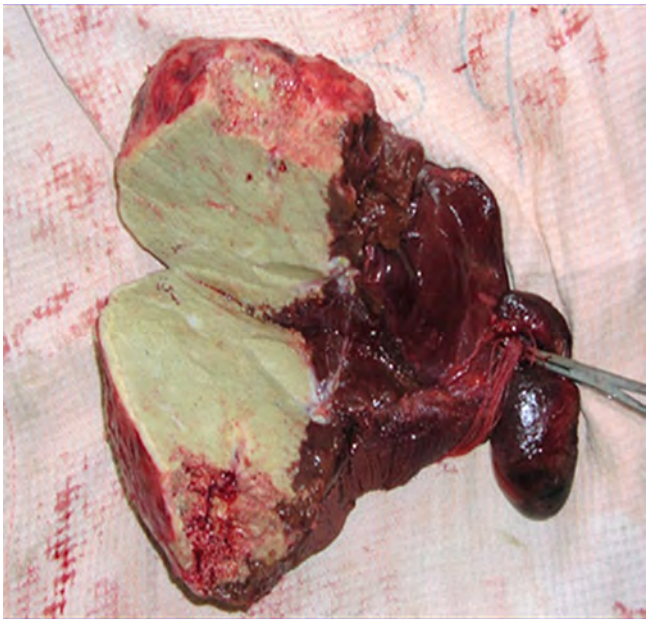

FIG. 2.
Atypical liver resection: gross specimen of "alveolar echinococcus tumor"

FIG. 3.
Atypical liver resection: alveococcus gross specimen with necrotic cavity and suppuration

TABLE 3
VARIANTS OF SURGICAL INTERVENTIONS IN GROUPS

Surgical intervention	Main group (n = 39) 100%		Comparison group (n = 33) 100%		p value	
	right lobe	left lobe	right lobe	left lobe	right lobe	left lobe
$P_1N_0M_0(R_{0-1})$ – atypical resection	6 (15 %)	5 (13 %)	6 (18 %)	4 (13 %)	$\chi^2 = 0.1$; df = 1; $p = 0.751$	$\chi^2 = 0.07$; df = 1; $p = 0.789$*
$P_{1-2}N_{0-1}M_0(R_{0-1})$ – anatomic resection	9 (23 %)	4 (10 %)	4 (12 %)	1 (3 %)	$\chi^2 = 0.8$; df = 1; $p = 0.370$*	$\chi^2 = 0.54$; df = 1; $p = 0.461$*
$P_{1-3}N_{0-1}M_0(R_{0-1})$ – extended resection	4 (10 %)	1 (3 %)	0 (0 %)	0 (0 %)	–	–
$P_{3-4}N_{0-1}M_{0-1}(R_{1-2})$ – cytoreductive resection	3 (8 %)	0 (0 %)	10 (30 %)	3 (9 %)	$\chi^2 = 4.74$; df = 1; $p = 0.029$*	–
Total	22 (56 %)	10 (26 %)	20 (60 %)	8 (25 %)	$\chi^2 = 0.13$; df = 1; $p = 0.719$	$\chi^2 = 0.02$; df = 1; $p = 0.891$
$P_{3-4}N_{0-1}M_0$ – liver transplant	3 (8 %)		0 (0 %)		–	
$P_{3-4}N_{0-1}M_{0-1}$ – palliative	4 (10 %)		5 (15 %)		$\chi^2 = 0.39$; df = 1; $p = 0.532$*	

Note. * – the Yates' correction was used in the comparison between groups; p values corresponding to statistically significant differences between groups are marked in bold.

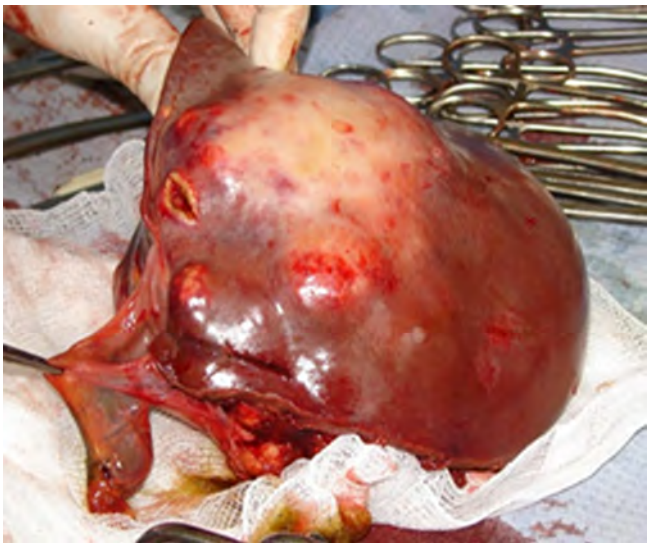

FIG. 4.
Right anatomic liver resection: gross specimen of resected organ with gallbladder and alveococcus

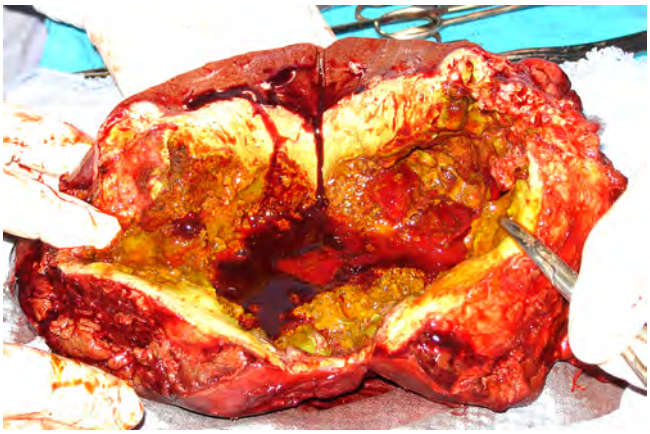

FIG. 5.
Right anatomic liver resection: gross specimen of "alveolar echinococcus tumor" with necrotic cavity

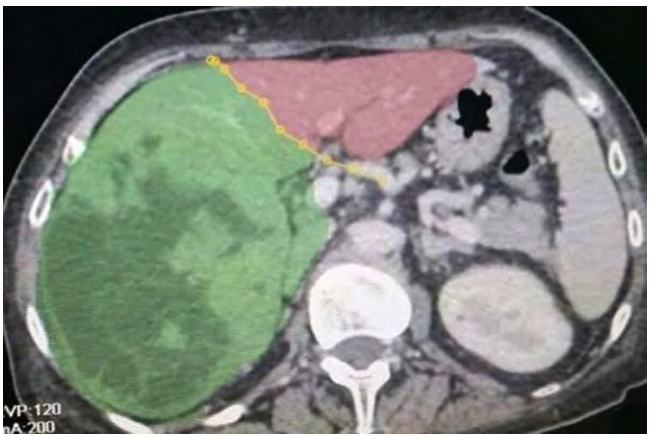

FIG. 6.
Computed tomography of the liver: the area of alveococcus lesion is marked in green

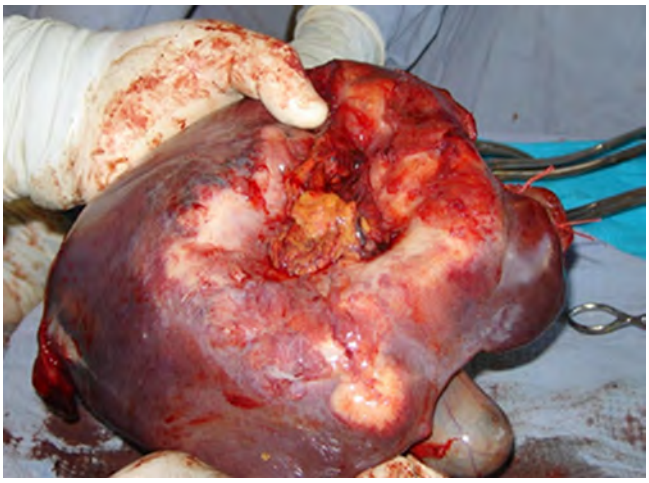

FIG. 7.
Right extended hemihepatectomy: gross specimen of a resected organ with gallbladder and alveococcus

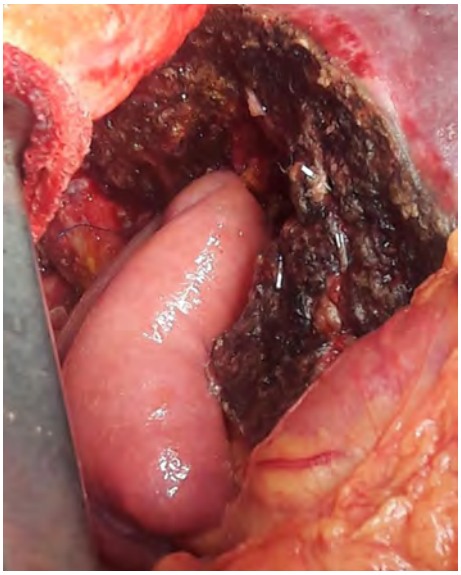

FIG. 8.
Formed hepaticojejunal anastomosis after extended hemihepatectomy (fragment of the surgery)

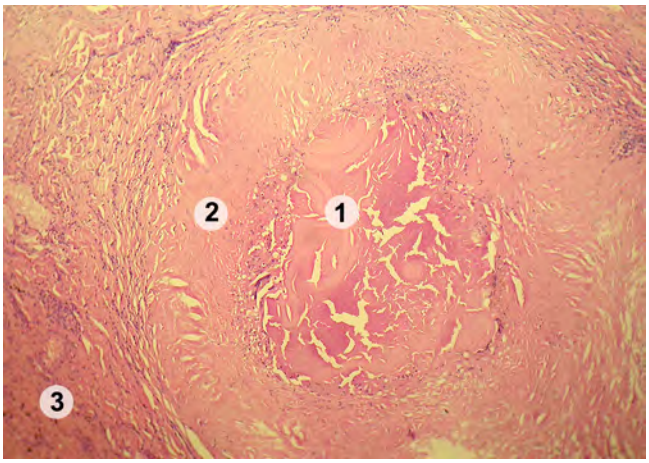

FIG. 9.
Alveococcosis of the liver: **1** *– daughter vesicles in the center of the parent bladder;* **2** *– parent cuticular membrane;* **3** *– fibrous membrane (productive inflammation)*

TABLE 4

AVERAGE DURATION AND AVERAGE VOLUME BLOOD LOSS IN RESECTABLE SURGERIES, ME [25 %; 75 %]

Parameters	Main group (n = 39)	Comparison group (n = 33)	p
Average surgery duration (min)			
Atypical resection	180.2 (132.5; 227.7)	220.4 (156.8; 284.0)	$p = 0.003$
Anatomic resection	247.2 (183.9; 385.8)	296.2 (233.9; 359.3)	$p = 0.002$
Extended resection	310.3 (273.7; 346.9)	0	–
Cytoreductive resection	200.1 (158.0; 242.3)	230.2 (187.8; 274.1)	$p = 0.004$
Average blood loss volume (ml)			
Atypical resection	480.0 (240.2; 720.5)	640.1 (409.8; 870.4)	$p = 0.005$
Anatomic resection	1150.3 (640.1; 1660.2)	1450.2 (909.8; 1990.5)	$p = 0.018$
Extended resection	1930.3 (1109.8; 2750.5)	0	–
Cytoreductive resection	670.4 (480.0; 860.8)	860.3 (590.1; 1130.6)	$p < 0.001$

TABLE 5

COMPLICATIONS, RECURRENT SURGERIES, MORTALITY IN IMMEDIATE POSTOPERATIVE PERIOD

Complications	Main group (n = 39) 100 %	Comparison group (n = 33) 100 %	Total (n = 72) 100 %
Liver stump bleeding / *relaparotomy*	2 (5.1 %) / 1 (2.6 %)	4(12.1 %) / 2(6 %)	6 (8.3 %) / 3 (4.2 %)
	$\chi^2 = 0.41$; df = 1; $p_1 = 0.521$*		
	$\chi^2 = 0.02$; df = 1; $p_2 = 0.882$*		
Liver stump bile leakage / *relaparotomy / ultrasound-guided puncture*	2 (5.1 %) / 0 / 1 (2.6 %)	5 (15.2 %) / 2 (6 %) / 0	7 (9.7 %) / 2 (2.8 %) / 1 (1.4 %)
	$\chi^2 = 1.10$; df = 1; $p_1 = 0.302$*		
Intra-abdominal abscess(s) / *relaparotomy / US-guided puncture*	1 (2.6 %) / 0 / 1 (2.6 %)	2 (6 %) / 1 (3 %) / 1 (3 %)	3 (4.2 %) / 1 (1.4 %) / 1 (1.4 %)
	$\chi^2 = 0.02$; df = 1; $p_1 = 0.882$*		
	$\chi^2 = 0.36$; df = 1; $p_3 = 0.549$*		
Portal vein thrombosis	1 (2.6 %)	0	1 (1.4 %)
	–		
Pneumothorax / *puncture* / *puncture* + *pleural drainage*	1 (2.6 %) / 1 (2.6 %) / 0	1 (3 %) / 1 (3 %) / 1 (3 %)	2 (2.8 %) / 2 (2.8 %) / 1 (1.4 %)
	$\chi^2 = 0.36$; df = 1; $p_1 = 0.549$*		
	$\chi^2 = 0.36$; df = 1; $p_3 = 0.549$*		
Liver failure	1 (2.6 %)	5 (15.2 %)	6 (8.3 %)
	$\chi^2 = 2.24$; df = 1; $p = 0.134$*		
Mortality	0	1 (3 %)	1 (1.4 %)
	–		
Total	8(20.6 %)	18 (546 %)	26 (36.1 %)
	$\chi^2 = 8.97$; df = 1; $p = 0.003$		

Note. US – ultrasound; * – the Yates' correction was used in the comparison between groups; p values corresponding to statistically significant differences between groups are marked in bold.

During histological examination of the material, there were occasional difficulties in differential diagnosis with malignant tumor of the liver. A typical picture of liver alveococcus and its growth are shown in Figure 9.

When comparing the surgery duration and blood loss during resectable surgical interventions, we obtained a statistically significant difference in all variants of liver resections, which is presented in Table 4.

We analyzed and compared all complications encountered in the immediate postoperative period and the surgical interventions performed to manage them. The complications identified and presented in terms of their characteristics when comparing the study groups had no statistically significant difference when compared individually. However, when combined, we obtained a significant difference between the groups in terms of the number of all complications and surgical interventions performed for their management: 8 (20.6 %) in the main group versus 18 (54.6 %) in the comparison group ($\chi^2 = 8.97$; df = 1; $p = 0.003$), as presented in Table 5.

CONCLUSION

Our long-term clinical experience of surgical treatment allowed us to develop and introduce into practice an algorithm of differential diagnostics in case of suspected liver alveococcosis, the application of which allows early detection of the disease, as well as its various complications. Comparative analysis showed differences in comparable groups, which in some indicators have a significant difference, which is primarily due to modern diagnostic capabilities and technical surgery support. Early detection of the disease allows to perform radical or conditionally radical surgery aimed at removing the «parasitic tumor», which leads to recovery or to a significant reduction in the subsequent possible manifestations of liver alveococcosis. Minimally invasive surgical interventions have a twofold significance: firstly, as a preparatory stage for radical surgery; secondly, as a definitive surgical intervention when resection surgery is not possible, which significantly improves the quality of life.

Conflict of interest

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

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EXPERIENCE IN SURGICAL TREATMENT OF ENTEROATMOSPHERIC FISTULAS IN THE LATE PERIOD OF POSTOPERATIVE PERITONITIS

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ABSTRACT

Background. *Enteroatmospheric fistulas (EAF) that occur during the use of the "open abdomen" surgical tactics are a complex surgical pathology with a high mortality rate.*

The aim. *To assess the effectiveness of treatment of various forms of enteroatmospheric fistulas in patients with postoperative peritonitis using vacuum aspiration technology.*

Methods. *We assessed the results of the surgical treatment of 46 patients with EAF in the late course of postoperative peritonitis (PP). Three clinical and morphological groups were distinguished: group 1 (n = 24) – EAF in small wounds of the anterior abdominal wall; group 2 (n = 15) – EAF opening into limited cavities; group 3 (n = 7) – EAF opening into laparostoma wounds. In group 1, a fistula was formed using continuous aspiration devices or VAC systems. In group 2, we used continuous aspiration of intestinal contents from the cavity. In group 3, laparostoma was treated using vacuum devices with isolation of the intestinal fistula and simulation of a floating enterostoma.*

Results. *Group 3 of patients with EAF was characterized by a high flow rate (1224.2 ± 210.3 ml), duration of treatment (87.3 ± 12.5 day), extensive laparostoma (335.4 ± 14.3 cm^2), high mortality rate (57.1 %). The best results of treatment were obtained in groups 1 and 2. The flow rate was 675.8 ± 154.3 and 541.3 ± 114.1 ml, the duration of treatment was 2 or 3 times less (37.7 ± 6.1 and 26.4 ± 5.2 days), the mortality rate was 8.3 % and 6.7 % respectively.*

Conclusion. *EAF that occur when using the "open abdomen" surgical tactics due to the impossibility of their isolation in extensive wounds of the anterior abdominal wall are complicated clinical and morphological forms. For their treatment, it is advisable to use VAC systems, aimed at the treatment of both the anterior abdominal wall wound itself and the intestinal fistula opening into it for its gradual extraterritorialization by modeling a floating enterostoma in a vacuum device.*

Key words: *postoperative peritonitis, enteroatmospheric fistula, aspiration, vacuum therapy, enterostoma*

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ОПЫТ ХИРУРГИЧЕСКОГО ЛЕЧЕНИЯ НЕСФОРМИРОВАННЫХ ТОНКОКИШЕЧНЫХ СВИЩЕЙ В ОТДАЛЁННОМ ПЕРИОДЕ ТЕЧЕНИЯ ПОСЛЕОПЕРАЦИОННОГО ПЕРИТОНИТА

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РЕЗЮМЕ

Обоснование. *Несформированные тонкокишечные свищи (НТКС), возникающие в ходе использования тактики «открытый живот», являются сложной хирургической патологией с высокой летальностью.*
Цель исследования. *Оценить эффективность лечения различных форм несформированных тонкокишечных свищей у больных послеоперационным перитонитом при использовании технологии вакуумной аспирации.*
Методы. *Проведена оценка результатов хирургического лечения 46 больных с НТКС в периоде позднего течения послеоперационного перитонита (ПП). Выделены три клинико-морфологические группы: 1-я группа (n = 24) – НТКС в небольших ранах передней брюшной стенки; 2-я группа (n = 15) – НТКС, открывающиеся в ограниченные полости; 3-я группа (n = 7) – НТКС, открывающиеся в лапаростомные раны. В 1-й группе формировали свищ с помощью аппаратов непрерывной аспирации или VAC-систем. Во 2-й группе использовалась непрерывная аспирация кишечного содержимого из полости. В 3-й группе с помощью вакуумных устройств проводилось лечение лапаростомы с изоляцией кишечного свища и моделированием плавающей энтеростомы.*
Результаты. *3-я группа больных с НТКС отличалась высоким дебитом (1224,2 ± 210,3 мл), длительностью лечения (87,3 ± 12,5 койко-дней), обширностью лапаростомы (335,4 ± 14,3 см^2), высокой летальностью (57,1 %). Лучшие результаты лечения получены в 1-й и 2-й группах. Дебит составил 675,8 ± 154,3 и 541,3 ± 114,1 мл, срок лечения был в 2–3 раза меньше (37,7 ± 6,1 и 26,4 ± 5,2 дня), летальность – 8,3 % и 6,7 % соответственно.*
Заключение. *НТКС, возникающие при использовании тактики «открытый живот» вследствие невозможности их изоляции в обширных ранах передней брюшной стенки, являются сложными клинико-морфологическими формами. Для их лечения целесообразны VAC-системы, направленные на лечение как самой раны передней брюшной стенки, так и открывающегося в неё кишечного свища для его постепенной экстерриторизации путём моделирования в вакуумном устройстве плавающей энтеростомы.*

Ключевые слова: *послеоперационный перитонит, несформированные тонкокишечные свищи, аспирация, вакуумная терапия, энтеростома*

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OBJECTIVES

To date, the problem of postoperative peritonitis (PP) continues to be one of the most important issues of practical surgery, because, despite all the recent achievements, it is the direct cause of death in 50–86 % of patients after abdominal surgery [1, 2]. The main strategy for surgical treatment of PP is currently semi-open (semi-closed) techniques, including "scheduled" and "open abdomen" (laparostomy) relaparotomies [3–7]. Along with the positive aspects, the use of open abdominal management undoubtedly leads to the development of various kinds of complications such as eventration, decreased protein, electrolytes, loss of integrity and structure of the anterior abdominal wall and the development of enteroatmospheric fistulas (EAFs), which constitute the main problem of postoperative peritonitis. The generalized EAF incidence using open abdominal tactics ranges from 1.5 % to 7 5% [8, 9]. Incidence of high EAF, including against the background of widespread peritonitis, is characterized by high mortality – from 19 % to 67 %. Lethal causes are attributed to fluid loss and electrolyte imbalance, protein loss, nutrient deficiencies, infection and sepsis [10]. There are two directions of surgical tactics in modern surgery of intestinal fistulas: radical surgical intervention in the acute period of the disease [11] and purely conservative treatment aimed at the fistula formation and its conversion into a chronic one [12]. However, the main and problematic issues in EAF treatment remain tactical approaches in cases of their late occurrence, especially at the time of their discovery in the laparostoma wounds – when active inflammation in the abdominal cavity has already ended. Such localization of fistula in the world literature is usually designated by the term "enteroatmospheric fistula", which implies an opening in the gastrointestinal tract of the open abdominal cavity without blocking it with tissues of the anterior abdominal wall [13]. The frequency rate of these fistulas increases with the duration of treatment of the patient with open abdomen and directly correlates with the number of repeated abdominal sanations, as well as with anastomotic dehiscence, intestinal ischemia, degree of distal intestinal obstruction, and adhesions [14–16].

In this regard, one of the most important components of the complex treatment of unformed intestinal fistulas are measures aimed at minimizing intestinal chyme loss [17–19]. It is difficult and sometimes impossible to reliably obturate an unformed intestinal fistula. As a rule, obturation of unformed intestinal fistulas with localizations on eventrated, protruding into the wound and covered with granulation loops does not lead to permanent success, but on the contrary, only increases the size of the fistula [20]. The best results in the treatment of patients with EAFs have been obtained using the active-aspiration system, but even here there are a number of difficulties associated with difficult to correct loss of chyme and severe destruction of abdominal wall tissue in the area of the fistula [21, 22]. The recently proposed method of vacuum therapy opens new perspectives in the treatment of patients with EAFs, first of all, allowing rapid sanation of the purulent-destructive process in the wound around the intestinal fistula, as well as promoting its rapid localization and formation [23–25].

Thus, methods of unformed intestinal fistula treatment continue to be developed and improved, including negative pressure therapy of fistula wounds, fistula obturation, use of surgical stents, etc. However, there is still no single universal method that can be applied to the treatment of certain unformed intestinal fistulas due to the peculiarities of their course. Therefore, there should be an individualized approach for each patient, depending on the clinical and morphologic form of EAFs, the level of fistula, features of development, nature and number of losses.

MATERIALS AND METHODS

Over the last 30 years, more than 350 patients with postoperative peritonitis have been treated in the clinic of hospital surgery on the basis of the purulent surgery department of the Barnaul Regional Clinical Hospital, in the treatment of which active surgical tactics of programmed abdominal cavity sanation, including the use of "open abdomen" technologies with temporary and final closure of the laparostoma wound were used. Bogota bags, negative pressure vacuum devices, and early dermal-aponeurotic sutures were used for this purpose. A total of 46 cases of unformed intestinal fistulas of the middle parts of the small intestine and ileum, opening in 3 different positions and arising after the use of the "open abdomen" technique were included in this study: on the eventrated loops of intestine in the midline wound; EAFs opening into the wound of the anterior abdominal wall; EAFs opening into the localized cavities (Table 1) (Atamanov V.V., 1985). In groups 1 and 2, patients with single incomplete intestinal fistulas prevailed, with a moderate flow rate of intestinal losses (from 200 to 400 ml per day), whereas in group 3, 4 out of 7 patients had multiple and complete enteric fistulas, and their flow rate always remained high – more than 800 ml per day [26]. The exclusion criteria included patients with unformed duodenal fistulas and colonic fistulas.

Among the patients with EAFs, 32 (69.6 %) were predominantly male. The mean age was 57.3 ± 2.6 years. All patients underwent from 2 to 5 scheduled abdominal cavity sanations for severe postoperative peritonitis. The time to fistula opening from the last surgical intervention was 12.4 ± 3.5 days. The major surgical diseases after treatment of which EAFs opened are summarized in Table 2.

In groups 1 and 2, patients with a moderate degree of protein-energy malnutrition dominated, and in group 3, the severity of these disorders was severe. Nutrition was carried out in a combined method.

TABLE 1
CLINICAL AND MORPHOLOGICAL FORMS OF ENTEROATMOSPHERIC FISTULAS

Groups	Clinical and morphological form of EAFs	*n*	%
Group 1	Fistula opening into anterior abdominal wall wounds	24	52.2
Group 2	Fistula opening into a localized purulent cavity	15	32.6
Group 3	Fistula on eventrated loops of bowel in a laparostoma wound	7	15.2
Total		46	100

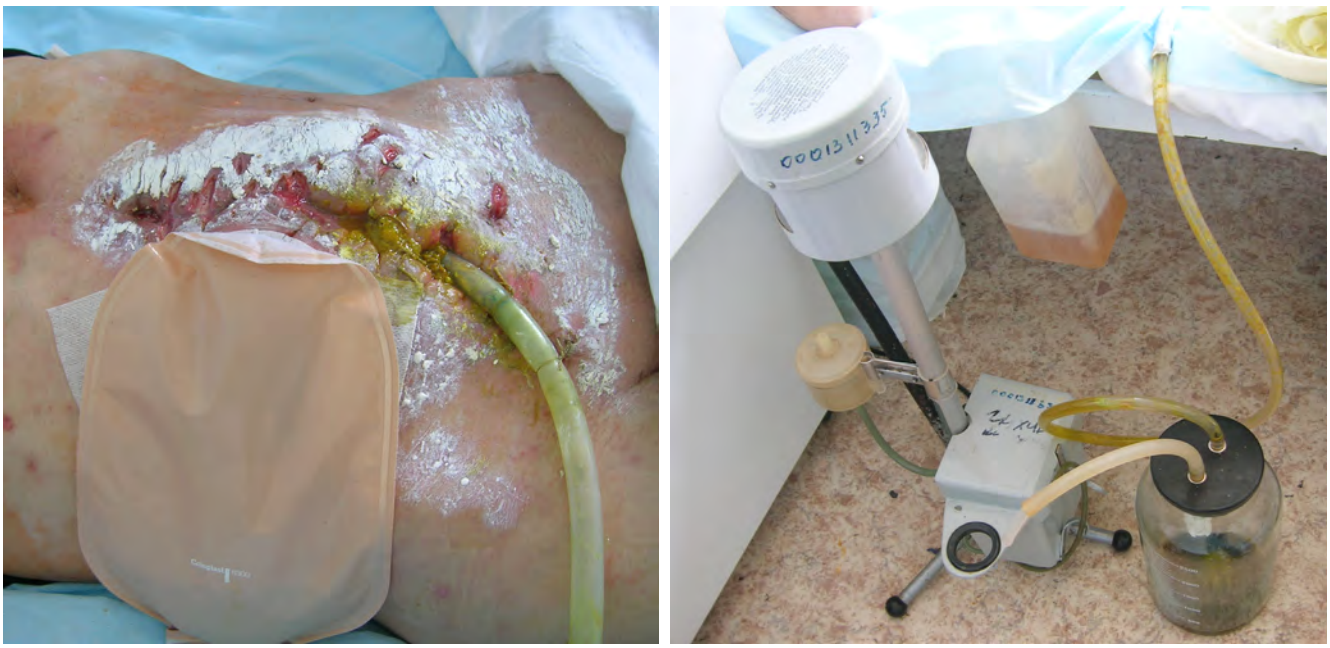
There were no significant manifestations of organ dysfunction in the groups of patients with EAFs. Surgical treatment of unformed enteroatmospheric fistulas in all three groups involved the use of different variants of vacuum aspiration.

TABLE 2
PRIMARY DISEASES CAUSED THE DEVELOPMENT OF POSTOPERATIVE PERITONITIS AND UNFORMED ENTEROATMOSPHERIC FISTULAS

Diagnosis	*n*	%
Acute adhesive intestinal obstruction	27	58.7
Pancreonecrosis	8	17.4
Closed abdominal trauma with damage to the small intestine	5	10.9
Colon cancer	3	6.5
Incarcerated hernia	2	4.3
Acute mesenteric ischemia	1	2.2
Total	46	100

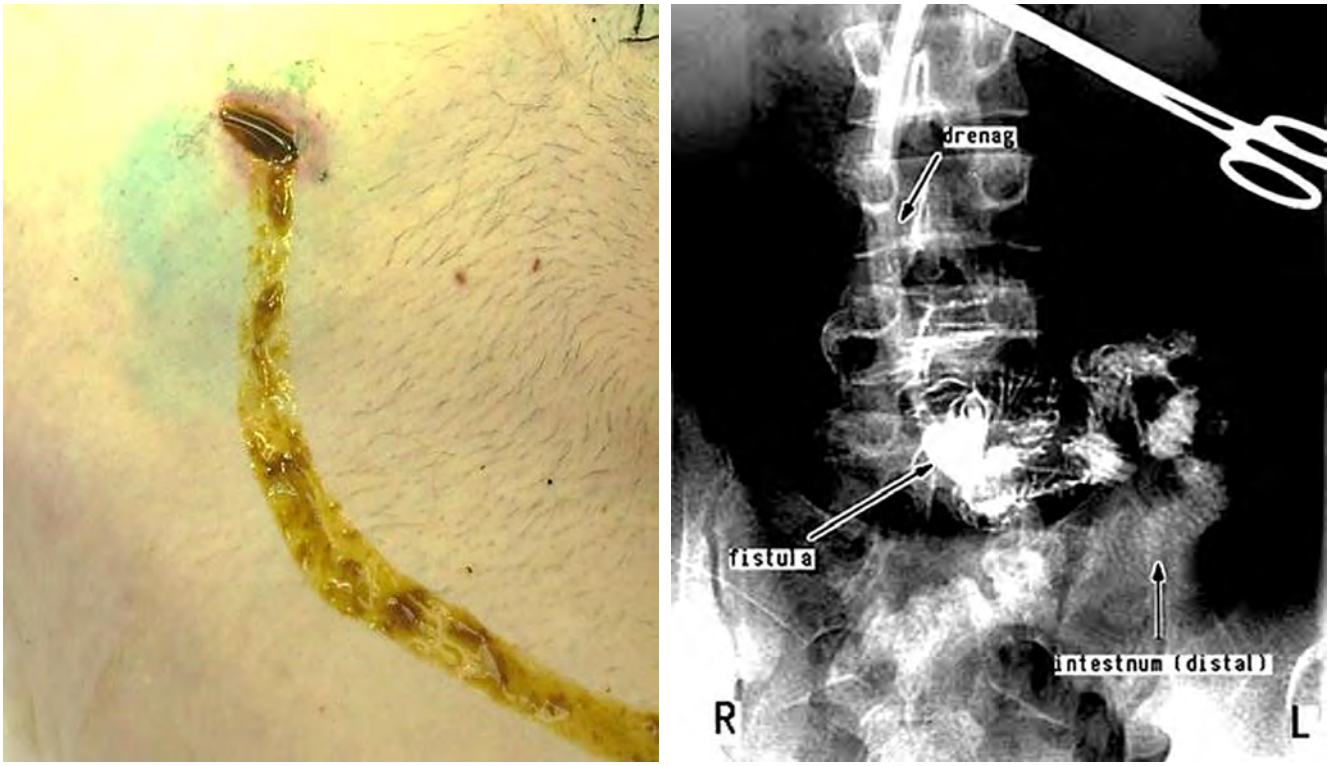
The opening of EAF into the wounds of the anterior abdominal wall ($n = 24$) occurred late in the course of peritonitis due to arrosion of the intestinal loop in a small laparostoma wound of the anterior abdominal wall in the presence of its purulent-necrotic changes with subsequent skin dermatitis due to irritation by intestinal enzymes. Continuous aspiration was used at local localization of the inflammatory process within the wound of the anterior abdominal wall and in the presence of enteroatmospheric fistulas, which was determined by the preservation of intestinal passage, small amount of small intestinal secretion, as well as the study data of barium passage through the small intestine and colon. The treatment algorithm consisted in clearing and reducing the purulent cavity where the intestinal fistula had opened, draining its contents outside the wound and reducing the phenomena of contact enzymatic dermatitis. The first stage of surgical treatment was necrectomy with secondary surgical treatment of the wound and skin suturing of its edges. This helped to reduce the size of the wound around the fistula and seal the single-lumen drainage (Fig. 1a). The continuous aspiration apparatus OP-01, creating negative pressure with a discharge range of 0.01–0.05 kgf/cm^2, was connected to it (Fig. 1b). In some cases, a modern vacuum system was simply applied to a laparostoma wound with a fistula. Aspiration devices were changed once every 3 days. All efforts were made to minimize the fistula wound by aspiration, additional secondary sutures and adapting it to the subsequent fixation of the colostomy bag.

Clinical EAF manifestations in the group 2 with fistulas opening into purulent cavities ($n = 15$), as a rule, occurred against the background of perforation of the intestinal loop in a localized purulent cavity, most often



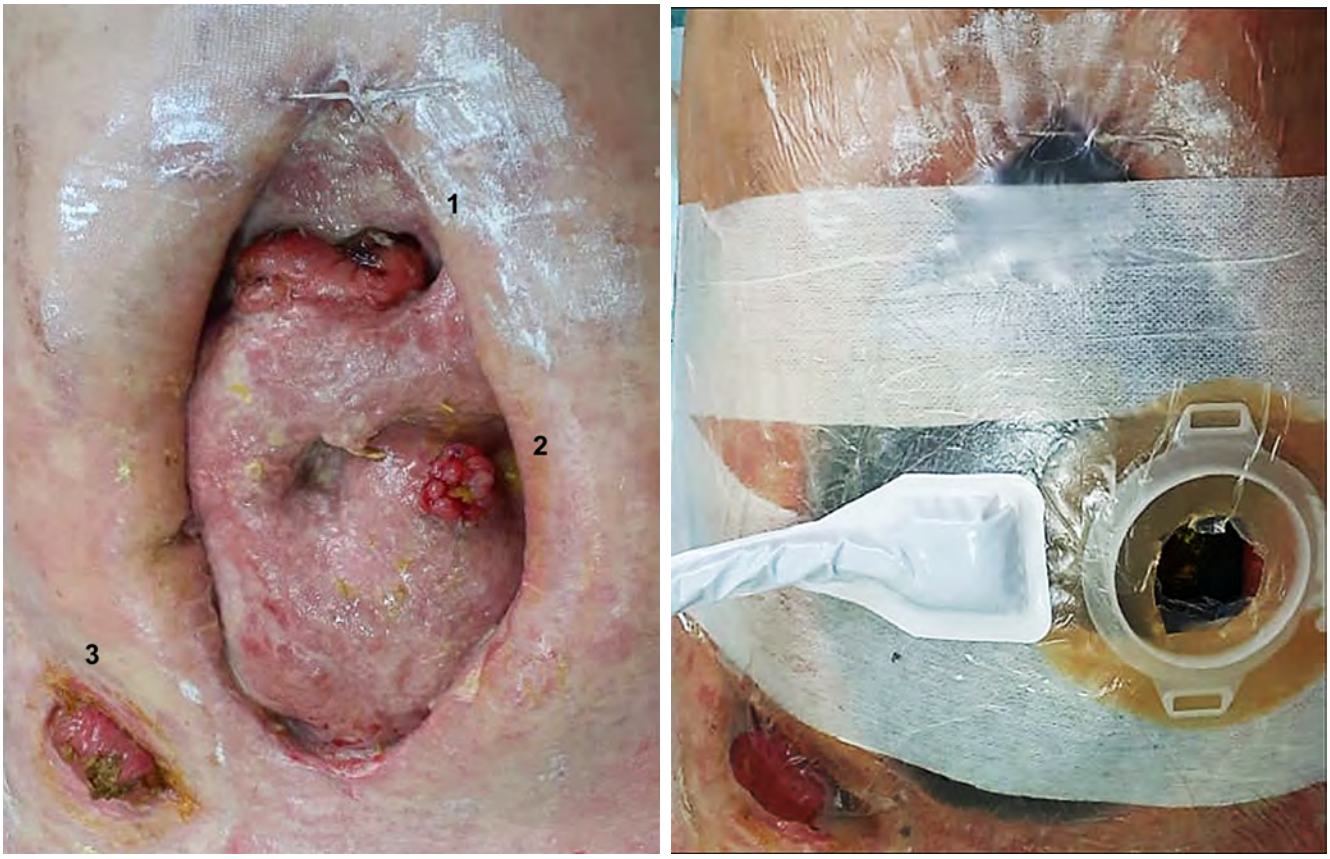
a ***b***

FIG. 1.
EAF opening into localized wounds of the anterior abdominal wall: ***a*** *– drainage in the postoperative wound and adaptation of the colostomy bag;* ***b*** *– device for continuous aspiration from a wound with a fistula*



a ***b***

FIG. 2.
EAF opening into a localized cavity: ***a*** *– intestinal contents leakage into the drainage wound of the right iliac region;* ***b*** *– fistulography (contrast of the distal loops of the small intestine, no contrast streaks)*



a *b*

FIG. 3.

*Open abdomen: **a** – view of the abdominal cavity ("frozen abdomen") 30 days after using the "open abdomen" technique (**1** – EAF of the transverse colon; **2** – functioning complete fistula of the small intestine; **3** – ileostomy); **b** – formation of a VAC system for the healing of a laparostoma with isolation of the EAF at the left edge of the laparostoma in a hole cut over the fistula in a polyurethane sponge for subsequent fixation of a two-piece colostomy bag on it [22]*

in the pelvic cavity; they were characterized by the flow of intestinal contents into the drainage wound (Fig. 2a) with no signs of widespread peritonitis and insignificant inflammatory changes. Preserved intestinal passage was recorded clinically and by enterography; in addition, no contrast streaks on fistulography implied the presence of a localized intestinal fistula (Fig. 2b). This facilitated conservative treatment using also continuous aspiration aimed at forming a tubular fistula.

When the patient was admitted after ultrasound examination of the abdominal cavity and fistulography in the surgical dressing room, the purulent cavity was revised, its size was determined, and the depth of intestinal fistula opening was investigated. Subsequently, a double-lumen drainage up to 1.5 cm in diameter was placed along the course of the wound channel towards the intestinal fistula. Formation of a impermeable cavity around the drain was achieved by applying secondary skin sutures. Aspiration was performed using a negative pressure apparatus (OP-01). When the flow rate of intestinal contents decreased, the drain was gradually replaced with a smaller diameter and removed. Daily instillations of Betadine solution were performed in the formed tubular drainage passage and gauze strips with Levomekol ointment were placed. According to the fistula localization, high enteric fistulas were noted in 3 patients, low enteric fistulas – in 12 patients.

The most problematic group of patients ($n = 7$) were patients with EAF opening on eventrated bowel loops. The occurrence of these intestinal fistulas also occurred late in the course of PP, when the "open abdomen" technique was used, and was due to perforation of intestinal loops that were in a rough infiltrative-adhesive process – "frozen abdomen" (Fig. 3a, b).

As already mentioned, according to the classification of M. Björck et al. [13], these EAFs belong to enteroatmospheric fistulas arising in the middle of the laparostoma. Usually such an intestinal fistula occurs in the presence of tight adhesions in the "frozen" abdominal cavity, it lacks a formed fistulous passage, and there is lateralization and retraction of the edges of the anterior abdominal wall, which makes it impossible to spontaneously close or seal it. Drying of intestinal loops and microtraumatization during abdominal sanations were the most important causes of these fistulas. The intestinal contents coming out of such a fistula were difficult to control, especially if the fistula was high (proximal), with a high flow rate, lead-

ing to multiple local complications (irritation, maceration, erosion, abdominal streaks, infection). In this situation, it was of high importance to clarify the level and anatomic location of the fistula in order to properly quantify fluid and electrolyte losses, and to use enterography to determine the total length of the remaining intestine and the maximum length of proximal intestine available for absorption. A hole was formed in the polyurethane sponge that was used for vacuum therapy of the laparostoma over the intestinal fistula (Fig. 3b), through which a soft round plastic pad with a diameter of 4–5 cm was placed on the fistula. Fixation of the sponge was performed with a patch. The two-piece colostomy bag was placed over a mouth of a fistula in the sponge. From above, the wound was sealed with adhesive films and an aspiration device was placed in the center. Small negative pressure (–80 mmHg) was created in the wound using the RENASYS GO device (Smith & Nephew, UK). Vacuum devices were changed once every 3–5 days. As a result, a directed collection of intestinal contents into the colostomy bag was gradually performed, and as the laparostoma was reduced, an enterostoma was formed.

STUDY RESULTS

In group 1 patients with EAF opening into purulent wounds of the anterior abdominal wall, the flow rate of intestinal contents ranged from 300 to 600 mL and averaged 675 ± 154.3 mL. The wound size reached 64.2 ± 9.8 cm^2. Continuous aspiration of intestinal contents in most cases contributed to intestinal passage improvement, reduction of flow rate from the fistula to 100–120 mL per day and motor adaptation of patients. Gradual wound reduction with secondary-delayed sutures allowed the intestinal fistula to continue to be managed conservatively. Reducing the size of the wound with intestinal fistula to 25 cm^2 in diameter made it possible to fit a colostomy bag with the widest opening (80–100 mm), which could be replaced by the patients themselves in the future.

The duration of aspiration was 25.7 ± 4.6 days and the mean hospital bed-day was 37.7 ± 6.1 days. Among the complications, 1 patient had arterial bleeding in the area of the fistula hole, 2 patients had abscess formation under the anterior abdominal wall, and 3 patients had transition of incomplete intestinal fistula to a complete one. In general, the process of further formation of such EAFs amounted to 2.5–3 months with subsequent planned surgical treatment of the formed enterostoma (resection of the fistula-bearing intestinal loop).

The main direction of surgical treatment in the group 2 of patients with EAFs was also the gradual formation of a tubular intestinal fistula due to continuous aspiration of intestinal contents, wound exudate (contents of the abscess cavity) from the sealed cavity formed from the wound edges above the fistula opening. Intestinal secretions aspirated from the cavity did not interfere with the healing processes. Against the background of vacuum-collaboration of the cavity, the defect in the intestinal wall decreased due to filling of the purulent cavity with granulation tissue, and the drainage tube served as a skeleton for the formation of a connective tissue fistulous passage, followed by a tubular fistula over the defect in the intestinal wall (Fig. 4).

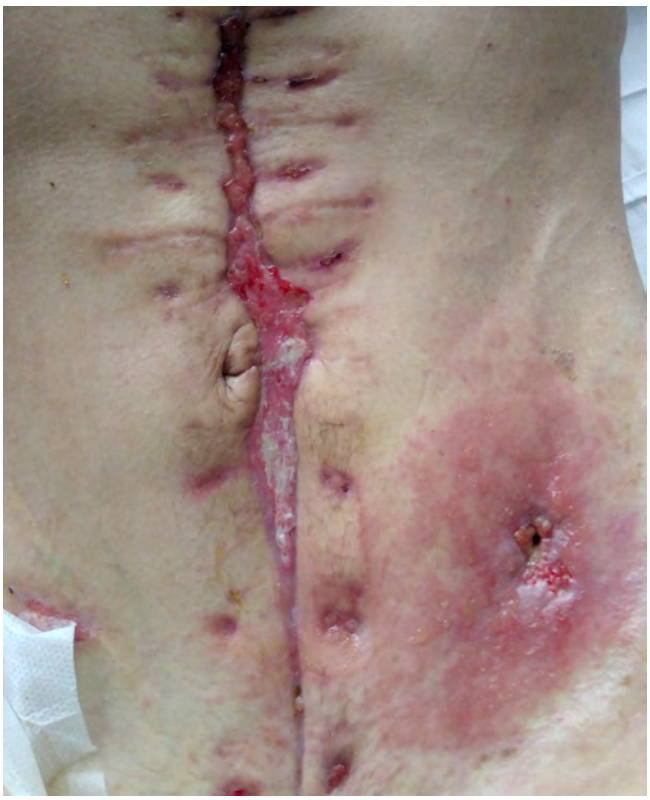


FIG. 4.
Formation of a tubular incomplete enteric fistula in the drainage wound of the left iliac region 3 weeks after continuous aspiration (the area of the wound has small manifestations of enzymatic dermatitis)

The duration of continuous aspiration was 19.4 ± 2.37 days, and the intestinal loss rate was 541.3 ± 114.1 mL. Due to the treatment 11 (73.3 %) out of 15 patients had a tubular enteric fistula with minimal flow rate (up to 20–30 mL), and in 3 (30 %) there was an independent closure of intestinal fistulas on the day 30–40. The mean bed day was 26.4 ± 5.2 days. Lethality was 6.7 % (1 patient); the cause of death was decompensation of cardiac activity.

In the group 3, treatment of extensive medial wound with negative pressure technique based on modern VAC systems was used. Considering that the laparostoma itself with intestinal fistula is also a wound, using vacuum aspiration, we also tried to reduce it. But the main challenge was to get the intestinal chyme outside the laparostoma wound. Generally, extra-territorialization of an intestinal fistula in this setting has been difficult,

but a floating stoma [27, 28] has been used in the construction, with the primary goal of forming a manageable fistula with collection of intestinal secretions separately into a colostomy bag in the laparostoma wound (Fig. 5). The mean size of the laparostoma wound where fistulas were opened was 335.4 ± 14.3 cm^2. The intestinal contents rate in group 3 EAF was 1224.2 ± 210.3 mL. Fistula formation time was 87.3 ± 12.5 days.

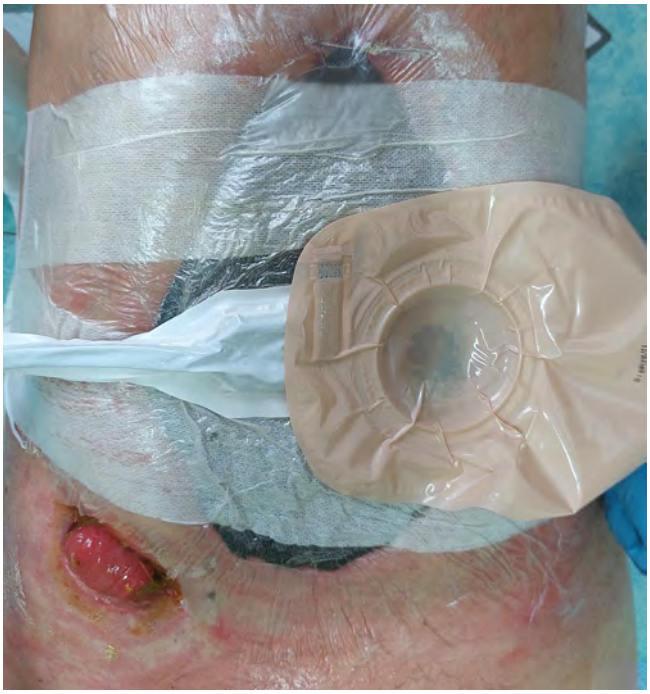


FIG. 5.
EAF opening into a vast laparostoma wound: extra-territorialization of the EAF at the left edge of the laparostoma wound with the installation of a colostomy bag on an enterostoma modeled in the VAC device (floating stoma) and on an ileostomy. Test of vacuum aspiration from a laparostoma (negative pressure – 120 mm Hg with its subsequent decrease to – 80 mm Hg)

Among the complications of the treatment period, arterial bleeding was noted in 2 patients, and in 2 observations – the appearance of additional intestinal fistulas in the laparostoma wound. In this group of patients, nutritional support was the most difficult task given the most commonly reported proximal location of the fistula and the large intestinal loss rate. Almost always the unformed intestinal fistula in the laparostoma was complete. It has been found that enteral nutrition can sometimes increase the flow rate of intestinal contents from EAF. Difficulties in enteral nutrition were noted when the driving small intestine was observed to be shorter than 75 cm according to enterography. However, combined nutrition with correction of the secretory function of the upper gastrointestinal tract was performed in these patients.

DISCUSSION

Given that the most important causes of unformed enteroatmospheric fistula formation in group 3 seem to be drying of the intestinal loops in contact with the external environment and microtrauma [29], all possible actions that could prevent them should be taken during treatment: 1) minimizing any rough or direct contact between the intestinal loops and the devices used for temporary abdominal closure (tissues, sponges, films); 2) avoiding prosthetic meshes, as they may cause perforation of the intestinal wall, leading to the formation of intestinal fistulas; 3) preventing drying of the intestinal loops; 4) early definitive closure of the abdomen; and 5) planning and performing dressing changes in the operating room. In addition, we should take into account the alteration of blood flow in the intestinal wall noted by us in earlier publications, leading to full blood flow in the mucous membrane of the small intestine [30]. It is caused not only by fixation and compression of intestinal loops in the wound of the anterior abdominal wall, but also by dysmetabolism associated with translocation of microflora into the intestinal wall. Therefore, we believe that the most important element in improving prognosis is the preservation of intestinal passage by enteral administration of nutrient mixtures and fractional enteral nutrition.

Treatment of the most difficult clinical and morphologic forms of unformed enteroatmospheric fistulas opening into extensive wounds of the anterior abdominal wall should be aimed at complete isolation of the fistula from the remaining open laparostoma wound; maximum atraumaticity of the materials used both for the fistula itself and for the underlying intestinal loops to avoid the occurrence of additional holes in the intestinal wall; ensuring the possibility of collection and quantification of intestinal losses, speed and ease of structures used change; protection of the surrounding tissues from the aggressive action of chyme; infection treatment and prevention.

CONCLUSION

Despite treatment, 7 (15.2 %) patients with EAFs died. The highest mortality was recorded in the group with EAFs opening into extensive laparostoma wounds – 4 (57.1 %) patients. Overall, the treatment results of patients with EAFs are summarized in Table 3.

The cause of a large number of fatal outcomes in group 3 was significant poorly managed intestinal losses in the presence of informed enteroatmospheric fistulas; repeated perforations of intestinal loops in the laparostoma. This group of patients with EAFs also differed in the duration of treatment, which was 2–3 months, resulting in the need not just to localize the intestinal fistula, but also to reduce the extensive laparostoma wound. In group 1,

TABLE 3
RESULTS OF TREATMENT OF PATIENTS WITH EAF

Parameters	Group 1 (n = 24)	Group 2 (n = 15)	Group 3 (n = 7)	p
Intestinal losses (ml)	675.8 ± 154.3	541.3 ± 114.1	1224.2 ± 210.3	$p_{1-2} > 0.05$ $p_{1-3} < 0.05$ $p_{2-3} < 0.01$
Wound size with intestinal fistula (cm^2)	64.2 ± 9.8 cm^2	12.5 ± 6.7	335.4 ± 14.3	$p_{1-2} < 0.01$ $p_{1-3} < 0.01$ $p_{2-3} < 0.0001$
Duration of aspiration (days)	25.7 ± 4.6	19.4 ± 2.3	67.3 ± 7.5	$p_{1-2} > 0.05$ $p_{1-3} < 0.001$ $p_{2-3} < 0.001$
Bed day (days)	37.7 ± 6.1	26.4 ± 5.2	87.3 ± 12.5	$p_{1-2} > 0.05$ $p_{1-3} < 0.01$ $p_{2-3} < 0.001$
Patients died, n (%)	2 (8.3)	1 (6.7)	4 (57.1)	$p_{1-2} > 0.05$ $p_{1-3} < 0.05$ $p_{2-3} < 0.05$

Note. p – statistical significance of differences between groups.

2 (8.3 %) patients died and in group 2, 1 (6.7 %) patient died. Death occurred as a result of the development of purulent-septic complications, formation of complete intestinal fistulas with the development of severe protein-energy malnutrition.

CONCLUSIONS

The use of "open abdomen" surgical tactics in patients with postoperative peritonitis is often accompanied by the opening of unformed enteroatmospheric fistulas. The most challenging clinical and morphologic forms among them are complete EAFs occurring in extensive laparostoma wounds, which are difficult to isolate in the stiff granulation tissue of vast wounds of the anterior abdominal wall, resulting in new perforations and total skin dermatitis. EAFs that open into small anterior abdominal wall wounds and localized purulent cavities while maintaining passage downstream of the fistula can be effectively treated conservatively with a variety of continuous aspiration options. In case of EAF in large laparostoma wounds, it is advisable to use VAC-systems aimed at treating both the wound of the anterior abdominal wall and the intestinal fistula opening into it with the aim of its gradual exteriorization by simulating a floating enterostoma in the device.

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

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THE FIRST TIPS SURGERY PERFORMED IN THE UDMURT REPUBLIC IN A YOUNG PATIENT WITH SECONDARY BILIARY CIRRHOSIS

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ABSTRACT

Treatment of patients with iatrogenic injuries of the biliary tract is the most difficult and important section in hepatobiliary surgery. When analyzing the causes, it was found that in 70–94 % of cases this type of injury is observed during cholecystectomy. We present a rare clinical case of the development of secondary biliary cirrhosis due to iatrogenic trauma of the biliary tract. This injury caused long-term suffering for the patient due to the further development of complications of cirrhosis, specifically of portal hypertension. The latter caused repeated recurrent profuse bleeding from varicose veins of the esophagus. The use of a minimally invasive transjugular intrahepatic portosystemic shunt procedure for the first time in the Udmurt Republic was of particular relevance in solving this problem. This procedure has become a key one in solving the abovementioned problems and will become the preventive measure for the cirrhosis progression in the future. It is also important to focus on prophylactic measures aimed at preventing iatrogenic injuries of the biliary tract, as this problem can cause irreversible complications. Prevention should include adequate examination and visualization of the bile ducts and gallbladder before surgery, their careful mobilization, compliance with the rules of operation with electrosurgical instruments, as well as the use of additional minimally invasive techniques such as choledoscopy, cholangiography and intraoperative ultrasound. Besides that, all manipulations should be carried out under strict control and clear visualization of instruments and anatomical structures of organs. The article provides a detailed description of the technique of transjugular intrahepatic portosystemic shunt surgery, as well as presents X-ray images obtained during this operation.

Key words: *biliary tract, iatrogenic trauma, cholecystectomy, biliary cirrhosis, transjagular intrahepatic shunting, TIPS*

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ПЕРВАЯ ОПЕРАЦИЯ TIPS, ПРОВЕДЁННАЯ В УДМУРТСКОЙ РЕСПУБЛИКЕ, ПО СПАСЕНИЮ МОЛОДОЙ ПАЦИЕНТКИ С ВТОРИЧНЫМ БИЛИАРНЫМ ЦИРРОЗОМ ПЕЧЕНИ

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РЕЗЮМЕ

Лечение лиц, получивших ятрогенные травмы желчевыводящих путей, является наиболее сложным и важным разделом в гепатобилиарной хирургии. При анализе причин установлено, что в 70–94 % случаев данный вид травмы наблюдается при холецистэктомии. На примере клинического случая нами представлено редко встречающееся в клинической практике развитие вторичного билиарного цирроза печени вследствие ятрогенной травмы желчевыводящих путей. Данная травма стала началом длительных страданий для пациентки ввиду развития в дальнейшем осложнения цирроза, а именно портальной гипертензии. Последнее стало причиной многократно рецидивирующих обильных кровотечений из варикозных расширенных вен пищевода. В решении данной проблемы особую актуальность имело применение малоинвазивной методики трансъюгулярного интрагепатического портосистемного шунтирования, которую использовали в Удмуртской Республике впервые. Данная операция стала ключевой в решении вышеизложенных проблем и в дальнейшем станет профилактической при прогрессировании цирроза печени. Также немаловажно сделать акцент на профилактические мероприятия, направленные на предупреждение ятрогенных травм желчевыводящих путей, ведь именно данная проблема стала причиной необратимых осложнений. К профилактике следует отнести адекватное обследование и визуализацию желчных протоков и желчного пузыря до оперативного вмешательства, тщательную их мобилизацию, соблюдение правил работы электрохирургическими инструментами, а также использование дополнительных малоинвазивных методик, таких как холедоскопия, холангиография, интраоперационное ультразвуковое исследование. Кроме того, все манипуляции должны производиться под чётким контролем и при ясной визуализации инструментов и анатомических структур органов. В статье приведено подробное описание техники трансъюгулярного интрагепатического портосистемного шунтирования, а также демонстрируются рентгеновские изображения, полученные в ходе данной операции.

Ключевые слова: *желчевыводящие пути, ятрогенная травма, холецистэктомия, билиарный цирроз, трансъягулярное интрагепатическое шунтирование, TIPS*

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INTRODUCTION

The factors that significantly increase the risk of iatrogenic trauma of extrahepatic bile ducts during laparoscopic cholecystectomy include congenital local anatomical features; variety of topographic-anatomical correlations; inflammatory and sclerotic changes in the area of the bladder neck, hepatoduodenal ligament; lack of adequate technological support; excessive increase in the number of electrocoagulation procedures; insufficient qualification and experience of the surgeon; incorrect traction and exposure; misidentification of anatomical structures of the hepatoduodenal zone; coagulation, clipping or crossing of structures without proper visual control of the instrument working part [1].

According to some reports, in 50 % of cases iatrogenic trauma is caused by the anomalies of biliary duct structure [2].

Secondary biliary cirrhosis of the liver is a diffuse proliferation of connective tissue of the hepatic parenchyma, followed by the organ architectonics disorder due to a long-term impairment in bile outflow and stagnation in the intrahepatic bile ducts system. The main etiological factor is the initial extrahepatic cholestasis that develops mainly due to biliary pathology, tumours and cholelithiasis (gallstones) [3]. However, this process can develop due to iatrogenic trauma of the choledochous duct, which is much less common than the above causes.

Among the life-threatening complications of cirrhosis is a portal hypertension syndrome, which can lead to the formation of a serious complication manifested as bleeding from varicose veins of the esophagus and upper third of the stomach. This complication takes 10–15 % in the structure of mortality of patients with cirrhosis [4]. In the present-day surgery, searching for effective methods of treatment of complications associated with a portal hypertension syndrome remains highly relevant. Minimally invasive techniques, namely X-ray endovascular interventions, are of great importance to solve this problem. This technique is transjagular intrahepatic portosystemic shunting (TIPS).

TIPS is a percutaneous minimally invasive technique through which it is possible to create a calibrated intrahepatic portosystemic shunt necessary for the treatment of portal hypertension. Self-expanding nitinol stent or coated stent is most commonly used as a shunt [5]. This shunt in modern practice is quite often used in the treatment of portal hypertension and represents a new way to connect the portal vein with hepatic veins. The operation provides good decompression of the portal system and is also characterized by its low invasiveness [6]. But this type of intervention has a number of potential complications, which include stenosis or shunt obturation. Balloon dilatation and placement of an additional stent are necessary to correct the complication. Another life-threatening complication of this technique is the development of hepatic encephalopathy. TIPS is commonly used as a short-term intermediate step before liver transplantation (LT), as this intervention preserves the anatomy of the liver and its gates [7].

CASE STUDY

Patient E., 31 years old, in 2009, she complained of repeated attacks of pain in the right subcostal area, periodic bloating after eating, nausea, weakness. On October 29, 2010 in the surgical department of one of the central district hospitals, the following operation was performed: laparotomy, cholecystectomy, drainage of the abdominal cavity. In the postoperative period bile secretion by drainage – up to 1 L per day. During this operation, iatrogenic trauma of the choledochous duct occurred. On November 05, 2010, relaparotomy, sanation, and drainage of the abdominal cavity were performed in the surgical department of the First Republican Clinical Hospital of the Ministry of Health of the Udmurt Republic. In May 2011, reconstructive surgery was performed in the First Republican Clinical Hospital of the Ministry of Health of the Udmurt Republic: transhepatic drainage of the left and right hepatic ducts on the small intestine loop switched off by Roux (hepaticojejunostomy). In 2013, drainage removal was performed with subsequent formation of a small enteroatmospheric fistula of the anterior abdominal wall, which closed after 1 month. Since June 2014, she noted worsening of her condition, jaundice, hepatic insufficiency; she was diagnosed with liver cirrhosis. On January 25, 2015 there was repeated bleeding from varicose veins of the esophagus; on January 30, 2015 – X-ray endovascular occlusion of the branches of the superior mesenteric artery (SMA). In 2015, 2017, the patient was treated in the gastroenterology department with the diagnosis: secondary biliary cirrhosis of the liver. Last hospitalization was in 2018 in the gastro department of the First Republican Clinical Hospital of the Ministry of Health of the Udmurt Republic for treatment of liver cirrhosis. After a while there appeared weakness, jaundice lasting about two months. She was bothered by skin itching, bleeding from varicose veins became more frequent, and in December 2021 there was an acute bleeding from the esophageal veins: the esophageal veins were dilated on fibrogastroscopy (FGS). On April 07, 2022, after a sharp deterioration of her condition, she was transported from the Republican Clinical Hospital by air ambulance to the First Republican Clinical Hospital of the Ministry of Health of the Udmurt Republic with bleeding for diagnosis and treatment.

According to the objective examination: the condition is severe. The patient is of an asthenic type. The position is active within the bed, paresis and paralysis are absent. The skin is pale, warm. Scleral icterus. Respiration is spontaneous, HR = 18/min. By auscultation: vesicular respiration, no rales. Percussion sound is pulmonary. Heart tones are rhythmic, clear. Blood pressure – 110/60 mmHg, pulse = 100 BPM. Tongue is dry, covered with gray plaque. The abdomen is soft, painless. Hepatic dullness is preserved. The gallbladder is not palpated. Rebound tenderness (Shchetkin – Blumberg's sign) is negative. Bowel peristalsis is audible. Tumor-like masses in the abdominal cavity are not palpated. Lumbar concussion symptom is negative on both sides. No edema.

According to clinical and biochemical blood tests, leukocytopenia (2.0×10^{12}/L), thrombocytopenia (64×10^{12}/L), anemia (3.09×10^{12}/L; HGB = 69), bilirubinemia (64 μmol/L) due to direct (25 μmol/L) and indirect (39 μmol/L) fractions, acid-base disorder (pH = 7.431) were noted. Transaminases (ALT, AST) are slightly elevated (250 u/l), total protein is within normal limits, as active infusion therapy was carried out.

Coagulogram from April 07, 2022: prothrombin time – 13.0; fibrinogen – 2.4; INR – 1.27; activated partial thromboplastin time (aPTT) – 37.2.

According to spiral computed tomography of abdominal cavity organs with intravenous bolus contrast enhancement: CT picture of liver cirrhosis with marked portal hypertension, splenomegaly, small peritoneal effusion. Postcholecystectomy condition, embolization of periapical portocaval anastomoses. Cholangiectasia.

As per FGS: erosive esophagitis, chronic gastritis with focal atrophy.

For technical and medical reasons, a liver biopsy was not performed.

Conservative treatment included hemostatic, infusion, gastroprotective and symptomatic therapy. Improvement of the condition was not observed, and repeated bleeding accompanied by decompensation could end in death.

This event occurred during the Plenum of hepatobiliary surgeons on portal hypertension, dedicated to the 110th anniversary of M.D. Patsiora's birth (Moscow, April 7-8, 2022), where the question of TIPS surgery was raised concerning this patient. After the critical deterioration of the patient's health condition, it was decided to invite postgraduate students of the Department of Intermediate-Level Surgery, Izhevsk State Medical Academy, an X-ray endovascular surgeon from the emergency hospital, and a general surgeon. The patient was prepared to undergo surgical treatment (TIPS). The surgery was performed under the supervision of the chief surgeon and professor of the Department of Intermediate-Level Surgery, Izhevsk State Medical Academy.

OP REPORT

Radiation exposure: absorbed dose – 15.2 mSv, maximum absorbed dose in the skin – 320 mGy, fluoroscopy time – 27.5 min. Under local anesthesia with 0.5 % lidocaine solution of 10.0 ml, and using ultrasound navigation equipment, puncture of the right internal jugular vein was performed. Afterwards, catheterization of the right intrinsic hepatic vein was performed with a Destination 7Fr Guiding Sheath (introducer) (Terumo Corporation, Japan) (Fig. 1). Under intravenous anesthesia with a Merit needle with a 30° angle for TIPS, puncture of the right branch of the portal vein was performed (Fig. 2). Next, a 0.035 guidewire was guided into the portal vein, and the needle was removed. In the area of the formed venous portosystemic shunt (PSS) in the liver parenchyma, predilation was performed with a balloon catheter of 5.5 mm diameter and 80 mm length to 10 atm. After balloon catheter removal, a delivery system with a mounted S.M.A.R.T. stent was installed in the shunt area. Flex Vascular Stent System (Cordis, USA) 10 × 60 mm (Fig. 3), which was deployed by inflating to 10 atm a balloon catheter with a diameter of 10.0 mm and a length of 40 mm (Fig. 4). The delivery system was removed, and phlebography was performed from a sheath (introducer) placed through the formed shunt into the portal vein (Fig. 5). The formed TIPS is functional, the stent is deployed. Further, phlebography of portal vein was performed, where an efferent dilated up to 10 mm venous network is visualized, coming from the loop of small intestine switched off by Roux, previously, before the operation, visualized on CT of abdominal cavity organs with intravenous bolus contrast enhancement. The system of guidewires and catheters was removed, aseptic dressing was applied to the area of puncture of the right internal jugular vein.

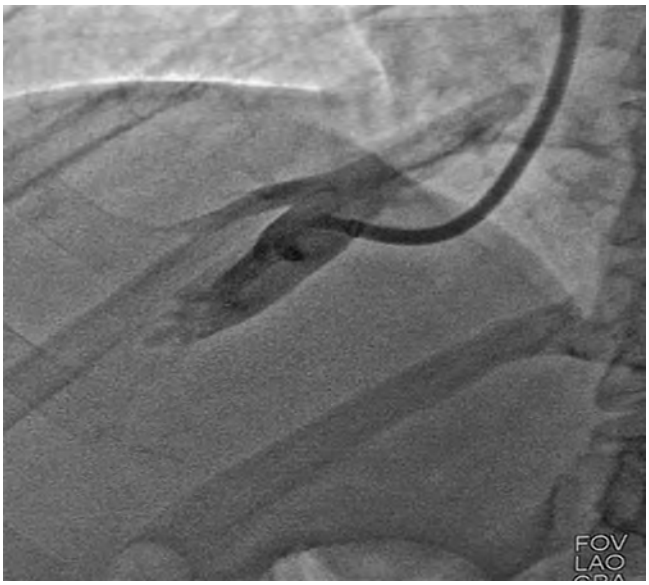

FIG. 1.
Patient E. Intraoperative X-ray image. Catheterization by an introducer and right hepatic vein angiography

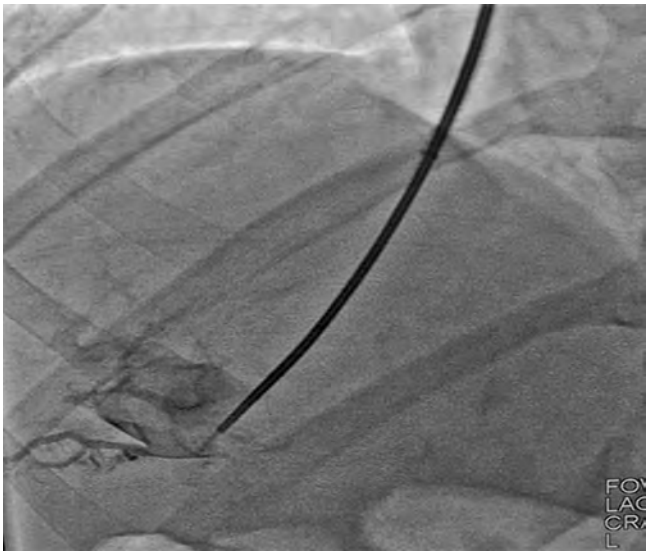

FIG. 2.
Patient E. Intraoperative X-ray image. The puncture stage, the needle is passed through the liver parenchyma into the portal vein

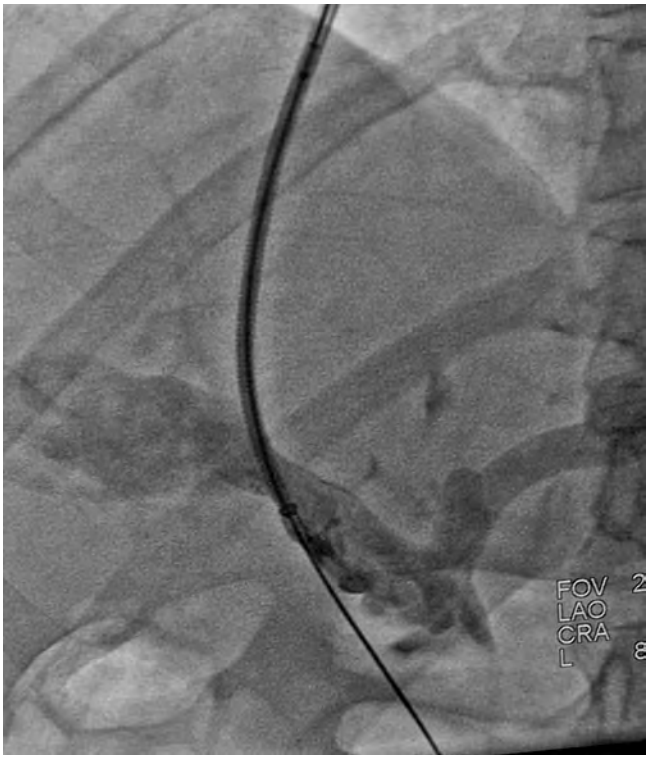

FIG. 3.
Patient E. Intraoperative X-ray image. Installation of the S.M.A.R.T. Flex Vascular Stent System

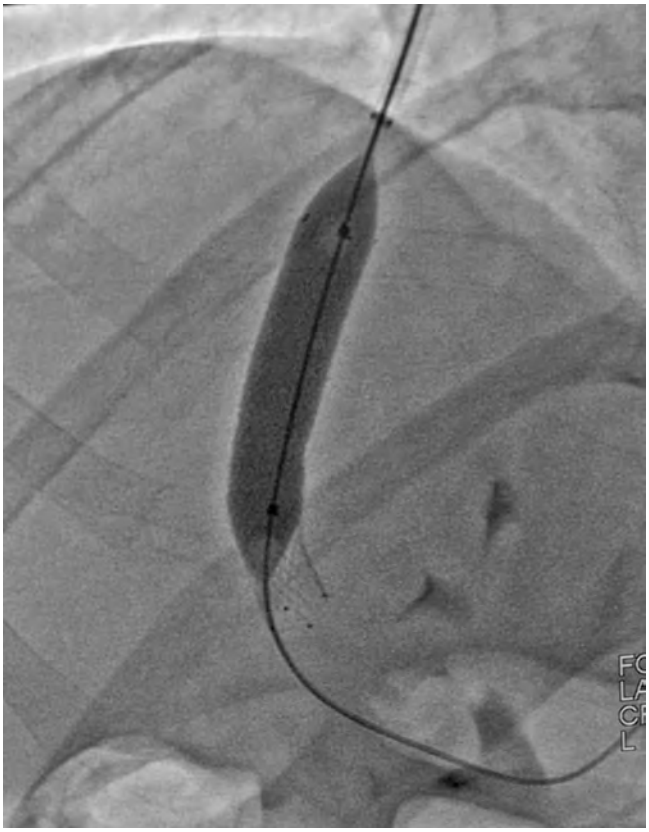

FIG. 4.
Patient E. Intraoperative X-ray image. Inflating the stent with a balloon

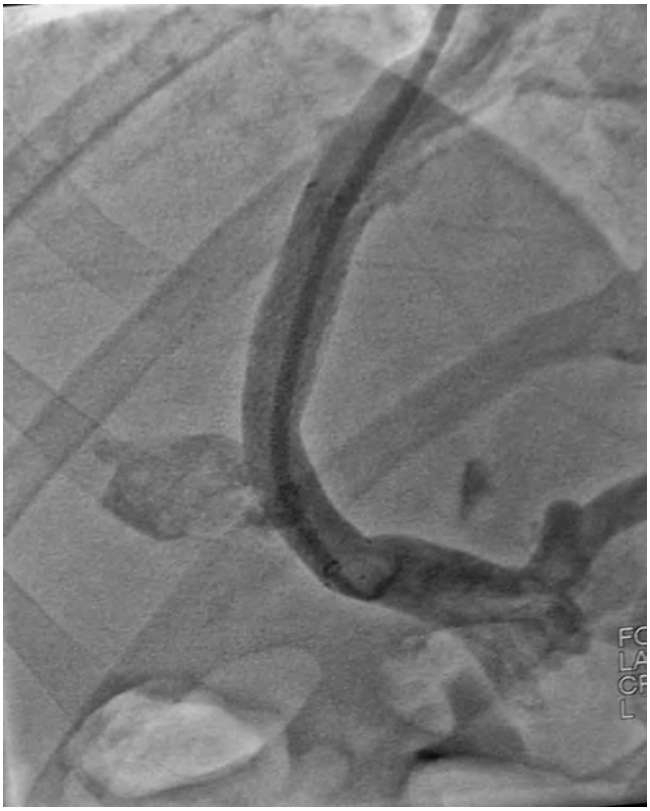

FIG. 5.
Patient E. Intraoperative X-ray image. Control angiography. TIPS visualization

As of April 12, 2022, the patient's condition has significantly improved, positive dynamics is observed. There was no evidence of recurrence and continued bleeding in the gastrointestinal tract. On April 15, 2022, the patient was successfully discharged. The patient was also prepared for the planned liver transplantation in Kazan under the supervision of the transplantology department head.

CONCLUSION

Thus, by the example of a clinical case, we presented a rarely encountered in clinical practice development of secondary biliary cirrhosis of the liver due to iatrogenic trauma of the biliary tract. This trauma caused long-term suffering for the patient due to the further development of complications of cirrhosis, specifically of portal hypertension. The latter was the cause of repeated heavy bleeding in the gastrointestinal tract, which could no longer be stopped by traditional methods of treatment. In this case, practitioners resorted to the use of minimally invasive techniques, namely, they successfully performed the first in the Udmurt Republic X-ray endovascular surgery on transjugular intrahepatic shunt. This procedure has become a key one in solving the above-mentioned problems and will become the preventive measure for the cirrhosis progression in the future. In modern surgical practice concerning portal hypertension syndrome, TIPS is a rather

effective method of decompression of the portal vein, allowing to achieve a decrease in the recurrence of bleeding from dilated veins of the esophagus and stomach.

It is also important to emphasize preventive measures aimed at prevention of iatrogenic traumas of biliary tract, because this very problem caused irreversible complications. Prevention should include adequate examination and visualization of bile ducts and gallbladder before surgical intervention, their careful mobilization, compliance with the rules of electrosurgical instruments, as well as the use of additional minimally invasive techniques, such as choledoscopy, cholangiography, intraoperative ultrasound. In addition, all manipulations should be performed under clear control and with clear visualization of the instruments and anatomical structures of the organs [8].

Conflict of interest

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

The bioethical standards in conducting the required research were complied with and approved by the organization's committee (Minutes No. 732/1 dated 08.02.2022).

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EXPERIMENTAL RESEARCHES

FEATURES OF THE INTEGRATION OF TWO-LAYER METAL KNITWEAR MADE OF TITANIUM NICKELIDE DURING THE REPLACEMENT OF A THORACOABDOMINAL DEFECT IN THE EXPERIMENT

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ABSTRACT

The aim *of investigation was to study experimentally the morphological features of tissue integration of two-layer titanium nickelide (TiNi) knitwear when replacing thoracoabdominal defects.*

Materials and methods. *The experiments were carried out on 40 Wistar rats. The experimental animals were divided into two comparison groups: in Group A (n = 20) the defect was replaced using a two-layer knitted tape made of TiNi, in Group B (n = 20) a polypropylene mesh implant was used. The technique of the operation and the peculiarities of keeping the animals did not differ. Animals were taken out after 14, 30, 60 and 90 days of experiment. The macroscopic structural features at the site of implant fixation to tissues and at the sites of contact with underlying organs were studied, and the inflammatory process was assessed. The histological and electron microscopic study was carried out with an assessment of the features of tissue integration through the mesh structure of knitwear.*

Results. *Thirty days after the surgery in four cases of Group B the appearance of the chest wall deformation at the site of implant fixation was noted, in one case the deformation site was located along the lateral edge of the abdominal wall. Among the animals of Group A no such changes were recorded. The histological and electron microscopy examination revealed that the porous structure of the TiNi wire, as well as the biomechanical and biochemical properties of the two-layer metal knitwear, ensure optimal integration of the endoprosthesis in the body tissues, forming an elastic frame close to natural. In Group B, on the contrary, the reaction of the body caused by the implanted polypropylene prosthesis was characterized by more pronounced fibrosis, and tissue integration through the mesh structure of the implant was not observed.*

Conclusion. *Two-layer TiNi knitwear in the replacement of complex structures of the thoracoabdominal zone showed promising results, which opens up prospects for further clinical research.*

Key words: *two-layer knitwear, titanium nickelide, mesh implant, replacement of post-resection defects, tissue integration, biocomparability*

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ОСОБЕННОСТИ ИНТЕГРАЦИИ ДВУХСЛОЙНОГО МЕТАЛЛОТРИКОТАЖА ИЗ НИКЕЛИДА ТИТАНА ПРИ ЗАМЕЩЕНИИ ТОРАКОАБДОМИНАЛЬНОГО ДЕФЕКТА В ЭКСПЕРИМЕНТЕ

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РЕЗЮМЕ

Цель исследования. *Изучить в эксперименте морфологические особенности тканевой интеграции двухслойного металлотрикотажа из никелида титана (TiNi) при замещении торакоабдоминальных дефектов.*

Материалы и методы. *Проведена серия экспериментов на 40 крысах линии Wistar. Экспериментальные животные были разделены на две группы сравнения: в группе А (n = 20) замещение дефекта осуществляли с применением двухслойной трикотажной ленты из TiNi; в группе В (n = 20) использовался сетчатый имплант из полипропилена. Техника операции и особенности содержания у животных в группах не отличались. Животных выводили из эксперимента через 14, 30, 60 и 90 суток после операции. Изучали макроскопические структурные особенности в месте фиксации имплантата к тканям и на участках контакта с подлежащими органами, оценивали воспалительный процесс. Проводили гистологическое и электронно-микроскопическое исследование с оценкой особенностей интеграции тканей сквозь сетчатую структуру металлотрикотажа.*

Результаты. *В ходе роста животных и набора веса через 30 суток в четырёх случаях в группе В отмечено появление деформации в месте фиксации импланта к грудной стенке, в одном случае участок деформации был расположен по латеральному краю брюшной стенки. Среди животных в группе А подобных изменений не зафиксировано. При гистологическом исследовании и электронной микроскопии отмечено, что шероховатая микропористая структура проволоки из TiNi, а также биомеханическое поведение двухслойного металлотрикотажа обеспечивают оптимальную интеграцию эндопротеза в тканях организма, формируя эластичный каркас, близкий к естественному. В группе В, напротив, реакция организма, вызванная имплантированным полипропиленовым протезом, характеризовалась более выраженным фиброзом, а интеграция ткани сквозь сетчатую структуру импланта не прослеживалась.*

Заключение. *Двухслойный металлотрикотаж из TiNi, используемый для замещения сложных структур торакоабдоминальной зоны, показал многообещающие результаты, что открывает перспективы для дальнейших клинических исследований.*

Ключевые слова: *двухслойный металлотрикотаж, никелид титана, сетчатый имплант, замещение пострезекционных дефектов, интеграция в тканях, биосовместимость*

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RELEVANCE

Improvement and progress of surgical technologies have significantly expanded not only operability but also resectability in various diseases of thoracic and abdominal cavity organs. Removal of a whole organ or part of it in some cases implies a reconstructive-restorative stage, where the use of implantation technologies is vital. Considering the variants of combined surgical interventions, the most common anatomical structures that require restoration or replacement are the abdominal wall, diaphragm, pericardium, and chest wall [1]. Vast experience of such operations shows that it is not always possible to replace extensive soft tissue defects with own tissues alone. Under conditions of significant tension, the intrinsic tissues do not meet the requirements of elasticity and resilience, which leads to tension at suturing, compartment effects and the risk of primary suture failure with the development of eventration [2, 3]. The disadvantages described have necessitated the development and implementation of alternative, mesh materials with the necessary biocompatibility.

A large number of different materials and tissues have been proposed as allografts during the long history of this field development. Materials such as mersilene (Dacron), polypropylene, lavsan (Marlex), Teflon, and titanium nickelide (TiNi) have been most widely used in the fabrication of mesh implants [4–6]. The core problems of known implanted devices are due to the biomedical properties of the material [7, 8]. The material introduced into the tissues for a long period of time should have the necessary implantation characteristics, such as biocompatibility, nontoxicity, resistance to the biological environment and infection, which determines the behavior of the material in the body. In an attempt to create a universal, areactive implant, the authors modified already known materials by changing their compositions and applying various elements to the surface, but the developments have not yet found wide application [9, 10]. Most studies focus on the adaptation of the implant in the body at the cellular level, while attention is paid to physical and mechanical properties only when it comes to bone replacement. However, when plasticizing mobile structures, the material must also possess a number of properties to ensure elasticity, hydrophilicity, and strength [11]. The vast range of presented implants is due to the lack of a universal material, which forces to continue the search for improving the results of replacement and prosthetics of various structures of the thoracic and abdominal cavities.

Currently, TiNi-based implants have shown optimal results, as evidenced by their widespread use in the clinic. The advantages include bioinertness, allowing the surrounding tissues to integrate through the implant to form a single biomechanical structure with the organ. It has also been found that a passive layer of titanium oxide group is formed on the surface of TiNi alloy, which functions as a physical barrier to the oxidation of nickel, making it harmless to the human body and protecting it from corrosion [12]. And while the material itself is well researched and favored and trusted among today's practicing surgeons, progress is moving forward, and more and more new forms of weave and tissue structures made of TiNi are emerging. One such form is a two-layer knitted tape made of superelastic TiNi-wires, whose behavior in tissues and features of its integration require further study.

THE AIM OF THE STUDY

To study experimentally the morphological features of tissue integration of two-layer metal knitted TiNi mesh when replacing thoracoabdominal defects.

MATERIAL AND METHODS

Prototype implants manufactured in the laboratory of superelastic biointerfaces of National Research Tomsk State University were used as a material for the study. The implant is a two-layer tape of low-modulus superelastic TiNi-wires with a diameter of 60 μm, woven by knitting technology (Jesi knitting). The study was conducted on 40 sexually mature male Wistar rats weighing 300–350 g and aged 2.5–3.0 months. The animals were kept in the conditions of the Central Scientific Laboratory of Siberian State Medical University of the Ministry of Health of Russia. The study was conducted under the approval and supervision of the local ethics committee of Siberian State Medical University of the Ministry of Health of Russia (minutes No. 732 dd. 10/06/2020), in compliance with international and native norms of humane treatment of laboratory animals (Directive 2010/63/EU of the European Parliament and of the Council of September 22, 2010 "On the protection of animals used for scientific purposes"; Order of the Ministry of Health No. 199n dd. April 1, 2016 "On approval of rules of good laboratory practice"; Order of the Ministry of Health of the USSR No. 755 dd. August 12, 1977 "On measures to further improve the organization of work using experimental animals") [13, 14]. The experimental animals were divided into two comparison groups: in Group A ($n = 20$) the defect was replaced using a two-layer metal knitted TiNi tape, in Group B ($n = 20$) a polypropylene mesh implant was used. The choice of polypropylene mesh implant as a comparison group is due to the most frequent use of the material in the clinic for prosthetic defects localized in the thoracoabdominal areas. Preoperative preparation, surgery, anesthetic management, and postoperative management were similar in all animals. All surgical interventions were performed using zoletil-xylanite anesthesia according to the following scheme: zoletil 3 mg/kg intramuscularly (i. m.), xylanite 8 mg/kg i. m., atropine sulfate 0.1 % solution – 0.1 ml/kg subcutaneously. After anesthesia, the animals were placed and fixed on the operating table in supine position with tissue ties. The skin was treated with antiseptic solution and dried with a sterile gauze pad. The surgical site was delineated with a sterile disposable sheet.

The surgery technique. Surgical intervention was started with a midline incision on the anterior abdominal wall with a transition to the chest wall (incision length was 3–4 cm), skin flaps and subcutaneous tissue were mobilized, the musculofascial and aponeurotic flap of the anterior abdominal wall was dissected, and the processus xiphoideus and cartilaginous fragments of the arcus costalis were resected extrapleurally. As a result, a 2 × 3 cm postresection defect was formed. The endoprosthesis was cut individually according to the shape of the defect with an allowance for its edge. Fixation was performed around the perimeter using a continuous suture with 4/0 polypropylene monofilament. When the needle was passed, the animal tissue along the defect line and the edge of the endoprosthesis were captured in the suture. The implant was fixed directly to the chest wall at the level of the resected processus xiphoideus and arches of the ribs. The surgical access was sutured layer by layer, tightly with polyglycolide (PGA) 4/0 thread on an atraumatic needle. In the postoperative period, prophylaxis of wound infection by antibacterial therapy with ceftriaxone 25 mg/kg per day was performed for 7 days.

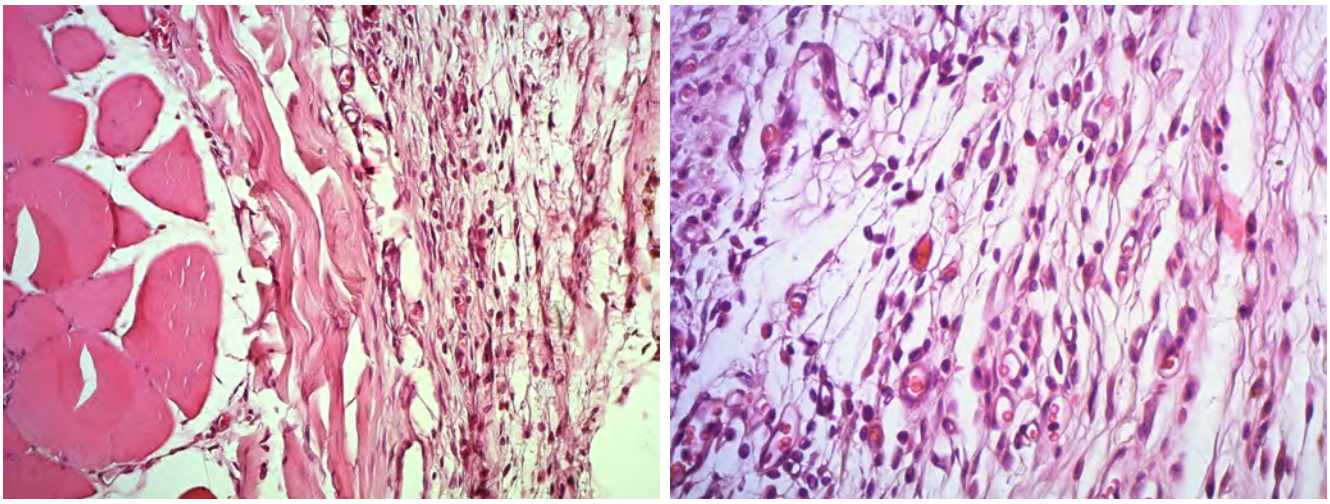
Animals were excluded from the experiment at days 14, 30, 60 and 90 after surgery by overdose of drugs used for anesthesia. After euthanasia, the abdomen and thorax of the animal were opened with a linear incision, and revision was performed. The implanted metal knitted mesh was dissected with the surrounding tissues, macroscopic structural features at the place of implant fixation to the tissues and at the areas of contact with the underlying organs were studied; the inflammatory process was evaluated. The obtained material was fixed in 10 % neutral formalin, embedded in paraffin, 5 μm thick sections were made and the preparations were stained with hematoxylin and eosin and Van-Gieson for connective tissue. Examination and microphotography were performed on an AxioLab.A1 microscope (Carl Zeiss Microscopy GmbH, Germany) with an AxioCamERc 5s video camera (Carl Zeiss Microscopy GmbH, Germany) and AxioVision Rel. 4.8 software (Carl Zeiss Microscopy GmbH, Germany). The features of tissue integration through the metal knitted mesh were evaluated by analytical scanning electron microscope. For this purpose, after preliminary fixation in 10 % neutral formalin solution, tissue fragments with implanted samples of the investigated materials were dissected. After freeze drying, the indicated samples were placed on conductive carbon tape in the electron scanning microscope chamber of Teskan Mira (Tescan Orsay Holding, Czech Republic).

RESULTS

The mean duration of surgery in Group A was 20.4 ± 3.2 min and in Group B was 21.2 ± 2.9 min. There were no statistically significant differences in terms of operation time in the groups. The animals were monitored throughout the experiment until the time of elimination. Decreased appetite and activity were clinically noted on the first day after surgery. On the day 2 after surgery, all animals had normalized motor performance and increased appetite. No intraoperative complications were noted; in both groups the surgical wound healed with primary tension, without signs of inflammation. During the growth of the animals at the standard weight gain after 30 days in four cases in Group B the occurrence of deformation at the place of implant fixation to the thoracic wall was noted; in one case the deformation area was located along the lateral edge of the abdominal wall. Among the animals of Group A no such changes were recorded.

Results of macroscopic examination. During macroscopic evaluation of the changes after the animals were eliminated from the experiment, it was noted that the deformation of the endoprosthetic zone recorded in the animals of Group B was due to hernia defects. The ligatures providing fixation of the implant to the cartilaginous parts of the ribs and in one case to the abdominal wall tissues were observed. A hernia sac containing omentum and loops of intestine was formed in the animals, in which one of the walls was represented by an implant. In addition, a significant adhesion between the walls of the sac and its contents drew attention. Among all animals in Group B, adhesions were recorded in 12 (60 %) cases and occurred after 14 days of experiment. The most frequent adhesions were located between the omentum and the implant fixation line, interintestinal adhesions were less frequent. The endoprosthetic area was characterized by stiffness, unevenness of the surface; there was a noticeable roll of scar tissue of various maturity levels along the implant fixation line, crawling along the edges on the inner surface of the mesh. In Group A, no hernia defects were detected in the endoprosthetic zone, adhesions were recorded in 3 (15 %) cases. Loose single adhesions were located between the omentum and the site of fixation of the mesh to the thoracic wall; no interintestinal adhesions were found. The endoprosthetic area was characterized by elasticity and was easy to deform. Starting from the day *14* of the experiment, the occurrence of a thin tissue film on the inner surface of the mesh was noted, evenly filling the pores of the mesh material, which hardly allowed to separate the implant from the tissue graft.

Results of histological examination. At the histological examination on the day *14* at the operation site in both groups there were still signs of acute inflammatory reaction manifested by infiltration with neutrophilic leukocytes, lymphocytes and macrophages; at that, in Group B the infiltration phenomena were more pronounced, the cells were concentrated not only around the mesh elements, but also diffusely. In Group A, the inflammatory infiltration was predominantly lympho- and macrophagal, and single neutrophils were found only near the mesh elements. A granulation tissue consisting of thin collagen fibers, small blood vessels and cellular elements, mainly fibroblasts, lymphocytes and macrophages, was formed between the mesh and the adjacent layers of the anterior abdominal wall muscles (Fig. 1). In both groups, edema was observed in the endomysium of the skeletal muscles in contact with the implants located in separate visual fields.



a ***b***

FIG. 1.

a *– granulation tissue around muscle fibers 14 days after implantation of the titanium nickelide metal knitted mesh.* ***b*** *– vessels and cellular elements (lymphocytes, macrophages, fibroblasts) of granulation tissue after implantation of titanium nickelide metal knitted mesh. Haematoxylin and eosin staining. Magnification ×400 (**a**), ×600 (**b**)*

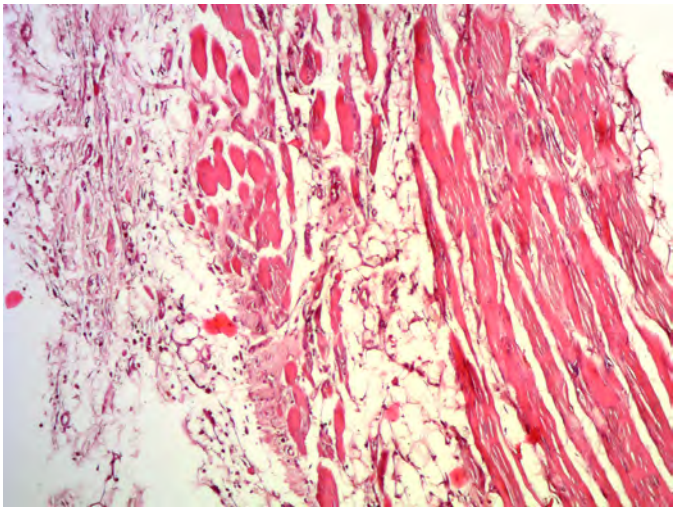


FIG. 2.

Tissue graft 30 days after implantation of the polypropylene mesh. Haematoxylin and eosin staining. Magnification ×400

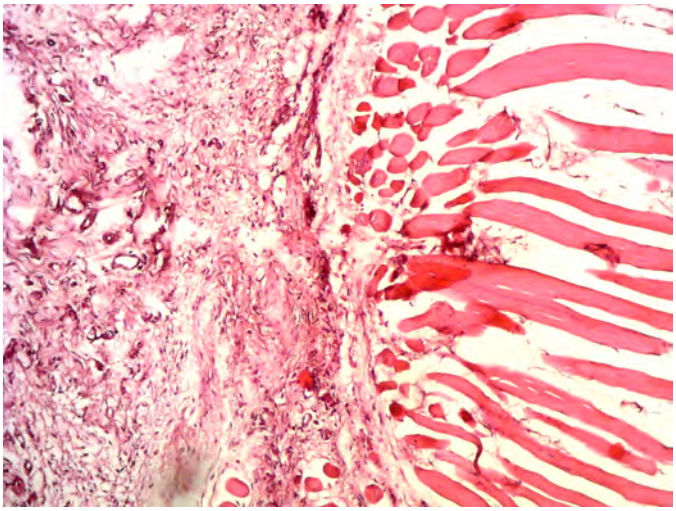


FIG. 3.

The tissue graft 30 days after implantation of titanium nickelide metal knitted mesh. Haematoxylin and eosin staining. Magnification ×400

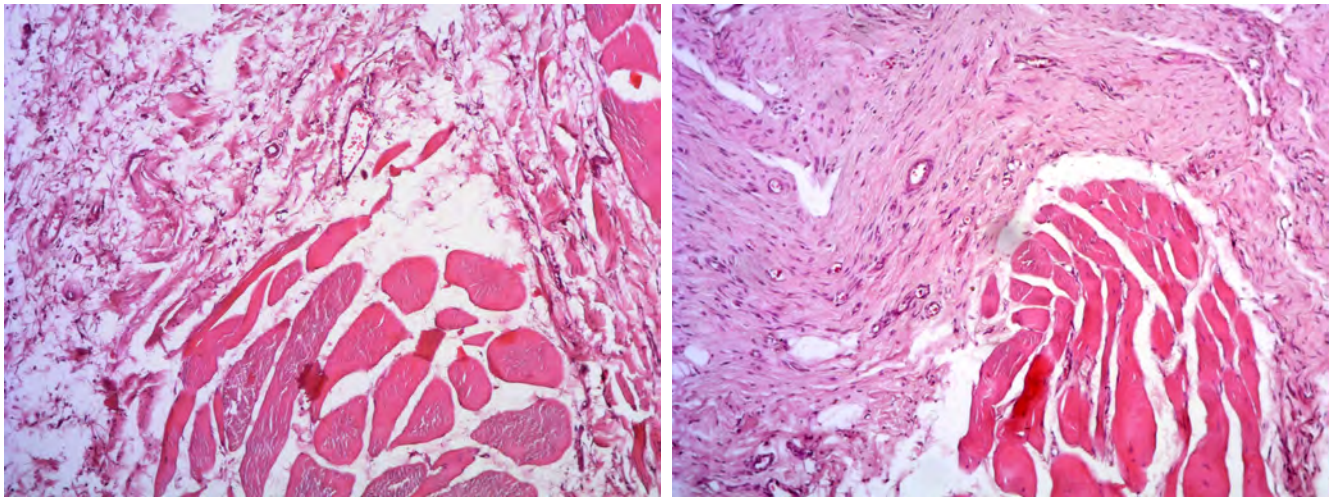
By the day 30 in both groups, the number of leukocytes in the infiltrate decreased and the number of fibroblasts increased, while the granulation tissue on the implant surface differed only in the degree of maturity. In Group B, areas of neutrophil aggregation persisted and collagen fibers were located along the mesh elements, the orientation was indistinct (Fig. 2).

In Group A, the number and thickness of collagen fibers increased, as a result of which they acquired a characteristic orientation along the TiNi strands and formed bundles, repeating the structure of the implant (Fig. 3). Growth of fibrocartilaginous tissue was observed along the edge of the resected cartilages.

On the day 60 in Group B there were still signs of edema and body response to foreign tissue, manifested by areas of lymphocyte and macrophage accumulation (Fig. 4a). In Group A, a graft of mature connective tissue formed around the implant (Fig. 4b).

In Group A, fibroblast growth was observed through the mesh knitted structure of the nickelide-titanium implant with tissue graft filling the thread pores (Fig. 5).

By the end of the study, a capsule of mature connective tissue was formed around the implants in both groups, which in Group B was characterized by the presence of areas of granulation tissue at the place of contact with the implant. In Group A the connective tissue capsule had a less pronounced thickness with the phenomena of filling with fibroblasts and collagen fibers of the implant mesh structure. The organ-specific differentiation of the tissues of the newly formed graft was observed along the implant fixation line.



a ***b***

FIG. 4.

a *– loose connective tissue between muscle fibers of the abdominal wall 60 days after the implantation of polypropylene mesh.* ***b*** *– mature connective tissue around the muscle fibers of the graft 60 days after the implantation of titanium nickelide metal knitted mesh. Haematoxylin and eosin staining. Magnification ×400*

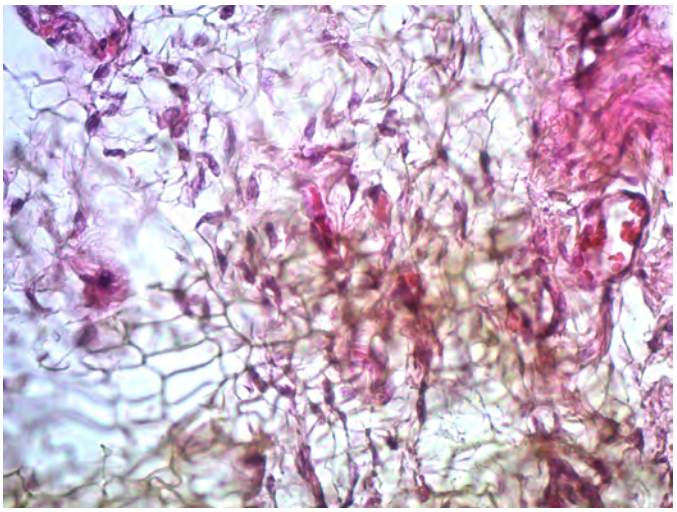


FIG. 5.

Fibroblasts filling the mesh structure of the nickelide-titanium implant on day 60 of the experiment. Haematoxylin and eosin staining. Magnification ×600

Mature connective tissue is visible between the muscle fibers. Muscle buds were observed at the site of muscle fiber incision as a manifestation of elements of skeletal muscle fiber regeneration.

Electron microscopy results. The study of preparations using electron microscopy in Group B indicated the absence of strong bonds between the connective tissue graft and the polypropylene mesh. The cells, as well as intercellular elements, were accommodated in the form of a dense roll around the perimeter of the implant with areas of crawling on the implant along the smooth filaments, ending with the intertwining of fibers to form a roll (Fig. 6). The integration of tissue elements through the mesh structure of the implant was not observed.

Group A showed a different microscopic pattern from day 14 onwards. The formation of connective tissue regenerate began at the areas of intersection and contact of TiNi-filaments as clusters of fibroblasts and bundles of collagen fibers forming plexuses of various types (Fig. 7). The surface of the tissue graft acquired a reticulated shape, repeating the contour of the implant. By the day 30 collagen fibers and fibroblasts in some areas completely filled the pores of the two-layer implant.

By the day 90 after surgery in Group A, the strength of the implant connection with the thoracic wall tissues increased significantly. At the level of the remaining ends of the resected cartilages, a roll was formed consisting of the formed fibrocartilaginous tissue, the embryo

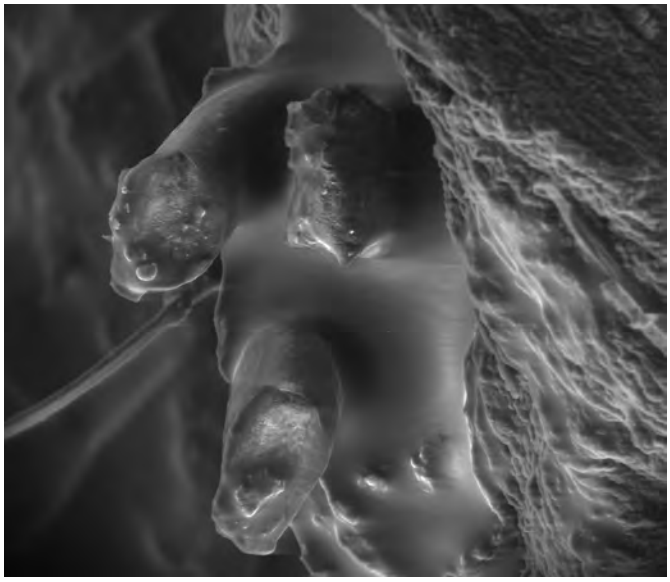


FIG. 6.
The formation of a cell roll along the polypropylene filaments in Group B on day 14. Scanning electron microscope. Magnification ×350

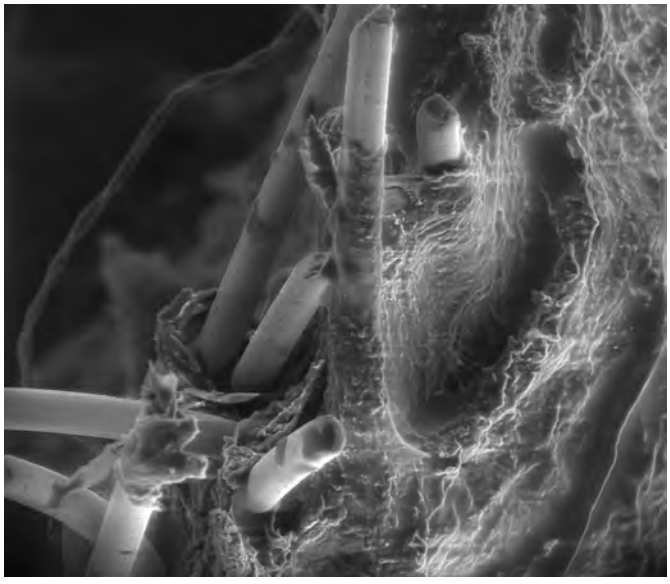


FIG. 7.
Formation of connective tissue graft in Group A on day 14. Scanning electron microscope. Magnification ×350

of which is the remaining supracartilage of the arcus costalis. It is observed that the cartilaginous tissue intimately adjoins and as if "crawls" as a part of the connective tissue graft on the implant surface, and the special form of fusion in this area due to the intertwining and sprouting of the directed connective tissue bundles through the mesh structure provides the stability and strength of the connection. In Group B no such effect was observed: fibrous cartilaginous tissue formed a regenerative roll and fixed the implant by means of a connective tissue bridge.

DISCUSSION

Choosing an endoprosthesis to replace complex body areas, especially those such as thoracoabdominal junctions, is challenging. For the replacement of small prolapses it is possible to use any type of endoprosthesis or enough own tissues, it is much more difficult to find an effective material for the elimination of extensive defects in which the edges are represented by different anatomical structures and tissues [15–17]. Advanced technologies in the development of medical-grade materials have increased the level of demands being imposed on the biomechanical properties of the material, namely the ability to withstand the varying load applied to the implant. Such a capability determines its functionality and affects immediate and long-term results. The difference in the stress-strain state between the implant and the body tissues causes complications manifested as hernia defects. In our study, no such complications were observed in Group A, which is determined by the extensibility of the tissue – implant complex. Such an effect, with its high tensile strength, plays an important role when the body area increases, as for example, when the patient gains excess weight or grows the body. However, despite all the physical and mechanical advantages, biocompatibility is the decisive factor when selecting a material as a bioprosthesis for defect replacement in humans.

The main task of any mesh implant is to become the basis for connective tissue graft, and in the absence of biocompatibility properties, its physical and mechanical qualities will not allow it to unfold. If the quantity and quality of collagen fibers depend on the biocompatibility properties during the formation of tissue graft, such parameters as the structure and thickness of the thread as well as the size of the weave mesh play a key role in the fixation of the implant and determine the risk of hernia defects. The results of our study convincingly prove that smooth surface architecture is not a favorable condition for the formation of a tissue graft united with the implant, preventing cell adhesion and reducing the overall biocompatibility. Such conditions cause the body to react to the implant as a foreign body, resulting in the formation of keloids, chronic pain syndrome and hernia defects. Similar behavior was demonstrated in the polypropylene mesh group in the form of long-lasting inflammation around the implant, stimulating the growth of excess connective tissue, isolating the implant in the body. At the same time, in Group A, the porous structure of the filament and the two-layer nature of the TiNi implant favored cell proliferation. This manifests itself as a cellular response to surface topography and is a primary feature of the formation of many tissues. In addition, the rough structure allows fibroblasts from the implant surface to be integrated into the bilayer structure, forming a single tissue graft that has elastic properties with an optimal safety margin.

CONCLUSION

Experimental administration of an endoprosthesis made of two-layer TiNi knitted mesh has shown promising results. In the group that used the polypropylene implant, more hernia defects were observed and adhesions were more frequently observed. In addition, the body response induced by the implanted polypropylene prosthesis was characterized by more pronounced fibrosis, and tissue integration through the mesh structure of the implant was not observed. The porous structure of the TiNi-wire and the biomechanical and biochemical properties of the two-layer knitted mesh provide optimal integration of the endoprosthesis in the body tissues and contribute to the formation of an elastic framework close to the natural one. two-layer TiNi knitted mesh in the replacement of complex structures of the thoracoabdominal area has shown promising preliminary results, which opens prospects for further clinical studies, including the use of methods of evaluative morphometry.

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Conflict of interest

The authors declare the absence of a conflict of interest.

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THE ROLE OF REACTIVE OXYGEN SPECIES AND REDOX-SENSITIVE PROTEIN KINASES IN THE INFARCT-LIMITING EFFECT OF OPIOID PEPTIDE DELTORPHIN II IN CARDIAC REPERFUSION IN RATS

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ABSTRACT

Background. *Mortality from acute myocardial infarction with ST-segment elevation in cardiac hospitals ranges from 4.5 to 7 %, and these data has not decreased in recent years. The most common cause of death in patients is cardiogenic shock, the likelihood of which directly depends on infarct size. It is quite clear that there is an urgent need to create drugs to limit the size of infarction and prevent the occurrence of cardiogenic shock.*

The aim. *To evaluate the role of reactive oxygen species and redox-sensitive protein kinases in the infarct-limiting effect of opioid peptide deltorphin II in cardiac reperfusion in rats.*

Materials and methods. *Coronary occlusion (45 min) and reperfusion (120 min) were performed in rats anesthetized with α-chloralose. The selective δ_2-opioid receptor agonist deltorphin II, a hydroxyl radical scavenger 2-mercaptoprpionyl glycine (2-MPG), a superoxide radical scavenger tempol, the protein kinase Cδ (PKCδ) inhibitor rottlerin, the PI3-kinase inhibitor wortmannin, the inhibitor of ERK1/2 kinase PD98059 were injected before of reperfusion of the heart.*

Results. *Deltorphin II contributed to a two-fold decrease in infarction size. Injection of 2-MPG, tempol, rottlerin, wortmannin, PD98059 alone had no effect on infarction size in rats. 2-MPG and tempol did not affect the infarction-reducing effect of deltorphin II. Rottlerin, wortmannin, and PD98059 eliminated the cardioprotective effect of deltorphin II.*

Conclusion. *The infarction-reducing effect of deltorphin II does not depend on the production of superoxide radical and hydroxyl radical. Superoxide radical and hydroxyl radical do not play a significant role in reperfusion injury of the heart after coronary occlusion (45 min). PKCδ, PI3-kinase, and ERK1/2 kinase are involved in the infarct-limiting effect of deltorphin II in myocardial reperfusion.*

Key words: *heart, ischemia, reperfusion, opioid receptors, reactive oxygen species, kinases*

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РОЛЬ АКТИВНЫХ ФОРМ КИСЛОРОДА И РЕДОКС-ЧУВСТВИТЕЛЬНЫХ ПРОТЕИНКИНАЗ В ИНФАРКТ-ЛИМИТИРУЮЩЕМ ЭФФЕКТЕ ОПИОИДНОГО ПЕПТИДА ДЕЛЬТОРФИНА II ПРИ РЕПЕРФУЗИИ СЕРДЦА У КРЫС

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РЕЗЮМЕ

Обоснование. *Смертность от острого инфаркта миокарда с подъёмом сегмента ST в кардиологических стационарах составляет от 4,5 до 7 %, и в последние годы этот показатель не снижается. Наиболее частой причиной гибели пациентов является кардиогенный шок, вероятность возникновения которого напрямую зависит от размера инфаркта. Вполне очевидно, что назрела настоятельная необходимость в создании препаратов, ограничивающих размер инфаркта и предотвращающих появление кардиогенного шока.*

Цель исследования. *Оценить роль активных форм кислорода и редокс-чувствительных протеинкиназ в инфаркт-лимитирующем эффекте опиоидного пептида дельторфина II при реперфузии сердца у крыс.*

Материалы и методы. *Коронарооккклюзию (45 мин) и реперфузию (120 мин) воспроизводили у крыс, наркотизированных α-хлоралозой. Перед реперфузией животным вводили: селективный агонист δ_2-опиоидных рецепторов дельторфин II, «ловушку» гидроксильных радикалов 2-меркаптопрпионил глицин (2-МПГ), «ловушку» супероксидных радикалов темпол, ингибитор протеинкиназы Cδ (ПКСδ) роттлерин, ингибитор PI3-киназы вортманнин, ингибитор ERK1/2 киназы PD98059.*

Результаты. *Дельторфин II способствовал двукратному уменьшению размера инфаркта. Инъекция крысам одного 2-МПГ, темпола, роттлерина, вортманнина, PD98059 не влияла на размер инфаркта. 2-МПГ и темпол не влияли на инфаркт-лимитирующий эффект дельторфина II. Роттлерин, вортманнин и PD98059 устраняли кардиопротекторный эффект дельторфина II.*

Заключение. *Инфаркт-лимитирующий эффект дельторфина II не зависит от продукции супероксидного радикала и гидроксильного радикала. Супероксидный радикал и гидроксильный радикал не играют существенной роли в реперфузионном повреждении сердца после коронарооккклюзии (45 мин). ПКСδ, PI3-киназа и ERK1/2 киназа вовлечены в инфаркт-лимитирующий эффект дельторфина II при реперфузии миокарда.*

Ключевые слова: *сердце, ишемия, реперфузия, опиоидные рецепторы, активные формы кислорода, киназы*

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INTRODUCTION

Mortality caused by acute myocardial infarction with ST-segment elevation in cardiological hospitals ranges from 4.5 % to 7 %, and in recent years this indicator has not decreased [1–3]. The most common cause of patient death is cardiogenic shock [4], the likelihood of which is directly related to the size of the infarct [5, 6]. Primary percutaneous coronary intervention provides almost 95 % recanalization of the infarct-related coronary artery [7], however, this still results in patients dying. One of the leading causes of death is reperfusion injury to the heart. It is quite obvious that there is an urgent need to develop drugs that can limit the size of the infarction and prevent the occurrence of cardiogenic shock.

According to our data, opioids have the ability to improve myocardial pumping function during cardiac reperfusion [8]. The opioid peptide deltorphin II is able to reduce the size of myocardial infarction when administered intravenously before reperfusion [9]. The infarct-limiting effect of this peptide has been found to be related to the activation of peripheral δ_2-opioid receptors (ORs) [9]. The intracellular signaling mechanism of the cardioprotective effect of deltorphin II has not yet been studied. It is generally accepted that mitochondrial K+channels (mitochondrial K^+channels (mitoK_{ATP}-channels) are the ultimate effector of the infarct-limiting effects of pre- and postconditioning [10, 11]. We have observed that the cardioprotective effect of deltorphin II is independent of the mitoK_{ATP} channel, but is mediated by activation of the sarcolemmal K_{ATP} channel (sarcK_{ATP} channel) [12]. In what way is the intracellular signal transmitted from δ_2-OR to the sarcK_{ATP} channel? We hypothesized that the role of intracellular messengers providing increased cardiac tolerance to reperfusion may be claimed by reactive oxygen intermediates (ROIs). What was this hypothesis based on?

In the 80s of the twentieth century, the prevailing assumption was that free radicals and ROIs play an exclusively negative role in cardiac reperfusion injury [13, 14]. Now a similar perspective is being held by some of the researchers [15–17]. The findings that free radical production in the myocardium increases manifold at the time of reperfusion compared to the preceding ischemic period were in favor of this view [18–20]. This perspective was supported by evidence of the infarct-limiting effect of antioxidants [21–23]. However, in these studies, antioxidants were generally used at high dosages that allow them to interact not only with free radicals but also with other molecules. As an example, the hydroxyl radical (•OH) "scavenger" 2-mercaptopropyl-glycine (2-MPG; 20 mg/kg) [24, 25] at a dose of 70 mg/kg injected intravenously can interact with peroxynitrite [26], and at a dose of 100 mg/kg limits the size of myocardial infarction by 2-MPG [27]. Consequently, the cardioprotective effect of 2-MPG may not be dependent on a decrease in •OH levels in myocardial tissue.

In the 90s of the XX century, the perception that free radicals and ROIs can not only be damaging but also protect the myocardium from the pathogenic effects of ischaemia and reperfusion was gradually formed. 2-MPG was found to eliminate the infarct-limiting effect of ischaemic preconditioning [11]. We have obtained evidence that ROIs play a key role in the cardioprotective effect of hypoxic preconditioning [28]. Free radicals and ROIs play the role of signaling molecules that activate redox-sensitive enzymes, primarily kinases [29]. For example, ROIs increase the activity of: protein kinase C (PKCδ and PKCε), PI3-kinase, and ERK1/2-kinase [29]. These kinases provide cardiac resistance to ischaemia and reperfusion [10]. There are reasons to believe that the role of such activators of kinases is claimed by: •OH, superoxide radical ($O_{2}^{-\bullet}$) and hydrogen peroxide [29].

THE AIM OF THE STUDY

To evaluate the role of reactive oxygen intermediates (ROIs) and redox-sensitive protein kinases in the infarct-limiting effect of the opioid peptide deltorphine II during cardiac reperfusion in rats.

MATERIALS AND METHODS

The study was performed on 144 male Wistar rats weighing 250–300 g. All procedures related to the housing and use of animals were undertaken in compliance with the European Parliament and Council of the European Union directives (2010/63/EU) governing the use of animals for scientific purposes. The study was approved by the Ethical Committee of the Cardiology Research Institute – branch of the Tomsk National Research Medical Center of the Russian Academy of Sciences (protocol No. 207 of 23.12.2020).

The animals were anesthetized with intraperitoneal administration of α-chloralose (60 mg/kg, intraperitoneal; Sigma) and connected to a SAR-830 Series artificial lung ventilation apparatus (CWE Inc., USA). Coronary occlusion (45 min) and reperfusion (120 min) were performed according to the method of J.E. Schultz et al. [30]. This procedure was performed by thoracotomy at the level of 2–3rd ribs and a ligature was applied to the left coronary artery a few millimeters below its exit from the aorta. After 45 minutes of ischemia, the ligature was removed to resume coronary blood flow. BP was recorded using a SS13L pressure-sensing means (Biopac System Inc., Goleta, USA) paired with an MP35 electrophysiological study device (Biopac System Inc., Goleta, USA). Blood pressure was measured by cannulation of the right carotid artery using a SS13L pressure-sensing means (Biopac System Inc., Goleta, USA) paired with an MP35 electrophysiological study device (Biopac System Inc., Goleta, USA) and a personal computer. This device was also used to register an electrocardiogram. After reperfusion, the heart was then removed from the thorax (chest) and flushed retrogradely through the aorta with physiological saline. To determine the area at risk (AAR), the ligature was retightened and the myocardium was stained by jet staining through the aorta with 5 % potassium permanganate. My-

ocardium subjected to ischemia-reperfusion is commonly referred to as the AAR. After being washed with physiological saline, 1 mm thick heart slices were made perpendicular to the longitudinal axis using an HSRA001-1 slicer (Zivic Instruments, USA). Visualisation of the necrosis zone from the area at risk was performed by staining with 1 % solution of 2,3,5-triphenyl tetrazolium chloride over a period of 30 minutes at 37 °C. The method is based on the ability of 2,3,5-triphenyl tetrazolium chloride to acquire a persistent colour when changing from the oxidized state to the reduced state under the action of dehydrogenases. Since no dehydrogenases were observed in the dead cardiomyocytes, the necrotic myocardium was not stained. After staining was completed, the slices were placed in 10% formaldehyde solution for 1 day. Slices were scanned on both sides using an HP Scanjet G4050 scanner. The size of the AAR and infarct size (IS) were determined by computerised planimetric method. The size of the infarct size was expressed as a percentage of the size of the hypoperfusion zone (area at risk) as the IS/AAR ratio.

Blockers were administered intravenously 10 min before reperfusion, and deltorphin II was administered 5 min before reperfusion. Each experimental group consisted of 12 specimens. Animals injected with physiological solution were included in the control group.

The following pharmacological agents were used in the experiment: δ_2-OR selective agonist deltorphin II – at a dose of 0.12 mg/kg [9]; hydroxyl radical "scavenger" 2-MPG – at a dose of 20 mg/kg [24]; superoxide radical "scavenger" tempol – at a dose of 30 mg/kg [31]; protein kinase Cδ (PKCδ) inhibitor rottlerin – at a dose of 0.3 mg/kg [32]; PI3-ki-

TABLE 1

HEART RATE (BEATS/MIN) AND SYSTOLIC BLOOD PRESSURE (MMHG) IN RATS WITH CORONARY OCCLUSION (45 MIN) AND REPERFUSION (120 MIN), Me [25%; 75%]

Group	Before ischemia	Before reperfusion	After 30 minutes of reperfusion	After 2 hours of reperfusion
		Heart rate		
Monitoring	367 [363; 371]	360 [358; 369]	354 [347; 360]	346 [340; 351]
Deltorphine II	364 [358; 369]	358 [353; 364]	352 [348; 355]	343 [338; 348]
2-MPG	361 [358; 366]	357 [352; 361]	353 [349; 358]	342 [337; 346]
Tempol	356 [351; 362]	351 [347; 355]	347 [344; 352]	339 [334; 343]
Rottlerin	370 [364; 374]	365 [360; 369]	358 [352; 363]	350 [343; 356]
Vortmannin	360 [356; 365]	354 [349; 360]	350 [345; 354]	340 [334; 345]
PD98059	363 [359; 368]	356 [352; 359]	352 [346; 358]	345 [341; 351]
		Systolic blood pressure		
Monitoring	124 [121; 127]	121 [117; 125]	118 [113; 121]	114 [109; 118]
Deltorphine II	121 [117; 125]	120 [118; 122]	116 [111; 119]	112 [107; 116]
2-MPG	125 [122; 129]	122 [119; 126]	119 [114; 123]	115 [111; 119]
Tempol	120 [116; 124]	116 [113; 121]	113 [110; 117]	107 [105; 112]
Rottlerin	125 [123; 129]	122 [119; 124]	117 [113; 120]	111 [108; 115]
Vortmannin	126 [122; 130]	121 [119; 126]	117 [114; 122]	113 [110; 117]
PD98059	128 [124; 132]	124 [120; 128]	120 [116; 125]	114 [109; 118]

nase inhibitor wortmannin – at a dose of 0.025 mg/kg [33]; ERK1/2 kinase inhibitor PD98059 – at a dose of 0.5 mg/kg [34].

Deltorphin II, 2-MPG, and tempol were dissolved in 0.9% NaCl, and the other inhibitors were dissolved in a mixture of DMSO/20% β-hydroxypropyl-cyclodextrin (1:9). As our preliminary experiments have demonstrated, a similar mixture that was infused at a dose of 1 ml/kg had no effect on infarction size.

Deltorphin II has been purchased from PolyPeptide Laboratories (USA), 2-MPG and rottlerin from Sigma-Aldrich (USA), tempol from Tocris (UK), wortmannin and PD98059 from LCLabs Company (USA).

Statistical data processing was performed with the use of "Statistica 13.0" software packages (StatSoft Inc., USA). The obtained data were verified for normality of distribution using the Shapiro-Wilk criterion; distributions that differed from normal were analyzed using the nonparametric Mann – Whitney criterion. Differences were considered statistically significant at $p < 0.05$. The results of all experiments are presented in the form of median and quartiles (Me [25 %; 75 %]).

RESULTS AND DISCUSSION

We have revealed that coronary occlusion and reperfusion as well as the selective δ_2-OR peptide agonist deltorphine II do not affect hemodynamic parameters (Table 1), which corresponds to our published data [9].

Rottlerin, wortmannin, PD98059, tempol, and 2-MPG also had no effect on hemodynamic parameters among rats with coronary occlusion and reperfusion (Table 1). The δ_2-OR agonist deltorphine II caused a two-fold reduction in the infarct size (Fig. 1).

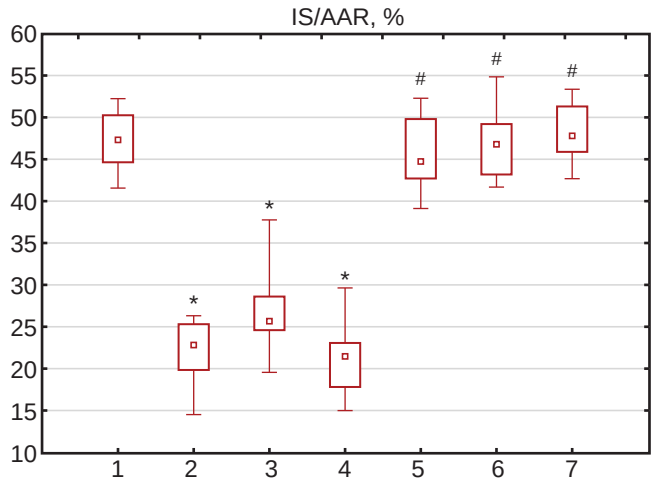

FIG. 1.

*The role of reactive oxygen species, the protein kinase Cδ, the PI3-kinase, and the ERK1/2 kinase in the mechanism of the cardioprotective effect of deltorphin II (Me [25%; 75%]). Groups: 1 – control; 2 – deltorphin II; 3 – deltorphin II + 2-MPG; 4 – deltorphin II + tempol; 5 – deltorphin II + rottlerin; 6 – deltorphin II + wortmannin; 7 – deltorphin II + PD098059. * – $p < 0.05$ vs control; # – $p < 0.05$ vs deltorphin II*

Injection of the PKCδ inhibitor rottlerin alone, the PI3-kinase inhibitor, or the ERK1/2-kinase inhibitor PD98059 had no effect on infarct size (Fig. 2).

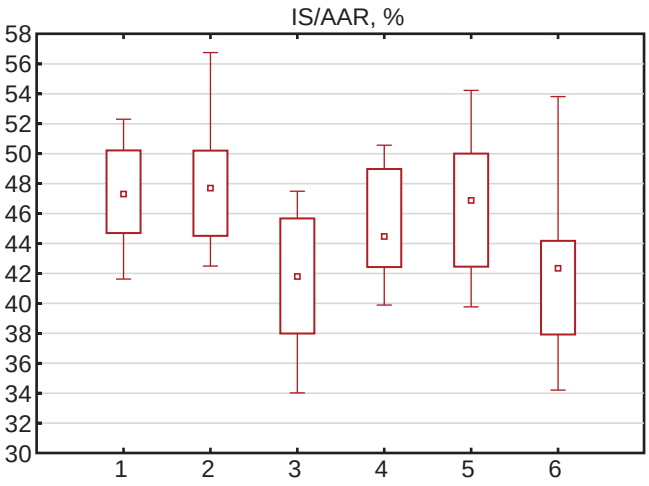

FIG. 2.

The effect of reactive oxygen species, inhibitors the protein kinase Cδ, the PI3-kinase, and the ERK1/2 kinase on infarct size as percentage of the area at risk after a 45-min ischemia and a 120-min reperfusion (Me [25%; 75%]). Groups: 1 – control; 2 – 2-MPG; 3 – tempol; 4 – rottlerin; 5 – wortmannin; 6 – PD098059

Consequently, these kinases are not involved in the formation of myocardial infarction in rats. An administration of the "scavenger" $^{\bullet}OH$ 2-mercaptopropionyl glycine or injection of the "scavenger" $O_{2}^{-\bullet}$ tempol also did not affect necrosis focus formation during cardiac reperfusion (Fig. 2). These data indicate that $^{\bullet}OH$ and $O_{2}^{-\bullet}$ are not involved in the pathogenesis of cardiac reperfusion injury.

We hypothesized that $^{\bullet}OH$ and $O_{2}^{-\bullet}$ do not damage the heart, but may increase cardiac resistance through activation of one of the isoforms of protein kinase C. PI3-kinase and ERK1/2-kinase may be involved in the cardioprotective effect of δ_2-OR agonist. Actually, we have previously observed that the infarct-limiting effect of deltorphin II is associated with the activation of protein kinases from group C; the inhibitor of all PKC isoforms chelerythrine eliminated the cardioprotective effect of the named peptide [12]. PKCs are known to be activated by ROIs [29], so it was reasonable to assume that ROIs are involved in the infarct-limiting effect of deltorphine II. However, it turned out that the "scavenger" $^{\bullet}OH$ 2-mercaptopropionyl glycine or the "scavenger" $O_{2}^{-\bullet}$ tempol did not affect the deltorphin-induced increase in cardiac reperfusion tolerance (Fig. 1). Consequently, $O_{2}^{-\bullet}$ and $^{\bullet}OH$ are not involved in the signaling mechanism of the protective effect of deltorphin II. It is possible that the activator of PKC and other redox-sensitive kinases is hydrogen peroxide, which is involved in intracellular and intercellular signaling [29].

Protein kinase C, PI3-kinase and ERK1/2-kinase are involved in the infarct-limiting effect of ischemic pre- and post-conditioning [10, 11]. These findings led us to sug-

gest that the above kinases are involved in the cardioprotective effect of deltorphin II. Indeed, the selective PKCδ inhibitor rottlerin was found to completely abolish the infarct-limiting effect of the named peptide (Fig. 1). After inhibition of PI3-kinase by wortmannin, we were unable to observe an infarct-limiting effect of deltorphine II (Fig. 1). After the blockade of ERK1/2 kinase with PD98059, we did not record the cardioprotective effect of the δ_2-OR agonist (Fig. 1). The presented data are consistent with the widespread viewpoint about the important role of protein kinase C, PI3-kinase and ERK1/2-kinase in ensuring the tolerance of the heart to the effects of ischemia and reperfusion [10, 11].

CONCLUSION

The presented data evidence that $O_{2}^{-\bullet}$ and $^{\bullet}OH$ are not involved in the pathogenesis of cardiac reperfusion injury after 45-minute coronary occlusion. These free radicals are not intracellular messengers mediating the cardioprotective effect of deltorphin II. PKCδ, PI3-kinase, and ERK1/2-kinase appear to play an important role in the formation of deltorphine-induced increase in cardiac tolerance to the pathogenic effects of reperfusion. Activation of the above kinases by deltorphin II occurs without the involvement of $O_{2}^{-\bullet}$ and $^{\bullet}OH$.

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Conflict of interest

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

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EPIDEMIOLOGY

EPIDEMIOLOGICAL MANIFESTATIONS OF TUBERCULOSIS INFECTION IN THE OMSK REGION: DYNAMICS AND TRENDS

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ABSTRACT

Background. *Tuberculosis (TB) infection remains relevant as one of the leading public health problems in Russia.*
The aim. *To characterize the dynamics and trends of epidemiological manifestations of TB infection in the Omsk region.*
Materials and methods. *An observational descriptive-evaluative epidemiological study was carried out in the Omsk region from 2009 to 2021. The data available in the Federal statistical observation forms NN 7, 8, and 33, and results of the bacteriological study of patients with respiratory TB were analyzed.*
Results. *In the Omsk region, an improvement in the epidemiological situation was observed from 2009 to 2021. It was associated with a decrease in TB prevalence by 3.7 times (from 325.6 to 86.7), mortality – by 5.8 times (from 21.1 to 3.6), incidence – by 2.7 times (from 130.7 to 48.4 per 100,000). At the same time, proportion of microscopy/culture-positive patients infected with multidrug-resistant Mycobacterium tuberculosis strains increased from 9.2 to 29.8 % among all patients, and from 15.5 to 30.6 % among newly diagnosed patients. There was a trend towards an increase in the number of cases with primary extensive drug resistance of M. tuberculosis. The incidence of tuberculosis associated with HIV infection has increased 10 times and reached 15.6 per 100,000 population.*
Conclusions. *In the Omsk region, there is a change in the structure of M. tuberculosis strains with a predominance of multiple and extensive drug resistance along with decrease in TB incidence and mortality. New approaches are needed to organize the system of epidemiological surveillance and control of TB infection.*

Key words: *tuberculosis, incidence, prevalence, drug resistance, M. tuberculosis, HIV infection*

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ЭПИДЕМИОЛОГИЧЕСКИЕ ПРОЯВЛЕНИЯ ТУБЕРКУЛЁЗНОЙ ИНФЕКЦИИ В ОМСКОЙ ОБЛАСТИ: ДИНАМИКА И ТЕНДЕНЦИИ

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РЕЗЮМЕ

Обоснование. *Туберкулёзная инфекция сохраняет свою актуальность как одна из ведущих проблем общественного здравоохранения России.*
Цель исследования: *характеристика динамики и тенденций эпидемиологических проявлений туберкулёзной инфекции на территории Омской области.*
Методы. *Проведено наблюдательное описательно-оценочное эпидемиологическое исследование на территории Омской области за период с 2009 по 2021 г. Исследованы данные статистических отчётных форм № 7, № 8 и № 33, результаты бактериограмм больных туберкулёзом.*
Результаты. *Наблюдалось улучшение эпидемиологической ситуации, связанное с сокращением распространённости туберкулёзной инфекции в 3,7 раза (с 325,6 в 2009 г до 86,7 в 2021 г.), смертности от туберкулёза – в 5,8 раза (с 21,1 до 3,6 на 100 тыс. населения), заболеваемости – в 2,7 раза (с 130,7 до 48,4 случая на 100 тыс. населения). Вместе с тем отмечено изменение структуры случаев инфекции – в контингенте больных туберкулёзом доля бактериовыделителей штаммов Mycobacterium tuberculosis с множественной лекарственной устойчивостью (МЛУ) возросла более чем в 3 раза (с 9,2 до 29,8 %), доля впервые выявленных бактериовыделителей штаммов с МЛУ возросла с 15,5 до 30,6 %. Отмечена тенденция к увеличению количества случаев с первичной широкой лекарственной устойчивостью M. tuberculosis. Заболеваемость туберкулёзом, сочетанным с ВИЧ-инфекцией, возросла в 10 раз (до 15,6 случая на 100 тыс. населения).*
Заключение. *В Омской области наблюдается изменение структуры бактериовыделения с преобладанием множественной и широкой лекарственной устойчивости на фоне тенденции к снижению заболеваемости и смертности населения от туберкулёза. Требуется совершенствование организации системы эпидемиологического надзора и контроля за туберкулёзной инфекцией.*

Ключевые слова: *туберкулёз, заболеваемость, распространённость, лекарственная устойчивость, M.tuberculosis, ВИЧ-инфекция*

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OBJECTIVES

Tuberculosis (TB) infection remains relevant as one of the leading public health problems. Worldwide, about 10 million new cases of tuberculosis have been reported annually in recent years, which were 127 (114–140) cases per 100,000 population in 2020 [1].

The World Health Organization has set a global goal of eliminating tuberculosis by 2035 [2], and efforts to improve the system of tuberculosis care and increase the coverage of the population with measures intended for preventing care and avoiding the spread of tuberculosis have allowed the world community to save an estimated 63 million lives by 2020 [3].

The estimated number of tuberculosis cases in the European Region of the World Health Organization has been consistently decreasing since 2000. The average annual decrease in TB incidence was 5.2 % in the period between 2011 and 2020 and 6.4 % in 2019–2020, which is much higher than the global rate of decrease in TB incidence (1.9 %) and is an example of the fastest rate of decrease worldwide compared to other regions [4].

The epidemiological situation in the Russian Federation has improved significantly, with a significant decrease in the incidence of tuberculosis among the population, which characterises the epidemic situation. However, the increasing number of patients with multidrug-resistant and extensively drug-resistant tuberculosis (MDR/XDR) and HIV infection has an extremely negative impact [5].

Molecular epidemiological studies conducted in Russia during the past 20 years emphasize the special role of the dominant strains of *Mycobacterium tuberculosis* of the Beijing genetic family, which is characterized by a generally strong association with multidrug resistance [6, 7]. The results of a study conducted in the Omsk region made it possible to characterize the genetic diversity of *M. tuberculosis* strains circulating in the region and also to establish the leading role of the Beijing genetic family (65.6 %) [8]. In the population structure of the Beijing *M. tuberculosis* genetic family in the Omsk region, strains of modern sublineage (86.3 %), in particular clusters 94–32 (60.7 %) and B0/W148 (25.0 %) prevailed [7]. Cluster 94–32 strains were characterized by a high level of multidrug resistance (53.8 %). The proportion of ancient sublineage strains was higher than in other regions of Russia and amounted to 13.7 %. Particular clinical relevance has been shown for ancient sublineage strains of genotype Beijing, 52.5 % of which were characterized by pre-extensive and extensive drug resistance [7]. Among the dominant strains of modern Beijing sublineage, the B0/W148 and 94-32 clusters can be observed, in which 33 and 12.4 % of the strains had pre-extensive and extensive drug resistance [8].

Considering the relevance of the co-epidemic processes of tuberculosis and HIV infection, criteria were previously proposed to assess the impact of HIV infection on the development of the tuberculosis epidemic process. Simultaneous development of epidemic processes of tuberculosis and HIV infection, high activity of epidemic processes of both infections with involvement of the general population, as well as positive statistically significant correlations of incidence and prevalence rates indicate the integration of the epidemic processes of HIV infection and tuberculosis and require additional assessment of the quality of preventive and diagnostic measures [9]. Among TB patients combined with HIV infection, the primary MDR rate reached 31.3 % in Russian regions with high HIV prevalence [10, 11]. Among the deceased patients with comorbidities, multidrug resistance represented 40 %, and the efficacy of treatment of such patients was low and did not exceed 7.3 % of patients [10].

The aim of this study aimed to describe the dynamics and trends of epidemiological manifestations of TB infection in the territory of the Omsk region over a multi-year period (2009–2021).

MATERIALS AND METHODS

The study is based on the observation of the epidemic process of TB infection in the Omsk region. Retrospective epidemiological analysis of incidence of tuberculosis among the population has been carried out using data from statistical reporting forms No. 7-TB "Information about newly diagnosed patients and relapses of tuberculosis disease", No. 8 "Information about incidence of active tuberculosis", No. 33 "Information about tuberculosis patients» over the period 2009–2021. Disease incidence and disease prevalence, as well as extensive indicators characterizing the structure of incidence have been evaluated.

Trends in the development of the epidemic process were determined by straight-line equalization of the dynamic series of indicators using the least squares method and calculation of the coefficient of determination (R^2).

Bacteriological examination of patients, determination of the sensitivity spectrum of isolated *Mycobacterium tuberculosis* strains to the anti-tuberculosis drugs was carried out in accordance with the standard procedure of microbiological examination [12].

To compare demographic and clinical and epidemiological characteristics of two cohorts of patients newly diagnosed in 2009 (n = 2638) and in 2021 (n = 922), the PR prevalence ratio and its confidence intervals (CI) were calculated. Among the factors evaluated were gender, age, place of residence, clinical form of the disease, HIV status, massiveness of bacteriuria, and drug resistance pattern observed in patients discharging bacteria.

Data analysis was performed using Microsoft Office 2010 application (Microsoft Corp., USA).

RESULTS

The dynamics of TB infection prevalence in the Omsk region was characterized by a pronounced trend towards a decrease in the number of cases. The TB prevalence rate decreased by 3.7 times (from 325.6 in 2009 to 86.7 in 2021), and the patient cohort under follow-up medical care included 1,651 patients at the beginning of 2022 (Fig. 1). It should be noted that in the last two years the number of TB patients has not significantly changed, in 2020 the prevalence rate decreased by 24.3 % as compared to 2019 and amounted to 84.3 per 100 thousand population, and in 2021 it increased by 2.8 %.

The multi-year dynamics of tuberculosis incidence in population have had a similar trend, in 2021 the lowest incidence rate was observed (48.4 per 100,000 population), which is 2.7 times lower than in 2009 (2,638 cases), and 1.7 times lower than the average annual rate (83.9 cases per 100,000 population).

The mortality rate of the population caused by tuberculosis decreased 5.8 times, from 21.1 cases per 100,000 population ($n = 289$) to 3.6 ($n = 68$) cases per 100,000 population.

The incidence of bacteriologically proven tuberculosis decreased less intensively; thus, during the analysed period, the incidence of bacillary forms of tuberculosis decreased from 47.4 to 26.3 cases per 100,000 popu-

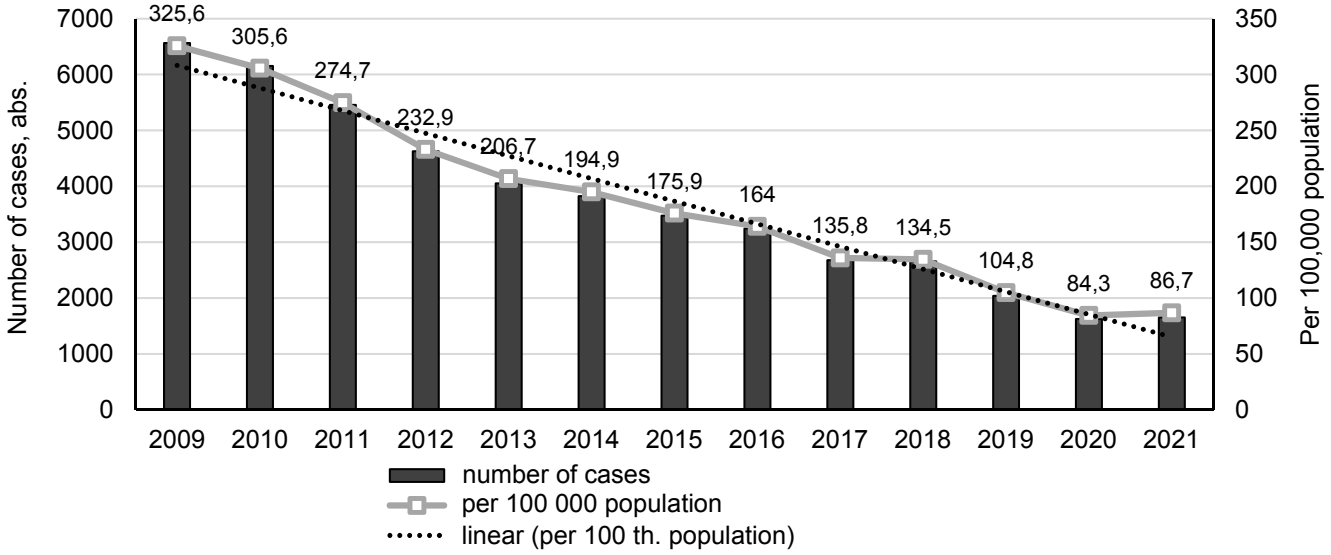

FIG. 1.
Dynamics of the spread of tuberculosis infection in the Omsk region (per 100,000 population, 2009–2021)

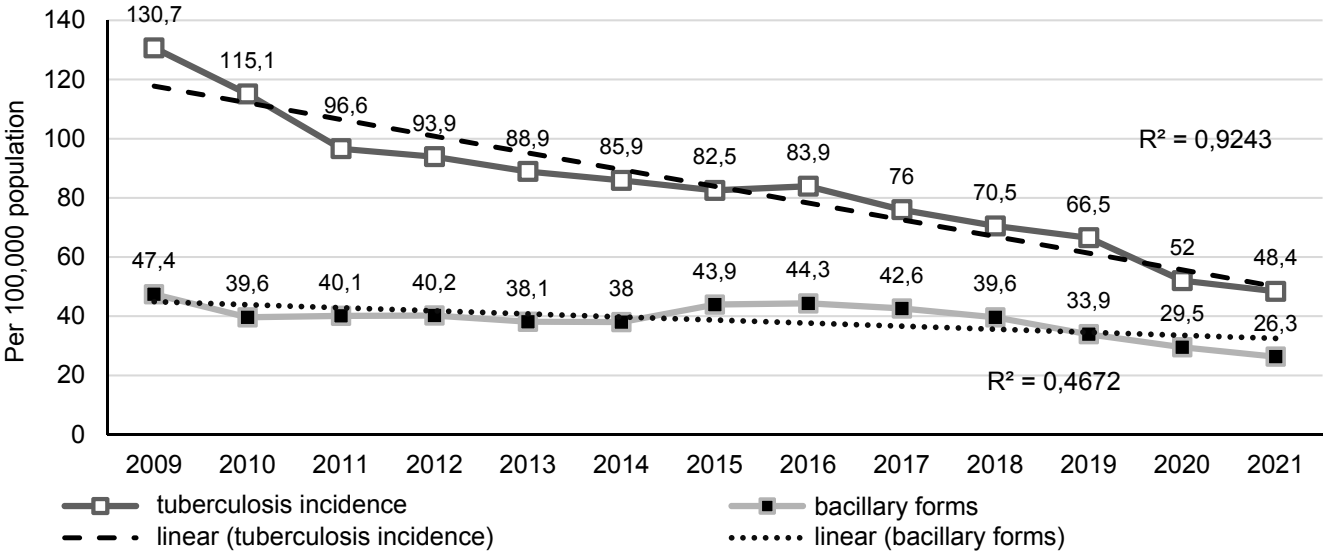

FIG. 2.
Dynamics of the incidence of tuberculosis in the population of the Omsk region (2009–2021)

lation, while the average annual rate was 38.7 cases per 100,000 population (Fig. 2).

The epidemic process of tuberculosis in the Omsk region was characterized by a number of qualitative changes. Thus, since 2009, the proportion of MDR bacterial excretion in the structure of bacteriological excretion among newly diagnosed TB cases has doubled from 13.1 % to 26.4 % (Fig. 3). Between 2016 and 2020, the proportion of MDR cases among newly diagnosed TB patients discharging bacteria averaged 30.5 %.

During the analyzed period, the average annual incidence of MDR-TB was 8.2 diagnosed in the population was 8.2 cases per 100,000 population. In the period between 2009 and 2014, the dynamics of MDR-TB incidence was stable (annual average – 7.2 cases per 100,000 population); in 2015–2016, the incidence increased to an average of 10.5 cases per 100,000 population. Incidence has been declining since 2017, with a yearly average of 8.6 cases per 100,000 population, reaching a rate of 6.5 TB cases per 100,000 population in 2021.

In the Omsk region, the prevalence of MDR-TB tended to decrease (Fig. 4). At the same time, the proportion of MDR strains of *M. tuberculosis* among patients with active tuberculosis under follow-up medical care more than tripled from 9.2 % (606/6565) to 29.8 % (478/1651), although at the end of 2021, only 66.2 % (478/721) of bacterial excretors under follow-up care were isolating MDR strains, twice as many as in 2009 (32.6 %).

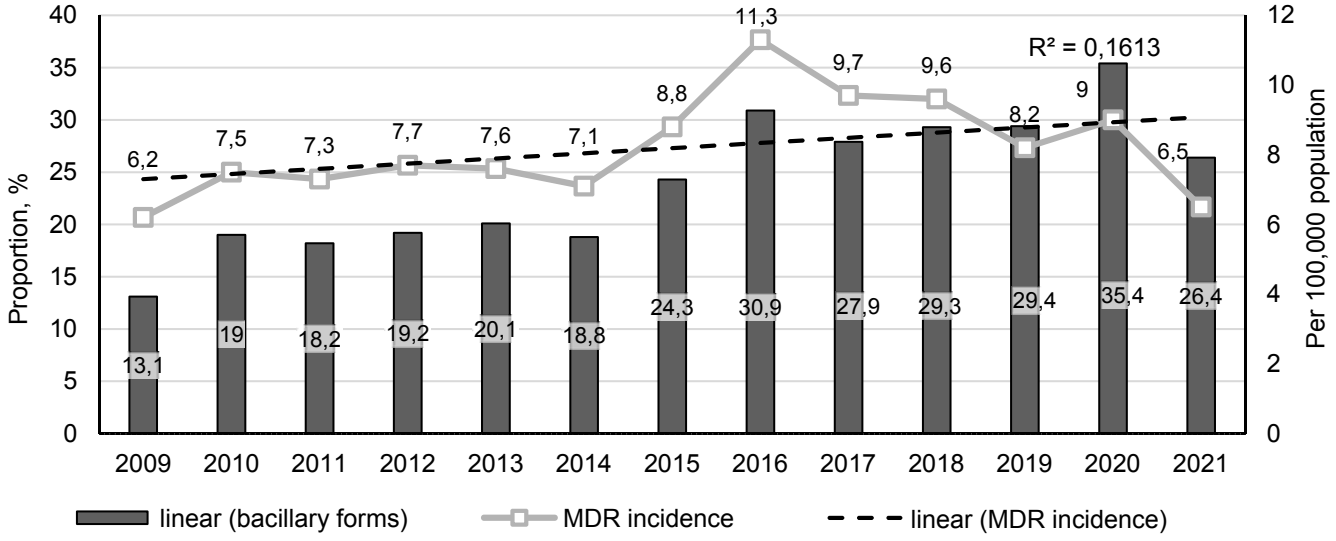

FIG. 3.

Dynamics of the incidence of multidrug-resistant tuberculosis in the population of the Omsk region (per 100,000 population)

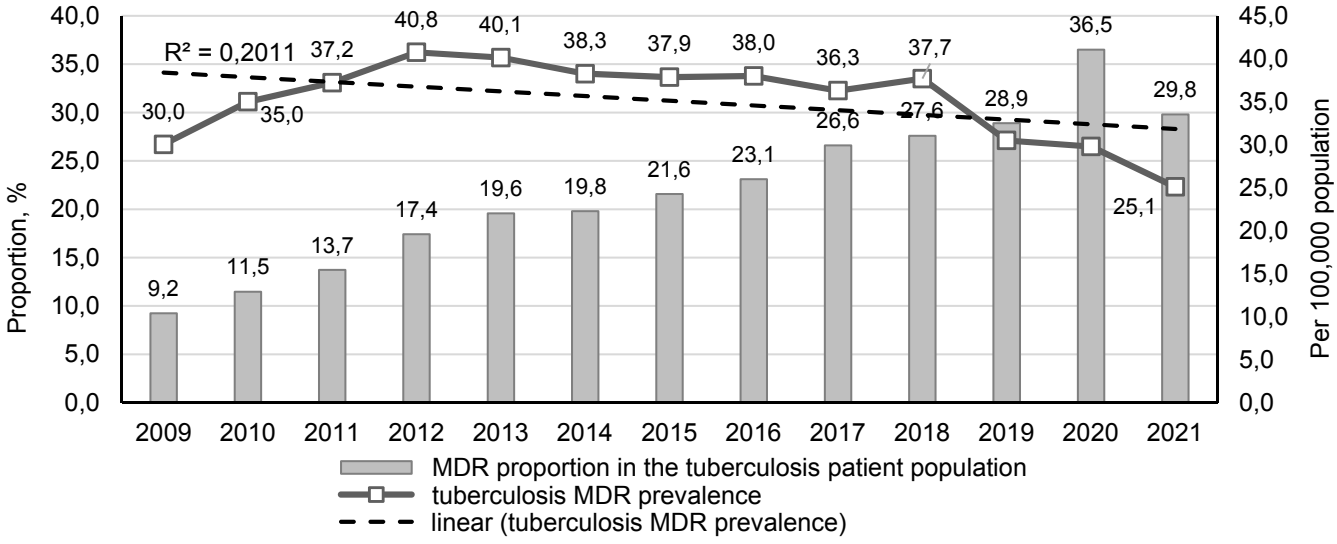

FIG. 4.

Dynamics of the prevalence of multidrug-resistant tuberculosis in the Omsk region (2009–2021)

Changes in the incidence pattern of TB infection were evaluated by comparing two patient cohorts who were first diagnosed with active TB in 2009 and 2021, i. e. at the beginning and end of the study period.

The evaluation revealed statistically significant differences in the groups of newly diagnosed patients. By the end of the study period, there was a statistically significant increase in the proportion of males among TB cases, rising from 67.1 % in 2009 to 71.9 % in 2021 (PR = 0.79; 95% CI: 0.67-0.94).

The age structure of TB patients has changed, the proportion of young population decreased: adolescents from 1.9 % to 1.0 % (PR = 0.56; 95% CI: 0.27–1.16), 18–24-year age group individuals from 28.6 % to 16.8 % (PR = 0.11; 95% CI: 0.07–0.18), 25–34-year age group from 15.6 % to 2.0 % (PR = 0.5; 95% CI: 0.41–0.61). However, there was a 2-fold increase in the proportion of the 35–44 years age group, from 17.9 % to 35.1 % (PR = 2.48; 95% CI: 2.1–2.9).

Newly diagnosed patients were represented predominantly by urban residents, with a statistically significant increase in their proportion over the period under study, from 68.5 % to 74.2 % (684/922) (PR = 0.46; 95% CI: 0.39–0.54).

Clinical forms of tuberculosis were characterized by an increase in the proportion of respiratory tuberculosis increasing from 97.6 % to 99.1 % (PR = 0.37; 95% CI: 0.17–0.77), with a decrease in the proportion of tuberculosis of extrapulmonary localizations to 0.9 % in 2021 from 2.4 % in 2009. The proportion of destructive forms of TB decreased from 42.2 % (840/1989) to 37.8 % (288/761) (PR = 1.2; 95% CI: 1.01–1.42). The proportion of fibrous cavernous tuberculosis in the structure of respiratory tuberculosis decreased by a factor of 8.6 to 0.5 % (4/761) compared to 4.3 % in 2009 (PR = 0.12; 95% CI: 0.04–0.35).

It should be noted that by the end of the follow-up period, the proportion of HIV-infected patients in the group of newly diagnosed patients significantly increased, which largely determined the clinical forms of tuberculosis and the characteristics of bacterial excretion. Among TB patients who underwent screening for human immunodeficiency virus antibodies, the proportion infected with HIV was 37.0 % (298/804) compared to 1.5 % in 2009 (34/2160) (PR = 36.8; 95% CI: 25.4–53.19). The incidence of tuberculosis combined with HIV infection has increased almost 10-fold since 2009, reaching 15.6 cases per 100,000 population in 2021 against 1.6 cases per 100,000 population.

The proportion of bacteria-excreting patients among newly diagnosed patients increased from 43.6 % to 54.5 % (PR = 0.64; 95% CI: 0.55–0.750), with bacterial excretion being diagnosed by simple bacterioscopy in 36.2 % of cases (334/922) at the commencement of treatment compared with 34.2 % in 2009 (904/2638) (PR = 1.09; 95% CI: 0.93–1.27).

The proportion of bacteriologically proven MDR *M. tuberculosis* strains doubled from 15.5 % to 30.6 % (PR = 0.42; 95% CI: 0.31–0.55).

In 2021, the pattern of drug resistance among newly diagnosed patients excreting bacteria who have been examined for drug susceptibility in 2021 is represented by 13.9% mono-resistant strains (33/237), 18.1 % poly-resistant strains (43/237), 54.8 % MDR strains (130/237), and 13.0 % XDR *M. tuberculosis* strains (31/237). The incidence of tuberculosis with XDR increased 4-fold in follow-up control for the period 2011–2021 and amounted to 1.6 cases per 100,000 population, while the average annual level was 1.1 cases per 100,000 population.

In the contingent of patients discharging bacteria examined for drug sensitivity, the proportion of bacteriologically proven strains with MDR was 15.7 % (152/966), pre-extensive drug resistance – 30.4 % (294/966), extensive drug resistance – 27.3 % (264/966).

DISCUSSION

The complex of measures to control TB infection actively implemented in our country over the past two decades has contributed to a decrease in the intensity of the epidemic process, which manifests itself in a decrease in the number of patients annually diagnosed, as well as in the patient cohort as a whole. In our study, the dynamics of population incidence of active forms of tuberculosis decreased by more than 3 times. By 2020, the target indicators of the first stage of implementation of the Strategy for TB elimination in relation to the baseline level of 2015 [2], which is the starting point for analyzing the effectiveness of the WHO global strategy implementation, were achieved in the Omsk region and reached 36.9 % (target indicator – decrease of TB incidence by 20.0 % by 2020), 85.8 % (target indicator – decrease of deaths by 35 % by 2020).

Although the worldwide incidence of tuberculosis has generally declined, the incidence of multidrug-resistant tuberculosis has increased by almost 10 % annually [3, 13].

The current trend of increasing incidence of multiple or rifampicin-resistant tuberculosis remains a hot public health problem. In 2020, the global rate of effective treatment of drug-resistant TB remained only 59 % of cases, which is a heavy economic burden on health systems, patients and their families, and reduces the effectiveness of TB control measures in different regions and countries worldwide [3, 14].

Russia has experienced a significant portion of the worldwide burden of drug-resistant tuberculosis [4, 5, 15]. Our study revealed a change in the nature of bacteriological excretion among newly diagnosed patients; despite the decrease in the number of detected cases in the structure of drug resistance, a more than 2-fold increase in the proportion of multidrug-resistant bacteriological excretion (up to 26.4 %) was observed, while the dynamics of the incidence of multidrug-resistant tuberculosis in the population remains sta-

ble with a long-term average of 8.1 cases per 100,000 population (R^2 = 0.161). In addition, at the end of the study period in the Omsk region, 66.2 % of culture-positive patients had MDR strains.

The results of the study showed that during the period of observation in the Omsk region the age group at risk has changed, the highest incidence rate was among persons between 35 and 44 years of age, the proportion of which was 35.1 %, and the incidence rate was 109.0 per 100,000 population of this age. In addition, those aged 35–44 years in the Omsk region are a group at risk of HIV infection; in 2021, the proportion of this age group in the age structure of HIV-infected people was 42.9 % [16]. The increasing burden of co-infection of tuberculosis and HIV infection is confirmed by a 10-fold increase in the incidence of comorbidity over the study period.

It should be noted that the epidemiological manifestations of TB infection were significantly influenced by the epidemic process of the new coronavirus infection COVID-19. A study of the clinical structure of newly diagnosed TB patients in the country during the COVID-19 pandemic revealed a worsening of the clinical forms of TB and an increase in their epidemic risk [17]. In our study, there was a statistically significant increase in the proportion of newly diagnosed patients discharging bacteria from 43.6 % to 54.5 % (PR = 0.64), including those detected by simple bacterioscopy to 36.2 % (PR = 1.09). At the end of the study, despite a statistically significant increase in the proportion of respiratory tuberculosis cases, no worsening of the clinical structure of the patients was observed, the proportion of destructive forms of respiratory tuberculosis and fibrous cavernous tuberculosis decreased significantly.

CONCLUSION

Despite the observed favourable trends in the development of the tuberculosis epidemic process, decrease in thetuberculosis incidence, prevalence and mortality, there is a change in the structure of bacterial excretion with the prevalence of multiple and extensive drug resistance, as well as active involvement of HIV-infected patients in the epidemic process. For further timely assessment and prognosis of the manifestations of the epidemic process of TB infection, as well as to characterize the quality and effectiveness of preventive and anti-epidemic measures under conditions of drug-resistant TB extension, it is necessary to improve the organization of the system of epidemiological surveillance and control of TB spread.

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Conflict of interest

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

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