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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 3, 2023

Sergey I. Kolesnikov

Member of RAS

This issue presents a wide range of articles that can attract the reader both by the novelty of approaches and data, and by their practical orientation. For the first time in recent years, articles on infectious pathology do not come out on top.

The first leading article of the issue is undoubtedly a study led by an outstanding scientist, member of RAS V.A. Tutelyan (S.A. Apryatin et al.; Moscow, St. Petersburg), who conducted a comparative analysis of changes in the liver transcriptome of two lines of rats and three lines of mice treated with excessive calorie content and lipogenic effect diets. In each animal species and sex studied, genetic variants with a diet-induced obesity tendency were identified. This justifies new approaches to personalized diet therapy for alimentary-dependent diseases.

The second editorial article is a review of the master of free radical processes research V.Z. Lankin et al. (Moscow), which provides evidence of the predominant role of low-density lipoproteins (modified with low molecular weight dicarbonyl compounds formed during free radical oxidation of lipids and carbohydrates) in the development of endothelial dysfunction and atherosclerotic vascular damage. It is assumed that pharmacological correction of free radical oxidation can slow down not only atherogenesis, but also diabetogenesis.

The section of **therapeutic orientation** is represented by a few works. A.N. Sumin et al. (Kemerovo, Novokuznetsk) devoted their article to the problem of diagnosing acute coronary syndrome in women. The author of the second work L.A. Ivanova (Irkutsk) identified risk factors for the development of vascular dementia, but concluded that they are potentially reversible, which can reduce the development of cognitive disorders. There are unexpected findings in the other publication. We are used to the fact that the troponin test has become a routine in the diagnosis of myocardial infarction. But it turned out, according to the article by M.S. Smirnova et al. (St. Petersburg) that troponin I increases 2–4 times (without myocardial damage) in cross-country skiers in response to training and may be an indicator of its intensity.

The only article on **epidemiology** came from Kazakhstan (Almaty, Uralsk), which presents an analysis of long-term data on the epizootic and epidemic activity of natural foci of tularemia and the created electronic map of settlements endemic for tularemia to determine the volume of preventive measures.

Surgical articles are represented by a large section of reviews, original studies, and descriptions of unique cases, including ones in the field of **oncology**. The review by E.A. Kravtsova et al. (Tomsk) might be of interest to the physicians in terms of education. The article showed that in countries implementing the National Program of Vaccination against Human Papilloma Virus of High Carcinogenic Risk, a decrease in the incidence of both cervical pathologies of varying severity and other cancers associated with the carriage of this virus was registered. The second review by K.V. Protasov and O.A. Barakhtenko (Irkutsk) systematized data on stratification of the risk of cardiovascular complications of surgical treatment of cancer patients by using different biomarkers. It seems that specialists will also be interested in the experience of V.A. Pelts et al. (Kemerovo) on successful liver resections in the treatment of hepatocellular carcinoma, as well as a description of a unique clinical case of diagnosis and treatment of Castleman tumor of rare retroperitoneal localization, given by M.B. Polyanskiy et al. (Kursk).

Two articles are devoted **to coloproctology.** The article by Yu.A. Churina et al. (Moscow) showed the possibility of expanding the indications for the treatment of high anorectal fistulas involving up to 2/3 of the sphincter complex. The authors of the second article, A.A. Zakharchenko et al. (Krasnoyarsk), described the case of successful treatment of recurrence of hemorrhoidal disease by endovascular occlusion of hemorrhoidal arteries.

Dentists may be interested in the article by A.A. Fefelov et al. (Chita, Irkutsk) who have shown the effectiveness of plasmolifting for the treatment of chronic periodontal disease, including restoration of endothelial function evidenced by its markers. **Ophthalmologists** will learn about a rare case of sclerochoroidal calcification, which can be mistaken for a malignant tumor, from the article by A.D. Chuprov et al. (Orenburg).

In this issue, a large number of articles are related to **the study of obesity** as a global epidemic and lipid metabolism disorders.

In the treatment of obesity, one of the problems is sarcopenia, and in this regard, I would advise you to read the review of D.P. Kurmaev et al. (Samara), who evaluated the need for branched-chain amino acids in the treatment and prevention of sarcopenia in geriatric patients and concluded that the addition of BCAAs may be justified in cases where it is not possible to consume sufficient amounts of high-quality protein from food.

The original and insufficiently studied topic of the influence of olfactory and taste perception on metabolic homeostasis in obese patients was revealed in the review by Yu.G. Samoilova et al. (Tomsk).

Ju.G. Birulina et al. (Tomsk) revealed a change in the reactivity of the respiratory tract and the formation of bronchospastic conditions and the induction of an inflammatory response in the respiratory system in the obesity modelling in rats. On another model, T.V. Brus et al. (St. Petersburg) showed that in nonalcoholic fatty disease in the experiment, the increased formation of cytokines IL-1, IL-6 inhibits the absorption of iron in the duodenum and blocks the release of iron processed from aging erythrocytes into the plasma, which may be the pathogenesis link of anemia.

More and more attention of researchers is attracted by **the problem of rehabilitation of patients** after diseases and vascular accidents. There are two such articles in the issue. In the first one, the authors from Rostov, S.S. Memetova et al., revealed that the availability of measures for medical rehabilitation of a patient with a stroke in the early recovery period in a specialized rehabilitation center is limited and about half of the respondents expected a referral of up to 6 months, which indicates the need to expand the network of such institutions. The second article by V.A. Beloglazov et al. from the Republic of Crimea regarding the rehabilitation of post-COVID syndrome is also far from optimistic – it is stated that the presented plan of sanatorium-resort treatment is ineffective and the need for deeper scientific research in the direction of studying the mechanisms of formation of low-intensity inflammation and methods of combating it.

I found the review by E.M. Kuklina and N.S. Glebezdina from Perm interesting. It is devoted to insufficiently studied markers of Th1-polarized Th17 cells, which can cross the histohematic barriers and play a key role in the pathogenesis of many inflammatory and autoimmune diseases.

Where are three articles devoted to investigations ***in silico***. The one by N.Yu. Nosov et al. from Moscow showed that chocolate agar with growth additives produced by the Russian manufacturer "Gem LLC" can be successfully used for the cultivation of pure culture of *N. gonorrhoeae*, instead of imported one, which reduces the domestic microbiology dependence on imports. V.I. Shlyapkina et al. (Saransk) developed emulsion forms of Sosnovsky hogweed furanocoumarins as promising photosensitizers for various diseases phototherapy.

The issue concludes with the article by T.A. Fominykh et al. (Simferopol) dedicated to the 17th century anatomist Jean Pecquet, who made a significant contribution to the development of this science.

We are waiting for new interesting articles and comments, and of course are welcoming new authors!

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ПРЕДИСЛОВИЕ ГЛАВНОГО РЕДАКТОРА К № 3 (2023)

Колесников Сергей Иванович

академик РАН

В данном номере представлен широкий диапазон статей, которые могут привлечь читателя как новизной подходов и данных, так и своей практической направленностью. Впервые за последние годы на первое место не выходят статьи по инфекционной патологии.

Первой передовой статей номера, несомненно, является исследование под руководством выдающегося учёного академика РАН В.А. Тутельяна. В своей статье С.А. Апрятин и соавт. (Москва, Санкт-Петербург) провели сравнительный анализ изменений транскриптома печени двух линий крыс и трёх линий мышей, получавших рационы с избыточной калорийностью и липогенным действием. У каждого изученного вида и пола животных выявлены генетические варианты со склонностью к диет-индуцированному ожирению, что обосновывает новые подходы к персонализированной диетотерапии алиментарно-зависимых заболеваний.

Второй передовой статьёй является обзор коллектива авторов под руководством мэтра исследований свободнорадикальных процессов В.З. Ланкина (Москва), приводящего доказательства преимущественной роли липопротеидов низкой плотности (модифицированных низкомолекулярными дикарбонильными соединениями, образующимися при свободнорадикальном окислении липидов и углеводов) в развитии дисфункции эндотелия и атеросклеротического поражения сосудов. Предполагается, что фармакологическая коррекция свободнорадикального окисления может замедлить не только атерогенез, но и диабетогенез.

Раздел **терапевтической направленности** представлен немногими работами. Одна из них – статья А.Н. Сумина и соавт. (Кемерово, Новокузнецк) – посвящена проблеме диагностики острого коронарного синдрома у женщин. Автором второй работы, Л.А. Ивановой (Иркутск), выявлены факторы риска развития сосудистой деменции, но сделан вывод о том, что они являются потенциально обратимыми, что может уменьшить развитие когнитивных расстройств. Есть и неожиданные находки. Мы привыкли, что тропониновый тест стал рутиной при диагностике инфаркта миокарда. Но оказалось, согласно статье М.С. Смирнова и соавт. (Санкт-Петербург), что тропонин I повышается в 2–4 раза (без повреждения миокарда) у лыжников-гонщиков в ответ на тренировку и может быть показателем её интенсивности.

Единственная статья по **эпидемиологии** поступила из Казахстана (Алматы, Уральск). В ней представлен анализ многолетних данных по эпизоотической и эпидемической активности природных очагов туляремии и создана электронная карта эндемичных по туляремии населённых пунктов для определения объёмов профилактических мероприятий.

Статьи **хирургической направленности** представлены большим разделом обзоров, оригинальных исследований и описания уникальных случаев, в т. ч. в области **онкологии.** Интересен для врачей и в плане просвещения обзор Е.А. Кравцовой и соавт. (Томск), показавших, что в странах, реализующих Национальную программу вакцинации против вируса папилломы человека высокого канцерогенного риска, зарегистрировано снижение заболеваемости как патологиями шейки матки различной степени тяжести, так и другими онкозаболеваниями, ассоциированными с носительством данного вируса. Второй обзор из Иркутска – авторства К.В. Протасова и О.А. Барахтенко – систематизировал данные по стратификации риска сердечно-сосудистых осложнений хирургического лечения онкологических больных с помощью биомаркеров. Представляется, что заинтересуют специалистов

опыт В.А. Пельца и соавт. (Кемерово) по успешным резекциям печени в лечении гепатоцеллюлярного рака, а также описание редкого клинического случая диагностики и лечения опухоли Кастлемана редкой забрюшинной локализации, приведённое М.Б. Полянским и соавт. (Курск).

Две статьи посвящены **колопроктологии** – статья Ю.А. Чуриной с соавт. (Москва), показавших возможность расширить показания для лечения высоких аноректальных свищей при вовлечении до 2/3 сфинктерного комплекса, а также статья А.А. Захарченко и соавт. (Красноярска) по успешному лечению рецидива геморроидальной болезни эндоваскулярной окклюзией геморроидальных артерий.

Стоматологов может заинтересовать статья А.А. Фефелова и соавт. (Чита, Иркутск), показавших эффективность плазмолифтинга для лечения хронического парадонтоза, в т. ч. восстановления функции эндотелия по его маркерам. **Офтальмологи** из статьи А.Д. Чупрова и др. (Оренбург) узнают о редком случае склерохориоидальной кальцификации, которую можно принять за злокачественную опухоль.

В этом номере большое количество статей связаны с **исследованием ожирения** как мировой эпидемии и нарушений метаболизма липидов.

При лечении ожирения одной из проблем является саркопения, и в этой связи я бы посоветовал прочитать обзор Д.П. Курмаева и соавт. из Самары, оценивших необходимость применения аминокислот с разветвлённой цепью в лечении и профилактике саркопении у гериатрических пациентов и заключивших, что добавление BCAA может быть оправдано в тех случаях, когда невозможно потреблять достаточное количество высококачественного белка с пищей.

Оригинальную и недостаточно исследованную тему влияния обонятельного и вкусового восприятия на метаболический гомеостаз у пациентов с ожирением раскрыли в своём обзоре Ю.Г. Самойлова и соавт. (Томск).

Ю.Г. Бирулиной и соавт. (Томск) выявлены изменение реактивности дыхательных путей, формирование бронхоспастических состояний и индукция воспалительной реакции в респираторной системе при моделировании ожирения у крыс. На другой модели Т.В. Брус и соавт. (Санкт-Петербург) показали, что при неалкогольной жировой болезни в эксперименте повышенное образование цитокинов ИЛ-1, ИЛ-6 ингибирует всасывание железа в двенадцатиперстной кишке и блокирует высвобождение железа, переработанного из стареющих эритроцитов в плазму, что может быть звеном патогенеза анемии.

Всё больше внимания исследователей привлекает **проблема реабилитации пациентов** после перенесённых заболеваний и сосудистых катастроф. Таких статей в номере две. В первой – статье С.С. Меметова и соавт. (Ростов-на-Дону) – выявлено, что доступность мероприятий по медицинской реабилитации пациента с перенесённым инсультом в раннем восстановительном периоде в условиях специализированного реабилитационного центра ограничена, и около половины респондентов ожидали направление до 6 месяцев, что свидетельствует о необходимости расширения сети подобных учреждений. Вторая статья – В.А. Белоглазова и соавт. (Республика Крым) – относительно реабилитации постковидного синдрома также далека от оптимизма: заявлено о неэффективности представленного плана санаторно-курортного лечения и необходимости более глубоких научных изысканий в направлении изучения механизмов формирования низкоинтенсивного воспаления и методов борьбы с ним.

Мне показался интересным обзор Е.М. Куклиной и Н.С. Глебездиной (Пермь), посвящённый недостаточно изученным маркерам Th1-поляризованных клеток Th17, которым отводится ключевая роль в патогенезе многих воспалительных и аутоиммунных заболеваний, а также в преодолении гистогематических барьеров.

Работы *in silico* три. Одна (Носов Н.Ю. и соавт., Москва) показала, что шоколадный агар с ростовыми добавками производства российского производства (ООО «Гем», Москва) может успешно использоваться как импорто-

заместитель для культивирования чистой культуры *N. gonorrhoeae*, что снижает зависимость отечественной микробиологии от импорта. В исследовании В.И. Шляпкиной и соавт. (Саранск) разработаны эмульсионные формы фуранокумаринов борщевика Сосновского как перспективных фотосенсибилизаторов для фототерапии различных заболеваний.

Завершает выпуск статья Т.А. Фоминых и соавт. (Симферополь), посвящённая известному учёному-анатому XVII века Жану Пеке, внёсшему серьёзный вклад в развитие этой науки.

Ждём новые интересные статьи и новых авторов!

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CONTENTS

СОДЕРЖАНИЕ

DISCUSSION PAPERS, LECTURES, NEW TRENDS IN MEDICAL SCIENCE

MODIFICATION OF LOW-DENSITY LIPOPROTEINS BY LOW MOLECULAR WEIGHT CARBONYL PRODUCTS OF FREE-RADICAL OXIDATION OF LIPIDS AND CARBOHYDRATES PLAYS A KEY ROLE IN ATHEROSCLEROTIC LESION OF THE VASCULAR WALL AND IN ENDOTHELIAL DYSFUNCTION

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ABSTRACT

The review presents evidence of the participation of low-density lipoproteins (LDL) modified by low molecular weight dicarbonyl compounds formed during free-radical oxidation of lipids (malondialdehyde) and carbohydrates in the development of endothelial dysfunction and atherosclerotic vascular lesions. The authors believe that it is they, and not oxidized (hydroperoxide-containing) LDL, that are the main factors of pathogenesis. The role of dicarbonyl-modified LDL in LOX-1 dependent induction of processes leading to the development of endothelial dysfunction is discussed. The results of studies proving that damage to the glycocalyx (a layer of macromolecules that prevent the development of endothelial dysfunction) covering the luminal surface of the endothelium is caused by hyperproduction of reactive oxygen species. Ways of pharmacological correction of free-radical oxidation processes are discussed, due to which inhibition of atherogenesis and diabetogenesis can be achieved.

Key words: *malondialdehyde, methylglyoxal, endothelial dysfunction, glycocalyx, low density lipoproteins, free radicals, atherosclerosis, diabetes mellitus*

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МОДИФИКАЦИЯ ЛИПОПРОТЕИДОВ НИЗКОЙ ПЛОТНОСТИ НИЗКОМОЛЕКУЛЯРНЫМИ КАРБОНИЛЬНЫМИ ПРОДУКТАМИ СВОБОДНОРАДИКАЛЬНОГО ОКИСЛЕНИЯ ЛИПИДОВ И УГЛЕВОДОВ ИГРАЕТ КЛЮЧЕВУЮ РОЛЬ В АТЕРОСКЛЕРОТИЧЕСКОМ ПОВРЕЖДЕНИИ СТЕНКИ СОСУДОВ И ДИСФУНКЦИИ ЭНДОТЕЛИЯ

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РЕЗЮМЕ

В обзоре приводятся доказательства участия липопротеидов низкой плотности (ЛНП), модифицированных низкомолекулярными дикарбонильными соединениями, образующимися при свободнорадикальном окислении липидов (малоновый диальдегид) и углеводов, в развитии дисфункции эндотелия и атеросклеротического поражения сосудов. Авторы полагают, что именно они, а не окисленные (гидропероксид-содержащие) ЛНП являются основными факторами патогенеза. Обсуждается роль дикарбонил-модифицированных ЛНП в LOX-1-зависимой индукции процессов, приводящих к развитию дисфункции эндотелия. Рассматриваются результаты исследований, доказывающих, что к повреждению покрывающего люминальную поверхность эндотелия гликокаликса – слоя макромолекул, препятствующего развитию дисфункции эндотелия, – ведёт гиперпродукция активных форм кислорода. Обсуждаются пути фармакологической коррекции процессов свободнорадикального окисления, благодаря которой может достигаться торможение процессов атерогенеза и диабетогенеза.

Ключевые слова: *малоновый диальдегид, метилглиоксаль, дисфункция эндотелия, гликокаликс, липопротеиды низкой плотности, свободные радикалы, атеросклероз, сахарный диабет*

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In the middle of the last century, Denhem Harman hypothesized that aging is associated with the accumulation of cell damage caused by products of spontaneous free-radical oxidation (FRO) [1, 2]. Since such pathologies as atherosclerosis and diabetes mellitus can be attributed to diseases of old age, D. Harman suggested that the emergence and development of these pathological conditions (he called them "free-radical diseases") are associated with the damaging effect of free-radical reactions [3]. J. Glavind et al. [4] in 1952 were the first to suggest that free-radical oxidation of lipids might be one of the triggering factors of atherosclerotic lesion of the vascular wall. Based on the analysis of autopsy materials, these authors concluded that the level of lipoperoxides in human aorta with atherosclerotic lesions is always higher than in the unaffected vascular wall. Unfortunately, a small number of samples were studied in this work, and an insufficient method of analysis was used to analyze the lipoperoxide content, which, as it was found later [5], is not specific enough. In spite of this, during the following decade the conclusions of the work of J. Glavind et al. did not raise doubts. Only in 1965, F.R. Woodford et al. [5] made an attempt to experimentally verify these results, using a highly specific method of iodometric titration with amperometric equivalence point determination developed earlier for the analysis of lipoperoxides [6]. The results obtained by F.R. Woodford et al. practically refuted the conclusion of J. Glavind et al. as statistically significant differences between the content of lipoperoxides in atherosclerotic lesion areas and intact areas of the aorta in autopsies could not be detected by these authors. The pessimistic conclusions of the publication by F.R. Woodford et al. cooled the interest in the free-radical theory of atherogenesis for a long time, despite its theoretical justification given in the articles by D. Harman [1–3]. At the same time, we detected an increase in the content of free radical oxidation products in the aorta of animals with experimental atherosclerosis [7]. Only two decades later our group using an adequate method of high-performance liquid chromatography (HPLC) proved a significant increase in the content of primary products of FRO – lipid hydroperoxides (LOOH) [8, 9] in the aorta damaged by atherosclerosis (compared to the unaffected area of the vascular wall), which increased with the progression of atherosclerotic lesion [8, 9]. It should be noted that these unique studies were performed using autopsy material during rapid autopsies of people who died in car accidents within 3 hours after the confirmation of death, i. e., when native samples were analyzed [8, 9]. (HPLC on a chiral phase column) with ratio of S- and R-stereoisomers proved that LOOHs detected in atherosclerotically damaged aorta are the result of spontaneous (non-enzymatic) free-radical oxidation of unsaturated lipids [8, 9]. Cholesterol esters is the major class of lipids accumulating in the areas of atherosclerotic lesions of the vascular wall [10, 11], and not only fatty acid residues [8, 9] but also the sterol part of the molecule are subjected to oxidation [10, 12]. A decrease in the activity of key antioxidant enzymes (Se-containing glutathione peroxidase (GSH-Px) and Cu,Zn-superoxide dismutase (Cu,Zn-SOD)) was also detected in the areas of human aortic atherosclerotic lesions, progressing with the increasing degree of damage [9, 13]. Then the hypothesis of imbalanced systems of FRO product formation and utilization in atherosclerosis was formulated [9, 14]. The same data provided convincing grounds for classifying atherosclerosis as a "free-radical pathology", i. e., a disease whose pathogenesis is strongly influenced by FRO processes [9, 14].

It should be noted that a significant increase in the level of primary and secondary products of lipid free-radical oxidation was detected in a representative epidemiological study in blood plasma of probands with diagnosed atherosclerosis [7, 9, 14]. In the same study, decreased activity of erythrocyte GSH-Px, LOOH utilizing enzyme, was found in patients with atherosclerosis [9, 14]. Based on these results, it could be assumed that nanoparticles of the lipid-transporting system – blood plasma lipoproteins – undergo oxidation during atherogenesis [7, 9, 14]. Indeed, it has been shown that "atherogenic" low-density lipoproteins (LDL) easily undergo oxidation both when incubated in the presence of vascular endotheliocytes and in the presence of free-radical oxidation initiators [15, 16]. It was found that chemical modification of blood plasma LDL particles with acetaldehyde makes them more «atherogenic» [17], i. e. capable of binding to the scavenger receptor and accumulating in macrophages of the vascular wall [17]. Later, numerous studies found that LDL particles subjected to free-radical oxidation also become «atherogenic» [18–25].

It is recognized that lipid FRO is a two-stage process: first, primary – unstable – LOOH oxidation products are formed. They further undergo oxidative degradation and form low molecular weight dicarbonyls, i. e. secondary products [26]. Consequently, with a sharp increase of LOOH in tissues, oxidative stress during atherogenesis must inevitably be accompanied by the accumulation of such active carbonyl products as hydroxynonenals and malondialdehyde (MDA), i. e., converted to carbonyl stress [14, 26]. In turn, aldehyde groups of dicarbonyls can easily react with the amino end groups of proteins by the Maillard reaction to form intra- and intermolecular cross-links in their molecules [26]. The possibility of MDA participating in modification of LDL apoprotein B-100 has been established [27], but nevertheless, the question of the mechanism of LDL oxidative modification, due to which LDL particles acquire "atherogenicity", has not been solved so far [14].

In strict terminology, "oxidized" LDLs contain hydroperoxy acyls in the phospholipids of the outer layer of the particles. Fundamentally, the accumulation of hydroperoxy acyls in the outer phospholipid monolayer of LDL can lead to changes in apoprotein B-100 conformation. Thus, during free-radical oxidation of unsaturated ("liquid") acyls of membrane phospholipids, an increase in membrane microviscosity is detected [9,

28] due to the "pushing" or "pulling" of more polar hydroperoxy acyls into the aqueous phase, as the relative content of saturated («solid») fatty acid residues increases in the membrane [9, 28]. It is highly likely that when fundamental properties of biomembranes such as microviscosity and polarity are significantly altered, the conformation of peripheral and integral proteins embedded in the phospholipid bilayer may be altered. In particular, we found a multidirectional change in the activity of membrane-bound enzymes in the same membrane during free-radical oxidation of liver microsomes biomembranes: the activity of some enzymes (sensitive to oxidation) decreased, while that of others (resistant to oxidation) increased [29], which can be explained by a physical change in the conformation of the molecules of these proteins when the physicochemical properties of membrane lipids change. Based on these results, it could be assumed that oxidation of phospholipids in LDL particles would lead to a change in the apoprotein B-100 conformation, as a consequence of which the efficiency of binding of such "oxidised" LDLs to the scavenger receptor of macrophages would also be changed.

The *in vitro* induction of LDL free radical oxidation using various initiators (such as azo initiators, hydrogen peroxide, superoxide anion radicals, metal ions of variable valency, etc.) leads to an increase in the concentration of both primary (LOOH) and secondary lipoperoxidation products (MDA) [30, 31]. Therefore, it is obvious that it is impossible to determine which lipid FRO products cause "atherogenic" modification of LDL particles using standard approaches. We were able to obtain truly oxidized LDLs without an admixture of MDA-modified LDLs [31] using a homogeneous preparation of rabbit reticulocyte 15-lipoxygenase capable of oxidizing polyene acyls of phospholipids [32]. At the same time, MDA-modified LDLs without an admixture of oxidized (LOOH-containing) LDL were obtained by incubation of LDLs with MDA [31]. When studying atherogenicity (efficiency of LDL particles capture by cultured human macrophages) of the two obtained LDL modifications, we experimentally proved that not oxidized (LOOH-containing LDLs) but exclusively MDA-modified LDLs bind to the scavenger receptors of macrophages [31]. Consequently, LDL particles modified by natural dicarbonyls rather than oxidized LDLs should be efficiently captured and accumulated in lipid vacuoles of vascular wall cells [31]. This leads to preaterosclerotic lesions of vascular walls and the transformation of macrophages and smooth muscle cells into "foam cells" forming lipoidosis zones [9, 14]. The obtained results do not just clarify the existing terminology, but are of principal character, since they substantiate the existence of a quite definite molecular mechanism of "atherogenic" modification of LDL particles with the participation of natural low-molecular carbonyl compounds. It was also found that the most cholesterol-rich LDL particles are also simultaneously MDA-modified [33]. Therefore, carbonyl modification of LDL particles may contribute to the efficient entry of cholesterol into the vascular wall [33]. In addition, there is evidence that increased accumulation of MDA-modified LDLs is characteristic of patients with certain mutations of apoprotein B-100, i. e. there is a possibility that carbonyl modification of LDLs may be genetically determined [34].

Protein molecules Cu,Zn-SOD and GSH-Px, similarly to LDL apoprotein B-100, also undergo modification during MDA accumulation during atherogenesis [35, 36], which is accompanied by suppression of their activity due to conformational changes in the structure of the active centre [35, 36]. It is obvious that dicarbonyl-dependent inhibition of antioxidant enzyme activity during atherogenesis must result in stimulation of oxidative stress. Thus, the development of oxidative (LOOH accumulation) and subsequent carbonyl stress (MDA accumulation) during atherogenesis leads to the formation of dicarbonyl-modified LDLs, which are the key factor causing preterogenic damage to the vascular wall and subsequent formation of atherosclerotic plaques [14].

Although the available literature attributes diabetes mellitus as a risk factor for atherosclerosis or a contributing factor to its development, a large number of diabetic patients die due to vascular incidents [37–39], no convincing pathophysiological explanation is provided. Nevertheless, an important role of FRO in the pathogenesis of diabetes mellitus has been hypothesized for quite some time [40]. The basis of this hypothesis is the assumption that in diabetes mellitus initially develops carbonyl rather than oxidative stress [41], in which active dicarbonyls such as glyoxal and methylglyoxal formed during oxidative transformations of glucose accumulate [41–43]. Glyoxylation during autoxidation of glucose and other hexatomic carbohydrates leads to the formation of glyoxal. Methylglyoxal is synthesized during enzymatic oxidation of glucose with the formation of triosophosphates [41, 44, 45]. Methylglyoxal, as we have shown, can also be formed when glucose derivatives are attacked by lipoperoxyl free radicals, i. e. non-enzymatically [46]. High blood glucose level in patients with type 2 diabetes mellitus contributes to LDL co-oxidation and a sharp increase in the rate of LDL lipid FRO accompanied by superoxide anion radical formation [47]. In the Maillard reaction, the interaction of methylglyoxal and amino end groups of apoprotein B-100 LDL can also generate superoxide radical [48]. Thus, diabetogenesis, unlike atherogenesis, is characterized by the primary development of carbonyl stress (accumulation of active carbonyl compounds), and at later stages, reactive oxygen species (ROS) generated by the reactions described above induce secondary oxidative stress.

On this basis, the stages of carbonyl stress development and subsequent oxidative stress characterized by the accumulation of various oxidation products should be distinguished during diabetogenesis. The accumulation of glyoxal and methylglyoxal in the blood plasma of diabetic patients has been repeatedly confirmed experimentally [41–43]. At the same time, the presence of oxidative stress in diabetes is evidenced by a de-

crease in telomere length in blood nuclear cells [49], as well as an increase in the level of 8-hydroxy-2'-deoxyguanosine, the end product of oxidative DNA destruction, in the blood and urine of type 2 diabetic patients [49]. It should be noted that 8-hydroxy-2-deoxyguanosine is a recognized biomarker of oxidative stress [50]. Its accumulation is not associated with the development of carbonyl stress. Increased levels of LOOH-containing LDLs [41] in the blood of type 2 diabetes patients also suggests that secondary induction of oxidative stress may indeed occur during atherogenesis. Similar to atherosclerosis, type 2 diabetes patients have increased carbonyl modification of LDLs [49] and a sharp drop in erythrocyte Cu,Zn-SOD and GSH-Px activity [49, 51], which is a characteristic reflection of carbonyl stress.

A significant increase in glyoxal and methylglyoxal levels in the blood of type 2 diabetes patients [41-43] can induce LDL modification, which is recognized by scavenger receptors of macrophages and thus can induce LDL accumulation in the vascular wall with subsequent development of lipoidosis lesions [41]. It has been shown that LDL modification by methylglyoxal significantly increases the "atherogenicity" of LDLs (increases their receptor capture by macrophages) [41, 52]. Based on the above data, we hypothesized a single molecular mechanism of vascular wall damage in atherosclerosis and diabetes mellitus, which includes increased chemical modification of LDL apoprotein B-100 by dicarbonyls accumulated during free radical oxidation of lipids in atherosclerosis or autoxidation of glucose molecules in diabetes mellitus [47]. This hypothesis satisfactorily explains the reasons for the stimulation of atherogenesis in diabetes and the fact that diabetes may increase the risk of atherosclerosis [47].

As it has been found in recent years, oxidized LDLs also play an important role in causing endothelial dysfunction [53–56]. The endotheliocyte scavenger receptor LOX-1 is thought to bind to oxidized LDLs, causing the expression of NADPH-oxidase, which generates superoxide anion radical, causing endothelial cell damage [57]. We found that strong expression of LOX-1 and NADPH-oxidase biosynthesis in human endotheliocytes is induced by culturing cells in the presence of dicarbonyl-modified (MDA-, glyoxal-, and methylglyoxal-modified) LDLs [58]. Consequently, the initial stages of vascular endothelial dysfunction, a process that plays a leading role in atherogenesis and diabetogenesis, are likely to be directly dependent on the formation of dicarbonyl-modified rather than "oxidized" LDLs. As a result, superoxide-dependent endotheliocyte damage provokes stimulation of apoptosis and endothelial cell death [53, 56, 57], which, in turn, obviously facilitates the penetration of modified LDLs into the vascular wall.

We have found that the enzyme antioxidant system of endotheliocytes is represented mainly by special classes of enzymes – peroxiredoxins [59], which, in accordance with our data, like Cu,Zn-SOD and GSH-Px [35, 51], are very sensitive to the inhibitory action of low-molecular-weight dicarbonyls accumulated under oxidative and carbonyl stress [60]. There is no doubt that suppression of peroxiredoxin activity attenuates the antiradical defence of endothelial cells, contributing to endothelial damage and dysfunction. Thus, the data obtained suggest that the formation of carbonyl-modified LDLs is a key factor in the development of endothelial dysfunction, a process that plays a leading role in atherogenesis and diabetogenesis.

Endothelial dysfunction must precede damage to the endothelial glycocalyx. The glycocalyx is a protective layer of macromolecules (such as proteoglycans and glycoproteins) covering the luminal surface of endotheliocytes [61, 62]. Damage to the glycocalyx is considered to be the earliest stage of vascular wall damage in various pathologies [63–66]. Glycocalyx controls the permeability of the vascular wall [67] and the adhesion of blood formed elements on endotheliocytes [68, 69]. In addition, the glycocalyx protects the endothelium from damaging factors such as viruses, pro-inflammatory cytokines and ROSs [70, 71]. It is likely that glycocalyx layer is the barrier preventing atherogenic LDLs (obviously, dicarbonyl-modified LDLs) from penetrating into the subendothelial space of the vascular wall [72]. A decrease in glycocalyx thickness due to its fragmentation has been observed in the process of ROS hyperproduction ("oxidative burst") during ischemia and/or ischemia/reperfusion [73–75], as well as an increase in the level of oxidized LDLs [76, 77]. These facts suggest that oxidatively modified LDLs (most probably dicarbonyl-modified LDLs), formed by oxidative and carbonyl stress, are the most important factors in atherogenesis. Consequently, preservation of the glycocalyx should prevent atherogenesis and diabetogenesis. Damage to the glycocalyx can be considered as the first step in atherosclerotic vascular damage.

The above proves that it is logical to use antioxidants to suppress lipoperoxidation in LDLs, and several clinical studies have used natural antioxidants such as vitamin E (α-tocopherol, α-TOH) for this purpose. In contrast to the very encouraging positive results obtained from studies involving animals with experimental atherosclerosis, trail data on antioxidant intervention (predominantly α-TOH, in some cases in combination with ascorbate and/or β-carotene) in cardiovascular diseases are quite ambiguous [78–82]. In randomized, double-blind, placebo-controlled trials, the use of antioxidant vitamins was found to statistically significantly reduce the risk of cardiovascular disease and cardiac mortality [83–85]. Moreover, angiography was performed as a control in one of the few studies [86], and the suppression of coronary stenosis in patients treated with antioxidants was documented [86]. Studies involving large cohorts of men [87] and women [88] have demonstrated that regular consumption of α-TOH for several years contributes to a statistically significant reduction in the risk of coronary artery disease (CAD) [87, 88]. More than 2000 patients involved in the Cambridge Heart Antioxidant Study (CHAOS) with angiographically confirmed atherosclerosis received high (400–800 IU/day) doses of α-TOH

for one year, and a statistically significant reduction in the risk of myocardial infarction was detected [89]. A statistically significant reduction in myocardial infarction incidents was noted during the SPACE study involving patients with CAD who were treated with hemodialysis and therapy including 800 IU/day of α-TOH for almost 1.5 years [90]. However, several other clinical trials have not demonstrated statistically significant reductions in cardiovascular complications and/or reductions in cardiac incident mortality with antioxidant administration [91–94]. For example, a study including a large number of male smokers who received α-TOH and/or β-carotene for 5–8 years did not show statistically significant increase in cardiovascular mortality [91]. In the GISSI-Prevenzione Trial, administration of 450 IU/day of α-TOH to patients with post-myocardial infarction (about 3 months) was not effective in reducing mortality, nor in reducing the incidence of new infarctions or strokes for 3.5 years [92]. In the Heart Outcomes Prevention Evaluation Study (HOPE), more than 1500 patients at high risk of cardiovascular disease who received 400 IU of α-TOH per day for 4.5 years showed no statistically significant reduction in cardiovascular mortality [93]. In the MRC/BHF Heart Protection Study, more than 20,000 patients with CAD who received an antioxidant vitamin complex (900 IU/day of α-TOH) for 5 years showed no increase in infarction- and stroke-related mortality [94]. The results of such studies, which, contrary to expectations, have not provided clear positive results regarding the use of antioxidants (including extremely high doses of α-TOH), gave a reason to believe that the antioxidants used had a negative effect [78, 79]. Obviously, it is not correct to interpret the lack of effect as a negative effect, but the ambiguity of results on the use of antioxidants in the clinic makes it necessary to critically analyze the reasons for this. It is important to note that no studies have found negative effects of antioxidants (e. g., increased mortality and/or cardiac complications), only a lack of expected positive effects. Based on the design of the conducted studies, the principles of selection of the used antioxidants and their doses, the criteria for the assessment of biochemical and clinical changes, it seems obvious that the results of such studies a priori cannot give an unambiguous answer to the questions posed in them. We might agree with the statements of ardent opponents of further research on the use of antioxidants in cardiology about the uselessness (or even pointlessness) of continuing such research [78, 79]. However, we should not radically change approaches to planning and conducting work.

It should be noted that the choice of α-TOH (vitamin E) as an antioxidant used in most of the above studies cannot be considered as sufficiently successful and justified. It is known that α-TOH, like other fat-soluble vitamins, is transported in the body as part of the hydrophobic lipid core of LDL particles [95]. Nevertheless, the protection of circulating LDL particles from free-radical oxidation in the bloodstream is performed not by α-TOH, but by the reduced (phenolic) form of coenzyme Q_{10} [9, 96–100]. Based on the fact that in 1 LDL particle only 1–2 molecules of coenzyme Q_{10} account for approximately 650 molecules of the substrate of free-radical oxidation – phospholipids [101, 102], effective inhibition of free-radical reactions in LDLs by this antioxidant is impossible without its bioregeneration, possibly involving radical intermediates α-TOH and ascorbate [102–107]. At the same time, it has been shown that administration of high doses of α-TOH does not affect LDLs oxidability in CAD patients [100]. Thus, it should be recognized that the use of α-TOH to inhibit LDL oxidability in clinical trials is not justified, and the use of coenzyme Q_{10} is more effective in protecting LDL from oxidation [9, 100] and other phenolic antioxidants, in particular, the non-toxic synthetic antioxidant probucol [9, 100, 108–110], whose efficacy in inhibiting LDL oxidation has been convincingly confirmed [9, 100]. It is obviously impermissible to apply generalizations about "negative" results obtained on the use of individual antioxidants such as α-TOH or β-carotene [91–93] to the whole rather heterogeneous group of antioxidants [79], which includes substances of different structure and mechanism of action. In addition, the data presented in this review suggest that in order to suppress atherogenesis and endothelial dysfunction it is necessary to inhibit not only the accumulation of primary products (LOOH) in LDLs, but also the accumulation of secondary products of free radical oxidation – low molecular weight dicarbonyls. There are already positive examples of effects on the intensity of free-radical oxidation using biguanides – dicarbonyl scavengers [100, 111–113] and imidazole-containing peptides [114, 115]. In particular, the use of biguanides significantly suppressed the manifestation of oxidative and carbonyl stress in diabetic patients without the administration of any antioxidants ("quasi-antioxidant effect") [100]. Obviously, preventive cardiology should aim to prevent the adverse effects of oxidative modification of LDL, because modified LDLs, as shown in this study, play an important role in the molecular mechanisms of atherogenesis and diabetogenesis. Currently, preventive cardiology is focused on the development of effective approaches to pharmacotherapy aimed at inhibiting the formation of primary and secondary products of free radical oxidation in order to control the level of potentially dangerous oxidized and modified LDLs.

Conflict of interest

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

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METABOLISM EFFECTOR LINKS IN DIET-INDUCED AND GENETICALLY-BASED OBESITY: A FULL-TRANSCRIPTOME STUDY OF LIVER TISSUE IN EXPERIMENTAL MODELS IN RODENTS

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ABSTRACT

Background. *When developing methods for personalized diet therapy of obesity, an urgent task is to study the molecular genetics features of the obesity pathogenesis using in vivo experimental models in laboratory animals.*
The aim. *To determine metabolism effector links in obesity based on a comparative analysis of full-transcriptome profiles of the liver tissue of mice and rats of various strains.*
Materials and methods. *We carried out a comparative analysis of the changes in liver transcriptome in rats and mice fed with diets of excessive energy value and exerting lipogenic effect. Data of full-transcriptome profiling using DNA microarray technology have been presented previously in 8 publications.*
Results. *In three strains of mice treated with a high-carbohydrate high-fat diet (HCHFD), a significant differential expression (DE) of 1849 genes was revealed, of which 74 genes responded jointly in at least two groups of animals. In Wistar and Zucker^{fa} rats, 2109 genes responded to the consumption of HCHFD, of which 242 genes responded jointly in two groups of animals. For rodents different in genetic predisposition to the development of diet-induced obesity, the groups of genes that responded with the opposite sign of DE (depending on the genotype) in reaction to the consumption of HCHFD were identified. Bioinformatical analysis allowed establishing the presence of 43 metabolic pathways, which are targeted for the applied experimental diets exposure, in rats and 77 pathways – in mice. Four of these pathways – the pathway of retinoid metabolism, PPAR signaling pathway associated with it the previous one, xenobiotics metabolism and drugs metabolism mediated by cytochrome P450 system – responded in all groups of animals (except for female mice). The importance of the expression of Tat gene encoding tyrosine aminotransferase in the modulation of biogenic amines synthesis in diet-induced obesity was shown, which may represent a new neurometabolic regulatory function of the liver in response to the consumption of high-calorie diets.*
Conclusion. *The analysis of the results of full-transcriptome studies showed that within each studied species (Rattus rattus and Mus domesticus) and animal sex, a number of genetic variants with a greater or lesser predisposition to the development of diet-induced obesity phenotype can be identified; and at the same time, within these variants, there is a largely similar pattern in the response of metabolism effector links to hypercaloric dietary intake. This pattern creates new prospects for translating the results of transcriptomic and metabolomic studies of laboratory animals into clinical practice in order to substantiate new approaches to personalized diet therapy of alimentary dependent diseases in patients with different genetic predisposition to obesity.*

Key words: *obesity, in vivo models, rats, mice, liver, transcriptome, metabolic pathways, neurometabolic function*

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ЭФФЕКТОРНЫЕ ЗВЕНЬЯ МЕТАБОЛИЗМА ПРИ ДИЕТ-ИНДУЦИРОВАННОМ И ГЕНЕТИЧЕСКИ ДЕТЕРМИНИРОВАННОМ ОЖИРЕНИИ: ПОЛНОТРАНСКРИПТОМНОЕ ИССЛЕДОВАНИЕ ТКАНИ ПЕЧЕНИ НА ЭКСПЕРИМЕНТАЛЬНЫХ МОДЕЛЯХ У ГРЫЗУНОВ

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РЕЗЮМЕ

Обоснование. *При разработке методов персонализированной диетотерапии ожирения актуальной задачей является изучение молекулярно-генетических особенностей его патогенеза с использованием экспериментальных моделей у лабораторных животных.*

Цель исследования. *Определение эффекторных звеньев метаболизма при ожирении на основе сравнительного анализа полнотранскриптомных профилей ткани печени мышей и крыс различных линий.*

Методы. *Проведён сравнительный анализ изменений транскриптома печени крыс и мышей, получавших рационы с избыточной калорийностью и липогенным действием. Данные полнотранскриптомного профилирования с использованием технологии ДНК-микрочипов были представлены ранее в 8 публикациях.*

Результаты. *У мышей трёх линий, получавших высокоуглеводный высокожировой рацион (ВУВЖР), выявлена достоверная дифференциальная экспрессия (ДЭ) 1849 генов, из которых 74 совместно ответили как минимум в двух группах животных. У крыс линий Wistar и Zucker^{fa} на потребление ВУВЖР ответили 2109 генов, из них 242 – в двух группах животных совместно. Для грызунов, различающихся по генетической предрасположенности к развитию диет-индуцированного ожирения, были определены группы генов, ответивших противоположной по знаку ДЭ (в зависимости от генотипа) в ответ на потребление ВУВЖР. Биоинформатический анализ позволил установить наличие у крыс 43, а у мышей – 77 метаболических путей, являющихся мишенями воздействия применяемых экспериментальных рационов. Из них 4 – путь обмена ретиноидов, сопряжённый с ним PPAR-сигнальный путь, метаболизм ксенобиотиков и метаболизм лекарственных препаратов под действием системы цитохрома P450 – ответили у всех групп животных (за исключением самок мышей). Показана важная роль экспрессии гена Tat, кодирующего тирозинаминотрансферазу, в модуляции синтеза биогенных аминов при диет-индуцированном ожирении, что, возможно, является новой нейрометаболической регуляторной функцией печени в ответ на потребление высококалорийных рационов.*

Заключение. *Анализ результатов полнотранскриптомных исследований показал, что в пределах каждого изученного вида (Rattus rattus и Mus domesticus) и пола животных можно выявить ряд генетических вариантов с большей или меньшей склонностью к развитию фенотипа диет-индуцированного ожирения; при этом в пределах каждого из этих вариантов отмечается во многом сходный характер ответа эффекторных звеньев метаболизма на потребление гиперкалорийного рациона. Эта закономерность создаёт новые перспективы для трансляции результатов транскриптомных и метаболомных исследований на лабораторных животных в клиническую практику для обоснования новых подходов к персонализированной диетотерапии алиментарно-зависимых заболеваний у пациентов, различающихся по генетической предрасположенности к ожирению.*

Ключевые слова: *ожирение, in vivo модели, крысы, мыши, печень, транскриптом, метаболические пути, нейрометаболическая функция*

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INTRODUCTION

Alimentary dependent diseases, the leading development cause of which is an excess in energy value consumed and (or) unbalanced diet, represent one of the main challenges for modern medicine. In recent years, a large amount of experimental data has been accumulated that the pathogenesis of alimentary dependent diseases is based on the influence of excessive or unbalanced intake of macronutrients – fats and carbohydrates – on the expression of a large number of genes functionally related to the processes of lipogenesis, carbohydrate energy and protein metabolism, thermoregulation, systemic inflammation, circadian rhythms, and control of the amount of food consumed. The amplitude and trend of gene expression changes due to dietary factors are determined both by the genotype of the organism (the presence of polymorphisms in the genes of key enzymes, regulatory and transport proteins) and epigenetic factors (DNA methylation, histone acetylation, etc.), at the last in turn are also influenced by the composition of the diet [1]. The totality of issues of interaction between the genotype of an organism and such an important environmental factor as the composition of dietary nutrients in the formation of phenotype at the molecular, cellular, tissue, organ and organismic levels is the subject of study of a new section of nutrition science known as nutrigenomics [2].

The data obtained in the course of nutrigenomics research play an important role in the development of methods of personalized diet therapy of alimentary dependent diseases, taking into account such factors as the patient's genotype, nutritional status, stage and severity of the pathological process. Since nutrigenomics research in the clinic faces certain difficulties associated with the choice of a suitable biosubstrate, preclinical studies using *in vivo* experimental models of relevant diseases in laboratory animals characterized by different genetically-based propensity to disorders of lipid and carbohydrate-energy metabolism are in demand. A series of such models was developed based on the use of both genetically modified (mutant or knockout) and conventional strains of animals receiving excessive fat, simple sugars, or a combination of both (the so-called "Western diet" or the "cafeteria diet") [3]. Using up-to-date methods of molecular genetics and transcriptomics, a significant amount of data was obtained that in such animals during the development of obesity there are persistent changes in the expression of key gene groups responsible for metabolic processes in the liver and adipose tissue. For example, mice treated with a high-carbohydrate high-fat diet (HCHFD) for 1 year show differential expression (DE) of genes responsible for metabolic pathways of β-oxidation of fatty acids, steroid hormone biosynthesis and degradation, PPAR signaling, antigen processing and presentation, and proteasomal protein degradation [4]. Transcriptomics methods have been used to identify effector genes that are targets of dietary factors in dogs treated with a hypercaloric diet [5], in mice with diet-induced obesity (DIO) and non-alcoholic fatty liver disease [6]. Epigenetic factors contributing to the transmission of obesity phenotype from pregnant female rodents to their offspring have been characterized [7]. If suitable biomaterial (e. g., white adipose tissue specimens obtained during bariatric surgery) is available according to the requirements of medical ethics, it is possible to translate transcriptomic diagnostic criteria into clinical practice [8].

At the same time, the nature of nutrigenomic mechanisms determining a greater or lesser predisposition of the organism to the development of diet-induced obesity, which can be determined by comparative analysis of the results of transcriptomic studies performed using animals of different species and strains, is currently insufficiently covered in the literature. In a series of studies carried out in the Federal Research Center of Nutrition, Biotechnology and Food Safety during 2017–2022, the method of full-transcriptome analysis of liver tissue on a DNA microarray was used to identify effector links of metabolism – targets of the effect of minor bioactive substances of the diet (BAS), such as polyphenolic compounds, L-carnitine, amino acids, on models of DIO and spontaneous (genetically determined) obesity in rats and mice [9–13]. A number of rat strains (Wistar and Zucker^{fa}) and mice (C57Bl/6J, DBA/2J male strains, terahybrid mice) used in this study differed in the severity of obesity phenotype when consuming diets with excessive caloric intake, but the question of what differences in transcriptome parameters are the most significant in animals more or less prone to the development of DIO has not been sufficiently covered in these publications.

The aim of this work is to analyze data from transcriptomic studies on the nature of the liver transcriptome response to hypercaloric diets from a comparative perspective in rats and mice of different strains varying in susceptibility to the development of diet-induced or spontaneous obesity.

MATERIALS AND METHODS

The materials of full-transcriptome studies performed in mice and rats of different sexes and strains fed for 8–9 weeks a balanced control diet according to AIN-93M or modified semi-synthetic diets with a relative excess of fat (high-fat diet (HFD)), simple carbohydrate fructose (HFrD)), their combination (HCHFD) or cholesterol (high-cholesterol diet (HCD)) were used in this paper. Table 1 summarizes the experiments with references to publications revealing their design. All studies were conducted in compliance with the rules of biomedical ethics and were approved by the decision of the Ethics Committee of the Federal Research Center of Nutrition, Biotechnology and Food Safety (FSBIS) (record No. 4 of 20.04.2017).

After removal of animals from the experiment, liver tissue samples were obtained from them, from which total RNA was isolated using reagents of Agilent Total RNA Isolation Mini Kit (Agilent Technologies, Inc., USA) and full-transcriptome analysis was performed on Gene Expression Hy-

bridization Kit DNA microarrays (Agilent Technologies, Inc., USA) according to the Agilent One-Color Microarray-Based Gene Expression Analysis Low Input Quick Amp Labeling, version 6.8, protocol (Agilent Technologies, Inc., USA), using authorized certified equipment of the manufacturer (Agilent Technologies, Inc., USA).

The used DNA microarrays of different series contained between 30,000 and 32,000 annotated individual complementary sequences of the rat or mouse genome, including untranslated DNA sequences and splice variants. In all microarray experiments, 4 independent matrix RNA (mRNA) samples from each group of animals were analyzed. Microarrays were scanned on a SureScan Microarray Scanner (Agilent Technologies, Inc., USA). The DE value of genes determined by microarray analysis was expressed as the binary logarithm of the change in fluorescence (log_2FC) compared to control groups of animals treated with a balanced diet or to internal microarray controls (Spike-In). DE data were loaded into the «R» environment and bioinformatic analysis was performed with quantile normalization and further analysis in the limma package. The AnnotationDbi, org.Rn.eg.db, pathview, gage, and gageData packages were used to identify metabolic pathways using the Kyoto Encyclopedia of Genes and Genomes (KEGGs) international resource and visualize them. Statistical significance of expression changes was assessed using the the Benjamini – Hochberg multiple-correction T-test. Additionally, linear regression analysis was performed with calculation of Pearson correlation coefficients (PCC) between DE values (R) and their statistical significance (α). Annotation of genes that responded with statistically significant DE values during the deve-

TABLE 1

GROUPS OF ANIMALS USED IN EXPERIMENTS TO STUDY THE EFFECT OF OBESITY AND DYSLIPIDEMIA ON THE LIVER TRANSCRIPTOME

Species, sex of animals	Strain	*In vivo* model	Experimental diet composition[2]	References to publications
Female rats	Wistar[1]	Metabolic syndrome (MetS)	Semi-synthetic according to AIN-93M with replacement of drinking water with 30 % fructose solution (HFrD)	[10]
Female mice	C57Bl/6J[1]	MetS	HFrD	[9]
Female rats	Wistar[1]	Dyslipidemia (Dis)	Semi-synthetic according to AIN-93M with replacement of 0.5 % fat by weight of diet with cholesterol (HCD)	[10]
Female mice	C57Bl/6J[1]	Dis	HCD	[9]
Female rats	Wistar[1]	DIO	Semi-synthetic according to AIN-93M with replacement of 20 % of starch by weight of diet with fat (HFD)	[10]
Female mice	C57Bl/6J[1]	DIO	HFD	[9]
Male rats	Wistar[1]	DIO	Semi-synthetic according to AIN-93M with replacement of 20 % starch by weight of diet with fat, drinking water – with 20 % fructose solution (HCHFD)	[11, 12]
Male rats	Zucker[fa 3]	DIO	HCHFD	[13]
Female mice	C57Bl/6J[1]	DIO	HCHFD	[9]
Male mice	C57Bl/6J[1]	DIO	HCHFD	[13]
Male mice	DBA/2J[1]	DIO	HCHFD	[12, 13]
Male mice	DBCB[4] tetrahybrids	DIO	HCHFD	[13]

Note. [1] – obtained from the farm of Stolbovaya branch of the Scientific Center for Biomedical Technologies of FMBA of Russia; [2] – animals of control groups of each species, strain and sex received a balanced macronutrient and basic micronutrient semi-synthetic diet AIN-93M; [3] – obtained from Charles River farm (Italy); [4] – DBCB mice, hybrid of the 2nd generation (F2), were independently bred by the author of the study (S.A.A.) in the vivarium of the Federal Research Center of Nutrition, Biotechnology and Food Safety by sequential hybridization of mice of parental strains DBA/2J, CBA/lac (females) and BALB/c, C57Black/6J (males), as described in [13].

lopment of DIO in animals was performed using the international online resource Genemania (https://genemania.org) integrated with the PubMed (https://pubmed.ncbi.nlm.nih.gov) scientometric database. Set-theoretic analysis, construction of Venn diagrams and heat maps were performed using Venny 2.1 network application (https://bioinfogp.cnb.csic.es/tools/venny/) and MS Excel 2007 spreadsheets (Microsoft Corp., USA).

RESULTS AND DISCUSSION

Response of the liver transcriptome to the development of DIO in mice

As a result of full-transcriptome analysis of the livers of mice of four groups belonging to three strains (females and males of the C57Bl/6J strain, males of the DBA2J strain, males of the DBCB tetrahybrid strain), when comparing animals treated with HCHFD, with mice of control groups revealed DE at the level of more than 0.5 units modulo $\log_2$(FC) (i. e. 1.41-fold or more up or down) for 1,849 genes, of which 74 genes responded in at least two groups of animals together. Full data on the experiments performed have been presented previously [9, 13]. The analysis of the DE heat map of these genes (Fig. 1a) showed that the greatest similarity in the DE profile in response to the consumption of HCHFD is characterized by linear male C57Bl/6J and DBA2J mice, which, according to earlier studies, have a relatively high phenotypic resistance to the development of DIO [13], to which female C57Bl/6J mice adjoin, and DBCB tetrahybrid mice are characterized by the lowest degree of similarity with the other groups. Notably, it was the terahybrid mice that were the most phenotypically prone to the development of DIO according to the data of body weight gain and morphological signs of fatty liver disease (steatosis) [13]. Regression analysis showed that there was a statistically significant positive linear regression ($R = +0.562$; $\alpha = 0.005$) between the DE values of genes that responded jointly in C57Bl/6J and DBA2J males (Fig. 1b), whereas for DBA2J males and DBCB tetrahybrids (Fig. 1c), in contrast, the corresponding regression was negative ($R = -0.689$; $\alpha < 0.001$), and for DE comparisons between DBCB mice and C57Bl/6J males and between males and females of the latter strain (Fig. 1d, e), there was no statistically significant regression ($\alpha > 0.1$).

Thus, the genetic expression profile in different strains of mice phenotypically similar in resistance to developing of DIO is largely consistent, whereas in mice that differ markedly in their propensity to develop DIO (such as DBA2J and DBCB), the DE trend appears to be largely opposite.

Annotation of genes that responded with DE in mice of different strains showed several groups of genes that responded with opposite sign values of DE to HCHFD intake in DBCB tetrahybrid mice and DBA/2J, C57Bl/6J linear animals, which are among the parental strains of the indicated tetrahybrid. Of these, the most representative were two groups of genes belonging to metabolic pathways of lipid metabolism, the first of which included *Tff3, Scd1, Pltp* (positive DE in DBCB and negative DE in DBA2J and/or C57Bl/6J mice), and the second group included *Pparg, Usp18, Crot, Ifi202b, Mvk, Sqle, Msmo1, Idi1* (the DE trend was opposite to the first group of genes). *Tff3* expression in rodent liver is highly susceptible to changes in models of early diabetes and fatty liver disease. TFF3 protein reduced hepatic steatosis induced by a high-fat diet by increasing PPARα-mediated fatty acid oxidation [14]. The function of *Scd1* is to synthesize oleate from stearate and palmitooleate from palmitate. Suppression of *Scd1* expression protects mice from developing hepatic steatosis and obesity [13]. The *Pltp* gene is responsible for the synthesis of phospholipids, which are part of lacrimal fluid, pulmonary surfactant, and others. Cholesterol, phospholipid, apolipoprotein A1 and apolipoprotein B, and high-density lipoprotein concentrations in plasma were decreased in mice with knockout of this gene. Besides, PLTP protected mice from the development of atherosclerosis without causing lipid accumulation in the liver [15].

Pparg encodes a nuclear transcription factor that affects genome elements called peroxisome proliferators and which are responsible for regulating a complex set of genes involved in metabolism and peroxisomal β-oxidation of fatty acids, adipocyte differentiation, and glucose homeostasis. Its expression is associated with other *Ppar* family genes as well as *Fabp4*, *Mapk1*, etc. PPARγ functions in an ensemble with retinoid X receptors (RXR). The role of *Pparg* in the activation of Ito fat-storing cells in the liver has been proven [16]. The *Crot* gene is responsible for the attachment of medium-chain fatty acid residues to carnitine during their peroxisomal β-oxidation [13]. *Usp18* stimulates lipolysis, fatty acid oxidation in transformed lung epithelial cells, and protects mice from hepatic steatosis and the development of insulin resistance [17]. The expression of the gene *Ifi202b*, which is involved in the differentiation of adipose stem cells into mature adipocytes, is closely associated with it. Increased expression of *Ifi202b* stimulates adipogenesis in mice and humans [18]. A similar expression profile in tetrahybrid mice compared to linear mice was observed in a functionally closely related group of genes *Msmo1, Mvk, Idi1*, and *Sqle1* involved in cholesterol and steroid metabolism. Of these, *Msmo1*, as reported in the literature, has the ability to suppress adipogenesis and differentiation of immature adipocytes [19]. On the other hand, *Sqle* has been identified as one of the genes that contribute to obesity in mice [20].

A group of genes involved in mineral metabolism responded differently to DE values in linear and tetrahybrid mice. Of these, *Mt1* and *Mt2* were characterized by negative DE in DBA/2J mice and positive DE in DBCB mice. The proteins encoded by these genes are metallothioneins, which play a vital role in the homeostasis of a number of essential (copper, zinc, manganese) and toxic (cadmium, lead, mercury) trace elements; the amount of synthesized metallothioneins is associated with the development of obesity and hepatic steatosis [13]. In connection with the revealed facts, it is appropriate to note that an earlier study on DBA/2J and DBCB mice treated with HCHFD revealed a sig-

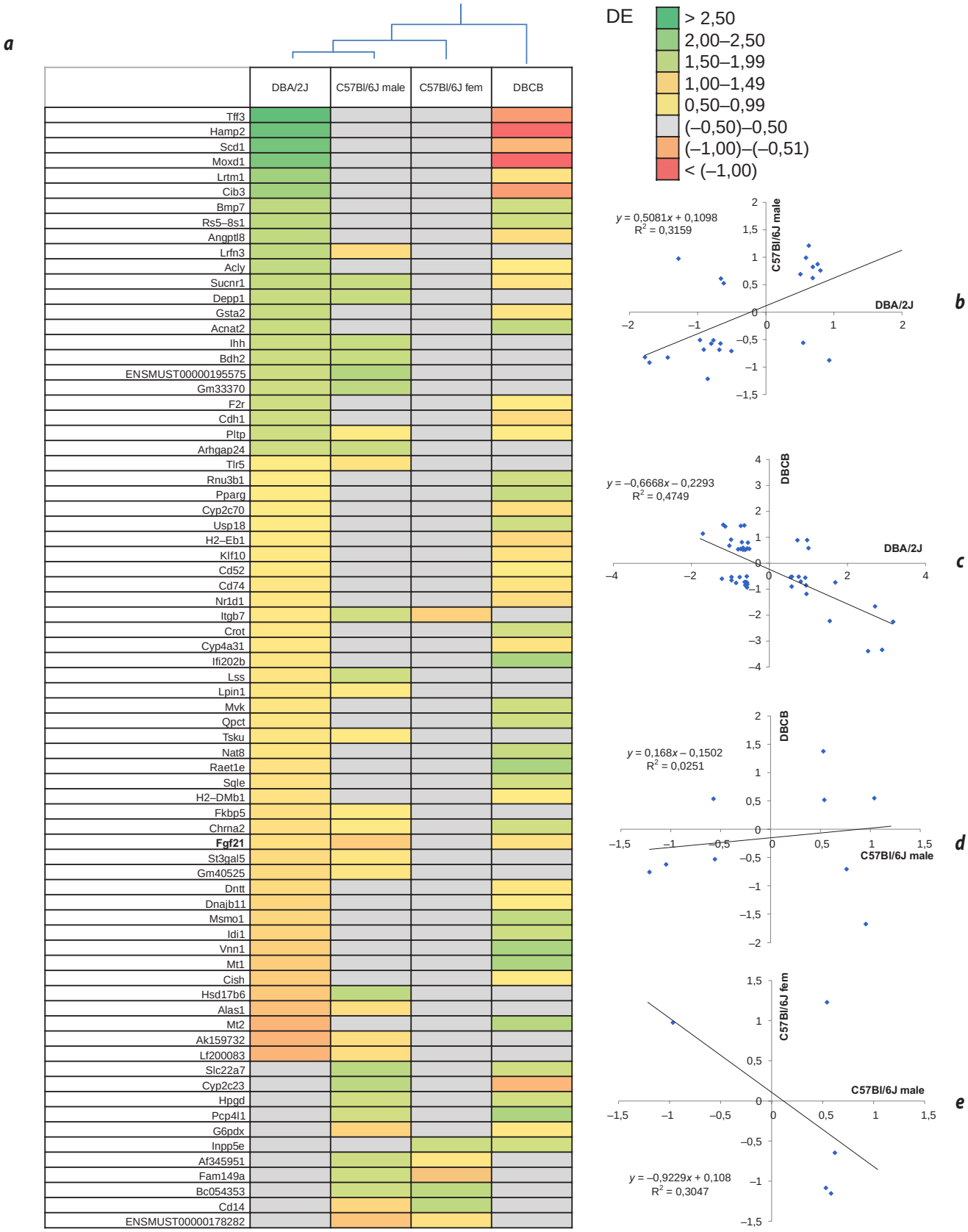

FIG. 1.

Comparative analysis of the differential expression values of genes that jointly responded to the consumption of high-carbohydrate high-fat diet in DBA/2J mice (males), C57Bl/6J (males and females), and tetrahybrid DBCB (males): ***a*** *– heat map of differential expression of genes in mice of 4 groups;* ***b*** *– DBA/2J – C57Bl/6J (males) regression;* ***c*** *– DBA/2J – DBCB regression;* ***d*** *– C57Bl/6J (males) – DBCB regression;* ***e*** *– C57Bl/6J (males) – C57Bl/6J (females) regression. The degree of similarity (X) was calculated according to the formula: X = N × (C – M), where N is the total number of jointly responding genes; C is the number of genes with the same sign of differential expression; M is the number of genes with the opposite sign of differential expression*

nificant effect of their genotype on the bioaccumulation indices of a number of divalent cations of trace elements (lead, manganese, copper, cadmium, etc.) [13].

The opposite profile of DE (positive in linear mice and negative in DBCB tetrahybrids) was characteristic of *Moxd1* and *Hamp2*. *Moxd1* encodes a copper binding protein that has dopamine β-monooxygenase activity and is involved in the biosynthesis of the biogenic amines octopamine and norepinephrine. The expression values of *Moxd1 and Dbh* (dopamine beta hydroxylase) are closely related to each other as analyzed by https://genemania.org. The significance of these data will be further discussed below. The product of *Hamp2* expression is the peptide hormone hepcidin, which regulates iron metabolism. Elevated plasma levels of this protein have been observed in obese children, which correlates with reduced iron status and the development of systemic inflammation as assessed by interleukin (IL) 6 production [21].

A number of genes involved in antigen presentation, cytokine signaling pathways, and inflammation development also responded with the opposite sign of DE to HCHFD consumption in DBCB tetrahybrid and linear mice. These include the *Sucnr1, Cdh1, Raet1e, and F2r* genes. The specific DE profiles of these genes may be correlated with differences in cytokine production in HCHFD-receiving DBCB tetrahybrids and linear DBA/2J mice in response to the consumption of a number of BAS [13].

Response of the liver transcriptome to the DIO development in rats

When analyzing the transcriptome of male Zucker^{fa}, female Wistar, and male Wistar rats (two repeats of the experiment) treated with HCHFD, the DE was detected for 2,109 genes at the level modulo more than 0.5 $\log_2$(FC) units compared to the group of animals of the same strains receiving the control diet. Of these, 174 genes responded jointly in males of the Zucker^{fa} and Wistar strains; 11 in males and females of the Wistar strain; 6 in males of the Zucker^{fa} and females of the Wistar strains; and 51 in males of the Wistar strain in two repeated experiments. Details of the liver transcriptome obtained in these experiments have been reported previously [10, 11, 13].

A heat map of the distribution of genes according to the value of their DE (Fig. 2a) shows that the greatest similarity in the DE profile is observed for males of the Wistar and Zucker^{fa} strains, whose difference from Wistar females is more significant. According to Figure 2b, there is a statistically significant (R = +0.422; α < 0.001), although not very powerful positive regression between DE values for Wistar and Zucker^{fa} males. The data in Figure 2c show that the regression between the DE of the genes reproduced in two repeats of the experiment on male Wistar rats is positive, highly statistically significant (R = +0.947; α < 0.001), and the regression line runs near the origin. For males and females of the Wistar strain (Fig. 2d), the regression relationship of the DE of jointly responded genes is statistically significant and negative (R = –0.651; α = 0.042), that is, the response of the transcriptome to HCHFD consumption is to a certain extent discordant between them.

Annotation of genes that responded with opposite sign of DE to HCHFD consumption in male Zucker^{fa} and Wistar rats identified several groups of genes responding during DIO development, including *Abhd2, Cpt1a, Kiss1, Myc, Myc, Prlr, Ppp1r3c, Tsc22d1*, and *Upp2*. *Cpt1a,* encoding carnitine palmitoyltransferase I, is characterized by negative expression in Zucker^{fa} males and positive expression in Wistar males. Positive expression of this gene, responsible for the process of β-oxidation of fatty acids, is considered as a normal response of the organism to the consumption of excess fat [13]; it can be assumed that this mechanism is disturbed in Zucker^{fa} rats.

A similar DE profile is characteristic of the *Kiss1* gene encoding the precursor neuropeptide kisspeptin, which is presumed to have an anorectic action (reducing appetite and food intake) [13]. According to numerous data in the literature, decreased *Kiss1* expression is observed during the development of diet-induced obesity [13]. The same trend in rats of the two strains has the expression of *Abdh2* (hormone-sensitive lipase subunit), *Upp2* (cofactor of hepatic X receptor, PPARα- and HNF-4α-signaling pathways), *Tsc22d1* (coregulator of PPARα), *Prlr* (prolactin receptor, which stimulates lipid oxidation and thermogenesis in brown adipose tissue, one of the cofactors of dopamine action [22]) and *Ppp1r3c* (a protein phosphatase responsible for the regulation of glycogen storage in liver cells).

The opposite trend, i. e. positive in Zucker^{fa} rats and negative in Wistar males, of DE was observed for the *Myc* gene. It encodes a multifunctional nuclear phosphoprotein that plays a role in cell cycle, apoptosis and malignant transformation. Its functions include triggering the development of liver fibrosis and regulation of glucose-responsive genes through the CHREBP signaling pathway [23].

The next group of genes that contrastingly responded to HCHFD consumption in rats of the two strains under study includes genes responsible for implementing the function of proto-oncogenes (i. e., intracellular protein phosphorylation signaling cascade) and stimulation of apoptosis. In addition to the analyzed *Myc*, these include *Jun, Atf3, Dusp6,7, Epcam*, and *Casp4*. In particular, Jun protein kinase, positively expressed in Zucker^{fa} and negatively expressed in Wistar, is a component of the JNK signaling chain responsible for the developmental consequences of oxidative stress, including apoptosis, and insulin resistance. According to [24], *Jun* expression is upregulated in mice with hepatic steatosis induced by HFD consumption. *Jun* is also functionally related to *Mlxipl*, which regulates glucose metabolism, and *Kiss1*, discussed above.

Among the differentially expressed genes involved in inflammation and cytokine signaling pathways, *Ccl3* (Mip-1a), *CD274,276* and *Cish* genes are characterized by positive DE in Zucker^{fa} and negative DE in Wistar, while *Ackr2, Bcl6* and *Bmf* genes have the opposite DE profile. Of these, *Bcl6* is a functionally relevant gene whose expression product suppresses IL-6-stimulated macrophage proliferation [25], IL-18 synthesis, and Th2 cell differentiation, which together may contribute to the control of systemic inflammation induced by excessive adipogenesis. In Zucker^{fa} rats, this molecular mechanism appears to be disrupted.

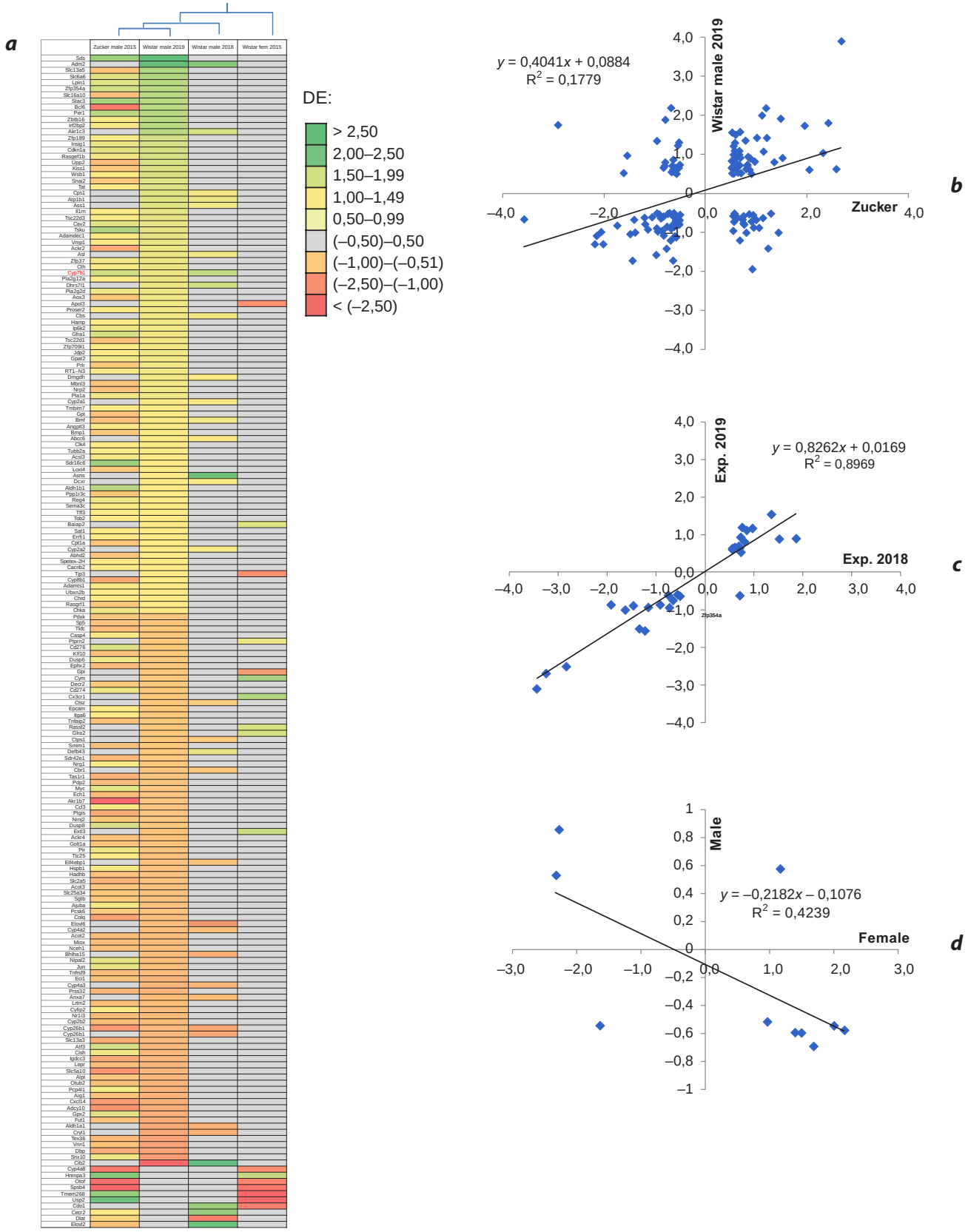

FIG. 2.

Comparative analysis of the differential expression values of genes that jointly responded to the consumption of high-carbohydrate high-fat diet in the Zucker^{fa} rat lines (males), Wistar rats (males (2 experiments) and females): ***a*** *– heat map of differential expression of genes in 4 groups of rats;* ***b*** *– Zucker^{fa} – Wistar (males, experiment of 2019) regression;* ***c*** *– regression for two groups of Wistar males in experiments of 2018 and 2019;* ***d*** *– Wistar females and males groups regression. The degree of similarity (X) was calculated according to the formula: X = N × (C – M), where N is the total number of jointly responding genes; C is the number of genes with the same sign of differential expression; M is the number of genes with the opposite sign of differential expression*

It is also necessary to point out the opposite trend in Zucker^{fa} and Wistar rats of the HCHFD-induced DE of a number of genes (*Aox3, Bmp1, Gpx2, Hspb1, Lox4, Pir*) responsible for the processes of mineral metabolism (binding of iron, copper), the function of bioantioxidants (selenium) and inhibition of oxidative stress. The same applies to genes of the steroid hormone metabolic pathway such as *Cyp8b1* and *Nrg1*, as well as amino acid metabolism and transport (*Gpt, Slc16a10*). At the same time, alanine aminotransferase (ALT), whose expression is decreased in Zucker^{fa} rats and activated in Wistar rats upon HCHFD consumption, is a gene suppressed by insulin and, like *Tat* discussed below, stimulated by glucocorticoids [26]. ALT plays an important role in protein catabolism and gluconeogenesis [13].

Comparing features of the liver transcriptome response to HCHFD between rats and mice

Genes in the PPARγ signaling pathway, xenobiotic metabolism by cytochromes P450, retinol metabolism, transamination, and other pathways of aromatic amino acid metabolism were common with respect to differential gene expression for both rodent species' metabolic regulatory processes.

Typically, genes related to transamination and apoptosis were activated in both rodent species.

The processes common to the two rodent species indicate that transamination and apoptosis are among the most relevant processes in the regulation of metabolic reactions in rodents in response to the consumption of a high-carbohydrate high-fat diet (HCHFD), determining the ratio of catabolic to anabolic reactions along with other transaminases such as aspartate aminotransferase (AST) and ALT. The differences between the two rodent species may be due to differences in ATP-dependent processes, regulatory cascades (PPARγ), mechanisms of energy metabolism (coenzyme A), oxidative stress (glutathione) and apoptosis between species and animal lines.

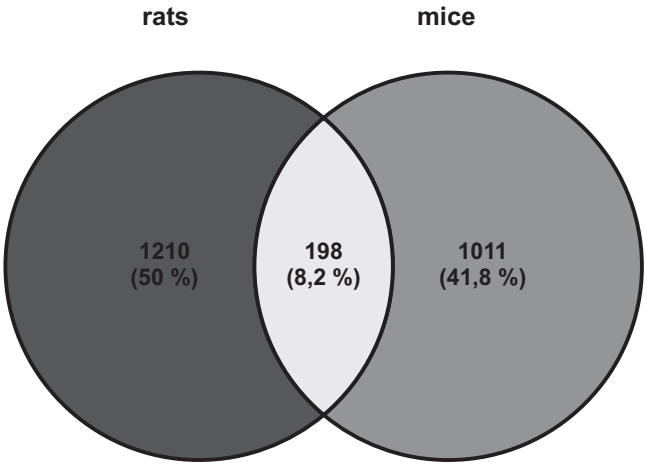

FIG. 3.
Venn diagram of differential expressions of genes in rats and mice of different strains that jointly responded to the consumption of high-carbohydrate high-fat diet

Comparative analysis of the results of studies of different mouse and rat strains revealed 198 DE genes common to both rodent species (Fig. 3). The genes that jointly responded by differential expression to HCHFD consumption in at least one of the studied mouse strains and in rats were *Cish, Lpin1, Nat8, Pcp4l1, Tsku, Bmp7, Cd52, Cd74, Depp1, Fgf21, Idi1, Ihh, Klf10, Lss, Nr1d1, Pparg, Tff3, Usp18, Vnn1*, with the first five of this list responding in both Zucker^{fa} rats and Wistar males. Some of the genes listed above play a key role in the regulation of lipid oxidation, lipogenesis, and adipose tissue proliferation.

For example, *Lpin1* encodes phospholipase C, which generates diacylglycerol, a coregulator of a large number of transcription factors. Overexpression of *Lpin1* in mice suppresses the development of alcoholic hepatitis by inhibiting lipolysis and reducing the amount of fatty acids entering the liver, and suppression of the activity of this gene is observed in the development of metabolic syndrome [13]. *Tsku* expression is associated with *Ppara* and is increased in non-alcohol related steatohepatitis [27]. *Fgf21* is an important hepatokine with pleiotropic function known as a metabolic regulator of glucose and lipid homeostasis with anorexigenic effects [28]. Finally, *Vnn1,* considered as a biomarker of toxic kidney injury and under the control of PPARα, plays a role in inflammation, oxidative stress and cell proliferation [29].

DE of the *Tat* gene encoding tyrosine aminotransferase was detected under HCHFD exposure in male Wistar and Zucker^{fa} rats, male DBA/2J mice and female C57Bl/6J mice, and spontaneously obese db/db mice compared with their parental C57Bl/6J strain (described in detail below). Other differentially expressed genes common to both rodent species were *Plekhf1* (pleckstrin homology domain), *Atp1b1* (Na^+/K^+-ATPase b1-subunit), and *Chka* (choline kinase alpha). The Na^+/K^+-ATPase b1-subunit is a plasma membrane pump with multiple physiological functions. It maintains ion homeostasis, which is crucial for cell survival, differentiation and apoptosis [30]. A similar pattern of positive differential expression in liver tissue to the *Tat* gene was obtained for the *Plekhf1* (*LAPF*) gene in the Wistar rat strain and the DBA/2J inbred mouse strain, but not C57Black/6J. The function of this gene in the pathogenesis of obesity is currently unclear. Chka is the second ATP-dependent protein to show directly opposite hepatic expression patterns in mice and rats. This may be due to differences in the rate of ATP-dependent energy metabolism processes between the two rodent species (it is higher in mice).

The role of genes such as *Idi1, Cish, Pparg*, *Tff3* and *Usp18* in adipogenesis and development of DIO has been discussed above.

Metabolic pathways of rats and mice of different strains responding to consumption of hypercaloric and hyperlipidemic diets

Bioinformatic analysis of DE genes identified metabolic pathways (KEGGs) statistically significantly altered by experimental hypercaloric diets. Details of the findings on KEGGs targets of different dietary exposures

in experiment are presented in previous publications [9–13].

In mice of the C57Bl/6J strains (male and female), DBA/2J males (in two repeats of the experiment) and DBCB tetrahybrids, the effect of HCHFD on 77 metabolic pathways was detected at the $p < 0.05$ level of statistical significance. Heat map analysis of these influences, plotted against the statistical significance of the effect value (p_{val}), showed (Fig. 4a) that male C57Bl/6J and DBA/2J mice and, on the other hand, DBA/2J and DBCB tetrahybrids form two similar clusters, and their difference from male C57Bl/6J mice appears to be more significant. Thus, mouse sex appears to be a stronger determinant of the trend of changes in KEGGs during DIO compared to genotype (strain). Venn diagram set-theoretic analysis (Fig. 4b) indicates that there were no metabolic pathways that responded simultaneously to HCHFD in mice of all sexes and strains. However, in male C57Bl/6J and DBA/2J mice, 9 metabolic pathways responded jointly to HCHFD consumption, 9 metabolic pathways also responded in DBA/2J and DBCB tetrahybrids, 5 in male C57Bl/6J and DBCB mice, and 1 in female and male C57Bl/6J mice. Four metabolic pathways (mmu00982 Drug metabolism – cytochrome P450; mmu00980 Metabolism of xenobiotics by cytochrome P450; mmu00830 Retinol metabolism; mmu00330 Arginine and proline metabolism) responded in all groups of mice except for C57Bl/6J females.

Noteworthy differences in the nature of the effects of HCHFD on the mmu00830 Retinol metabolism and associated mmu03320 PPAR signaling pathways in male DBA/2J and DBCB mice contrastingly differ in the development of the DIO phenotype in response to HCHFD consumption. Figure 4c shows that DBCB tetrahybrids have a partially bypassed metabolic block at the stage of conversion of vitamin A to its active all-trans retinal form by retinol dehydrogenase (RDH), which may lead to reduced production of 9-cis-retinoate. The latter, in turn, is a ligand for the RXR receptor of the PPAR signaling pathway (Fig. 4d), differentially expressed in mice of the two strains. Along with the presence of differential expression of PPARβδ receptors, this leads to a series of multidirectional changes (partial or complete metabolic blocks) in the processes of transport and β-oxidation of fatty acids, which is probably reflected in the differences in the phenotype of these animals discussed above.

In male Zucker^{fa} and Wistar rats in two replications of the study (the experimental data are presented in detail in publications [11, 13]), the intake of HCHFD caused a total of changes in 43 metabolic pathways, of which 7 were common to all conducted studies (Fig. 5a). When comparing two parallel tests on male Wistar rats [11, 13], 18 out of 39 (46 % of the total) identified KEGGs were observed to match, which is a fairly good indicator for the reproducibility of transcriptomic studies according to the MAQC Consortium (cited in [13]) in light of the fact that the studies were performed on different cohorts of animals and using DNA microarrays of different series.

All 7 metabolic pathways, including rno00830 Retinol metabolism, rno00980 Metabolism of xenobiotics by cytochrome P450, rno00982 Drug metabolism – cytochrome P450, rno03320 PPAR signaling pathway, rno00590 Arachidonic acid metabolism, rno00140 Steroid hormone biosynthesis, rno01040 Biosynthesis of unsaturated fatty acids, that responded to HCHFD consumption in all groups of rats responded simultaneously to this exposure in at least part of the mouse groups, with the first four of these KEGGs responding simultaneously in three of the four mouse groups studied (all males). This indicates a fairly high measure of confidence in the identification of these KEGGs as targets of the effects of hypercaloric diet in various models of DIO in rodents. In total, 31 metabolic pathways responded jointly in rats and mice in at least two experiments to HCHFD consumption (Fig. 5b).

Using the heat map method (Fig. 5c), the measure of similarity in the response of different metabolic pathways to different hypercaloric (HFD, HFrD, HCHFD) and hyperlipidemic (HCD) diets in rats and mice was analyzed to identify possible dietary, genotypic, and gender patterns in this. The results of clustering by animal groups are shown in Figure 5d in the form of a graph ("phylogenetic tree"). They show a clear separation of female C57Bl/6J mice from all other animal groups (both rats and mice). On the other hand, two other clusters are evident in the response to the investigated diets, of which one is represented by male C57Bl/6J and DBCB mice on HCHFD and female Wistar rats on HFD and the other by male DBA/2J mice on HCHFD and female Wistar rats on HFrD and HCD. Female Wistar rats and male Zucker^{fa} rats, the most prone to develop DIO, do not fall into any of these clusters, demonstrating high specificity of their metabolic response.

When comparing the features of metabolic pathways in male Wistar and Zucker^{fa} rats contrasting in the severity of the obesity phenotype, it was noted that in the rno00830 Retinol metabolism pathway in Wistar rats treated with HCHFD, there is a partially overcoming metabolic block in the enzymatic pathways for the formation of all-trans-retinoate and the thermodynamically irreversible formation of 9-cis-retinoate from it, whereas in Zucker^{fa} rats both of these metabolic blocks apparently cannot be overcome. On the other hand, a metabolic block of glucuronidation of all-trans-retinoate occurs in Zucker^{fa} rats, which can lead to inhibition of its clearance. Taken together, these effects could hypothetically affect the ratio of cis- and trans-isomers of retinoic acid, which play different roles in the regulation of intracellular processes through interaction with RXR receptors (see above for a mouse example). However, proof of the possibility of such a mechanism is impossible without kinetic modeling of the corresponding enzymatic reactions, for which there is currently insufficient experimental data.

The rno00590 Arachidonic acid metabolism pathway may also play a significant role in the difference in response to HCHFD consumption between Zucker^{fa} and Wistar rats. Namely, Wistar rats receiving HCHFD have a metabolic block (which cannot be overcome or partially overcome) in the biosynthesis of PGF2α, 5-HETE and 15(S)-HETE, in contrast to Zucker^{fa} rats in which these reactions are, in contrast, activated. The consequence of these differences may

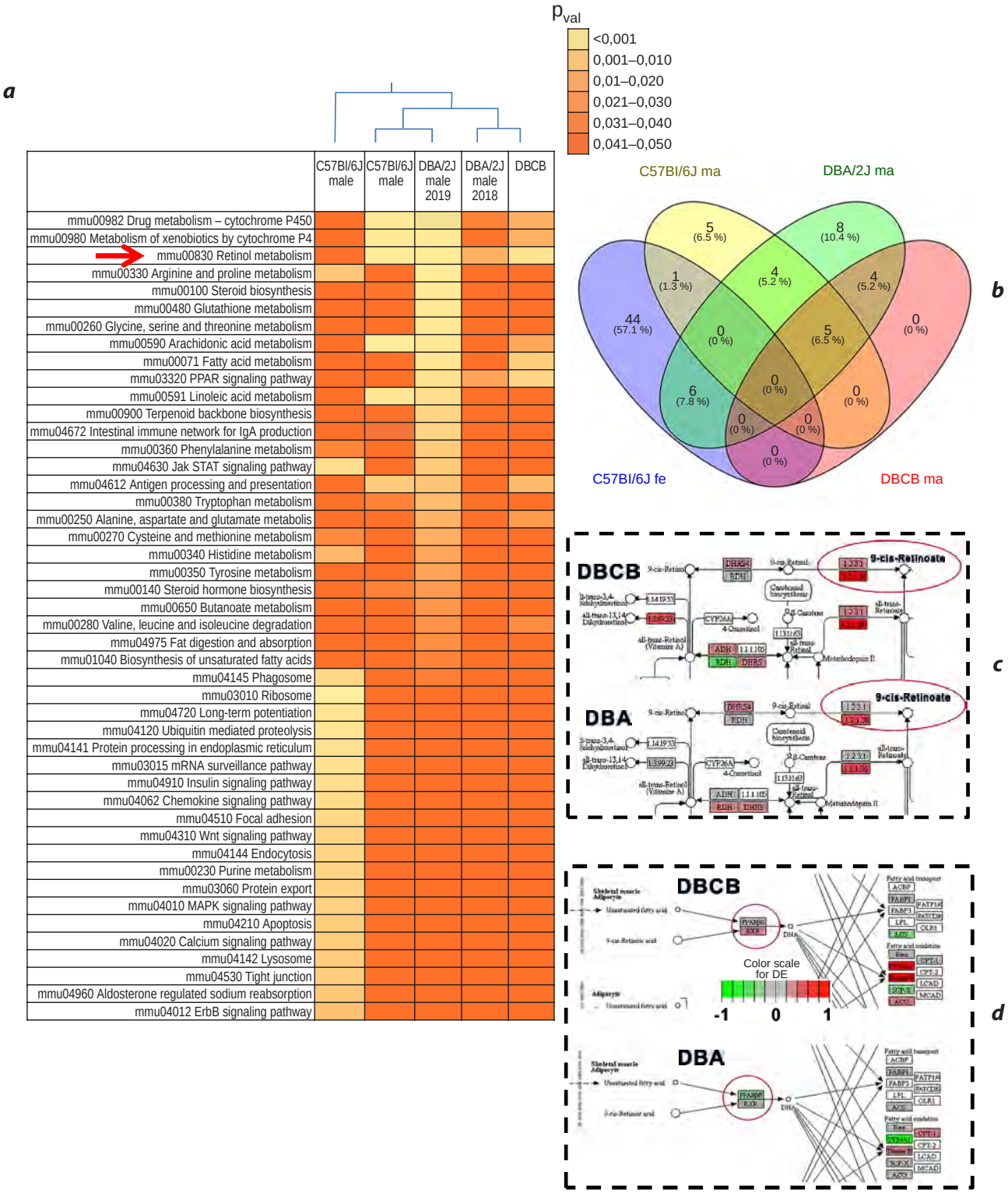

FIG. 4.

Comparative analysis of metabolic pathways (KEGGS) significantly ($p_{val} < 0.05$) responding to the consumption of high-carbohydrate high-fat diet in C57Bl/6J mice (males and females), DBA/2J (males, 2 experiments) and DBCB tetrahybrids: ***a*** *– heat map of the p_{val} values of the metabolic pathways in 5 groups of mice;* ***b*** *– Venn diagram of the distribution of metabolic pathways that jointly responded in different groups of mice;* ***c*** *– fragments of the metabolic pathway mmu00830 Retinol metabolism in DBCB and DBA/2J mice;* ***d*** *– fragments of the metabolic pathway mmu03320 PPAR signaling pathway in DBCB and DBA/2J mice. C57Bl/6J fe – C57Bl/6J females; C57Bl/6J ma – C57Bl/6J males; DBA/2J ma – DBA/2J males; DBCB ma – DBCB males. The measure of similarity was calculated by the criterion of the number of significantly ($p < 0.05$) jointly responded KEGGS*

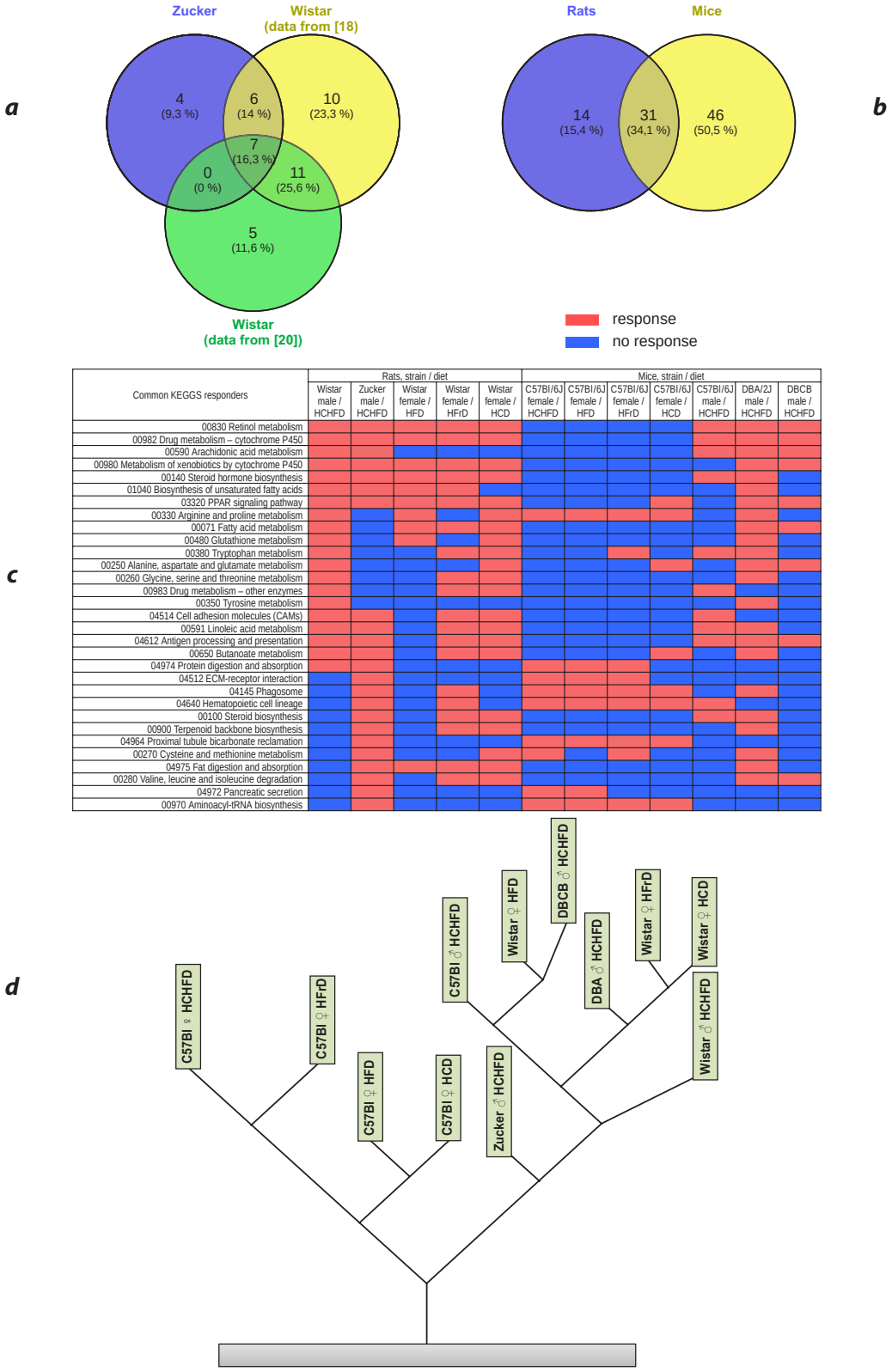

FIG. 5.

Comparative analysis of metabolic pathways (KEGGS) that jointly responded in rats and mice of different sexes and strains to the consumption of high-carbohydrate high-fat diet, as well as to the consumption of high-fructose, high-fat and high-carbohydrate diets (the last three – only in female C57Bl/6J mice and female Wistar rats): ***a*** *– Venn diagram of the distribution of metabolic pathways that responded to the consumption of high-carbohydrate high-fat diet by the groups of rats;* ***b*** *– Venn diagram of the distribution of the number of metabolic pathways that jointly responded to the consumption of high-carbohydrate high-fat diet in rats and mice (all studied sexes and lines);* ***c*** *– heat map of the responses of 31 metabolic pathways which jointly responded in rats and mice fed with various types of hyperlipidemic and hypercaloric diets;* ***d*** *– graph demonstrating a measure of similarity in the KEGGS response profile in different strains of rats and mice fed with the experimental diets*

be the different nature of the response in rats of these two strains of the oxylipin profile – derivatives of polyunsaturated fatty acids, which play an essential role in the expression regulation of a large number of genes, including those involved in the processes of lipogenesis, lipid (fat) metabolism, immune response and inflammation [13].

A significant limitation of the approach used in this paper is the incomplete coverage of diet-induced transcriptomic changes in mice and rats of both sexes and different strains, which is determined by the totality of data presented in the original studies [9–13] under comparable conditions. However, even on such a fragmentary sample of combinations of species, sex, and strains of animals, it became possible to reveal a general pattern consisting in the fact that under the influence of feeding hypercaloric diets in both rats and mice, the same groups of genes that respond oppositely with DE values to one and the same dietary factor, depending on the degree of genetic predisposition to the DIO development, are allocated. With a certain degree of schematic characterization, they may include 1) genes involved in the lipogenesis regulation and lipid metabolism; 2) genes of signaling pathways of proto-oncogenes and intracellular messengers, including those responsible for cell differentiation and apoptosis; 3) genes of inflammation factors, regulatory molecules of immune cells, cytokines and their receptors; 4) genes of proteins involved in the binding, transport and biological function of trace elements; 5) enzyme genes of amino acid metabolism, especially those capable of controlling the availability of substrates for the synthesis of biogenic amines (including trace amines and classical neurotransmitters) involved in the regulation of metabolic rate, physical mobility, eating behavior and appetite, which will be further discussed below in relation to the *Tat* gene.

It is particularly important to note a pattern common to both rodent species, consisting of the effect of an energy-abundant diet on the expression of genes involved in the regulation of metabolism via the PPARγ signaling pathway, xenobiotic metabolism by cytochromes P450, retinol metabolism, amino acid metabolism (including TAT transamination), and regulation of apoptosis. In contrast, the remaining differentially expressed genes were characterized not only by interspecies differences between mice and rats, but also by interstrain differences within mice of the two inbred strains and the tetrahybrid.

Role of *Tat* gene expression in metabolic effects of DIO in rat and mouse models

The *Tat* gene encodes the tyrosine aminotransferase enzyme (EC 2.6.1.5), which catalyzes the reversible reaction of transferring the amino group from the amino acid tyrosine to α-ketoglutarate to form *p*-hydroxyphenylpyruvate and glutamate, respectively. The TAT enzyme, like other aminotransferases, is vitamin B_6-dependent and plays an important role in the metabolic pathway of tyrosine biotransformation. The enzyme can also utilize phenylalanine as an amino group donor and phenylpyruvate as its acceptor [31]. In fact, through a metabolic link catalyzed by hepatic TAT, there is regulation of the amount of the conditionally essential amino acid tyrosine available for the synthesis of the biogenic amine dopamine and its derivatives, including adrenaline, epinephrine, and norepinephrine. A genetic defect in *Tat* in human's results in tyrosinemia type 2 accompanied by profound neurological disorders.

As follows from the data of Table 2, a significant DE of the *Tat* gene was detected under the influence of HCHFD in male Wistar and Zucker^{fa} rats, male DBA/2J mice and female C57Bl/6J mice, as well as in spontaneously obese db/db mice compared to their parental strain C57Bl/6J (the experimental data are presented in the monograph [13]). In all cases, except for db/db mice and C57Bl/6J females, factors leading to the development of obesity induced a positive DE of *Tat*. Interestingly, the differential expression rate of the *Tat* gene was more than twofold higher for Wistar rats ($\log_2$(FC) = 1.212; p_{val} = 0.002; adj.p_{val} = 0.067) compared to the control diet, while it was only 46 % higher for rats of the Zucker^{fa} strain ($\log_2$(FC) = 0.553; p_{val} = 0.041; adj.p_{val} > 0.1). Moreover, *Tat* expression was reduced almost twofold in db/db mice ($\log_2$(FC) = –0.962; p_{val} = 0.001; adj.p_{val} = 0.006).

The role of transamination reactions and tyrosine metabolism as effector links of metabolism in DIO and obesity caused by genetic factors is indicated by changes in AST activity and De Ritis ratio (AST/ALT) values in blood plasma of not only homozygous (*in vivo* model of hyperactivity accompanied by increased resistance to DIO development) but also heterozygous DAT-KO rats [13]. These results indicate the effect of excessive amounts of extracellular dopamine in the synaptic cleft of dopaminergic neurons in the striatum on the regulation of metabolic processes through activation of catabolic processes, including lipid and carbohydrate metabolism. As a consequence, the DAT-KO knockout rats receiving HCHFD showed a decrease in key parameters of the obesity phenotype (body weight, relative liver weight, white retroperitoneal adipose tissue, etc.) in comparison with «wild-type» animals, which were the parental Wistar strain for DAT-KO rats.

As in the case of *DAT* gene knockout, diet-induced increases in TAT expression and activity can reduce extracellular dopamine levels in synapses of dopaminergic neurons, acting in particular on the nigrostriatal and mesolimbic dopamine systems of the brain. The observed changes in TAT expression, along with ALT and AST transaminases, may affect the severity and trend of catabolic and/or anabolic organism reactions, the features of eating behavior and the ability to control the amount of food consumed.

Consistent with these findings are the results of a transcriptomic study indicating that both rats and mice more prone to develop an obesity phenotype (Zucker^{fa} rats, db/db mice, female C57Bl/6J mice) show less *Tat* expression compared with animals phenotypically relatively more resistant to developing obesity (male Wistar rats, DBA/2J mice). This includes results showing the presence of DE in mice of different strains of *Moxd1 and Dbh* genes responsible for some steps in the metabolism of dopamine and its derivatives. The expression of the *Hamp2* (hepcidin) gene discussed above may apparently be associated with dopa-

mine metabolism through the role of this gene in the regulation of tissue iron status, which is an important cofactor in a number of stages of metabolism of this biogenic amine.

Based on these findings, it can be concluded that there is a TAT-dependent effect on the activation of transamination processes in the liver by altering the rate of catabolic reactions, as well as regulation of dopamine levels via tyrosine utilization via the non-dopamine pathway, possibly representing a novel neurometabolic regulatory function of the liver in response to the consumption of high-calorie diets.

Analysis of intergenic interactions using the "genetic networks" tool implemented in Genemania resource shows the relationship of *Tat* expression with other aminotransferases, including Gpt (ALT), *Got* (AST), as well as *Vnn1, Fos, Jun* and some other genes involved in lipogenesis and response to HCHFD. This indicates a legitimate role for *Tat* as a metabolic link in the response of the body phenotype to the consumption of a calorie-abundant diet. *Tat* expression increases in mice receiving hypercaloric diet in the setting of metabolic correction by ingestion of *Luffa cylindrica* dietary fiber, and, according to the authors, the mechanism of this effect is mediated by a favorable effect of intestinal microflora metabolites on gene expression in the host liver [32]. In our study on Wistar rats, the content of TAT protein was increased in the liver cells of rats treated with HCHFD by immunohistochemical analysis [33].

Another putative mechanism of metabolic regulation of *Tat* expression is attributed to the systemic effects of glucocorticoids. Thus, *Tat* is known to be stimulated by corticosterone administration or by immobilization stress accompanied by massive glucocorticoid release [34]. In this regard, it is relevant to analyze the relationship between *Tat* expression and metabolic pathways (KEGGs) of steroid hormone biosynthesis and metabolism, which, according to transcriptomic studies, are the targets of the effects of HCHFD and other hypercaloric diets in different rodent species and strains.

CONCLUSION

Thus, the use of the method of full-transcriptome profiling made it possible to reveal on models of diet-induced obesity, hyperlipidemia and metabolic syndrome in rats and mice the nutrigenomic effects associated with the different character of the influence of excessive consumption of dietary fat and (or) carbohydrates on the transcriptome of liver tissue in animals more or less hereditarily predisposed to the development of DIO or hereditarily determined obesity, steatosis and dyslipidemia. A significant role of *Tat* gene expression, encoding tyrosine aminotransferase, in the regulation of metabolic reactions in rodents in response to HCHFD consumption and in the development of obesity was shown.

The results obtained in a large number of cases are reproducible, coincide in animals of different species and strains characterized by similar phenotype, and agree with the data of analysis of integral, biochemical, traceelement and morphological parameters. At the same time, within each studied species (*Rattus rattus* and *Mus domesticus*) and a certain sex of animals, it is possible to identify a number of genetic variants with a greater or lesser propensity to develop the DIO phenotype; in addition, within these variants, a largely similar trend of the transcriptome response to dietary influence is noted.

This summarizing result allows one to remember the scientific position of the outstanding geneticist Nikolai I. Vavilov, expressed in the 1st half of the XX cen-

TABLE 2

DIFFERENTIAL EXPRESSION OF THE *Tat* GENE IN DIFFERENT GROUPS OF ANIMALS IN RESPONSE TO THE DEVELOPMENT OF SPONTANEOUS OR DIET-INDUCED OBESITY AND DYSLIPIDEMIA

Models	Species, sex of animals	Strain	Log(FC)	p_{val}	adj. p_{val}	References to publications
MetS (HFrD feeding)	Female mice	C57Bl/6J	–0.554	< 0.001	0.08	[9]
DIO (HCHFD feeding)	Female mice	C57Bl/6J	0.775	< 0.001	**0.043**	[9]
Dyslipidemia (HCD feeding)	Female mice	C57Bl/6J	0.403	0.002	–	[9]
Spontaneous obesity (balanced diet)	Male mice	db/db[1]	–0.962	0.001	**0.006**	[13]
DIO (HCHFD feeding)	Same	DBA/2J	1.449	< 0.001	**0.004**	[12]
DIO (HCHFD feeding)	Male rats	Zucker	0.553	0.041	–	[13]
DIO (HCHFD feeding)	Male rats	Same	1.212	0.002	0.067	[13]

Note. [1] – mouse strain with leptin receptor gene knockout. Mice of the C57Bl/6J strain, which is the "parental" strain for this knockout strain [13], receiving the same diet served as a control in this experiment.

tury, that "Species and genera that are genetically close are characterized by similar series of hereditary variability with such accuracy that, knowing a number of forms within one species, one can foresee the finding of parallel forms in other species and genera". This postulate, known as the Law of homologous series in hereditary variability, was empirically derived from studies on genetic variants of bread cereal plants. However, N.I. Vavilov himself noted that the «law of homologous series» can also be applied to animals. If the assumption that the different responses of the transcriptome and its associated metabolome to the consumption of energy-dense food are similar across species (including humans), this improves the prospects for translating results obtained from *in vivo* experiments into clinical practice.

The general trend of the transcriptome response and its derived metabolome may contribute to both the development of DIO and the formation of resistance to it by increasing energy expenditure and controlling the amount of food consumed. Which of these variants is most likely to be realized depends on the genotype of the organism, i. e., the presence of allelic polymorphisms of key "obesity genes". The search for such genes is still a crucial task of nutrigenetics, but it is obvious from the available literature data that not all problems in this field have been solved. In particular, there are insufficient data on gene expression products that could be targets of planned personalized dietary interventions. Of the genes examined in this paper, the most numerous clinical evidences of association with the pathogenesis of obesity are known for polymorphisms of the *Pparg* gene. At the same time, there is reason to believe that the scope of work on the search for «candidate obesity genes» can be significantly narrowed, and these studies can be further structured and targeted to take into account genes that are part of metabolic pathways that respond contrastingly depending on the obesity phenotype. In accordance with the data presented in this paper, it is reasonable to search for such genes as part of the metabolic pathways of retinoid metabolism, RRAR signaling, steroid hormone metabolism, oxylipins (including eicosanoids), trace elements, aromatic amino acids, which are precursors of trace amines and neurotransmitters with known neuroregulatory function.

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Conflict of interest

The authors of this article confirmed that there is no conflict of interest to be reported.

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OBSTETRICS AND GYNAECOLOGY

HPV-ASSOCIATED CERVICAL CANCER: CURRENT STATUS AND PROSPECTS

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ABSTRACT

Every year, 570,000 new cases of cervical cancer (CC) are diagnosed in the world, and 311,000 people die from this disease. CC is the fourth most common type of cancer and therefore the fourth leading cause of cancer death in women worldwide. Numerous data on the occurrence and development of cervical cancer indicate an association in most cases (up to 90 %) with human papillomaviruses (HPV) of high carcinogenic risk (HCR).

CC prevention strategies are based on screening, and deaths from this oncopathology can be prevented through vaccination and treatment with early detection of the disease.

In this review, much attention is paid to current issues of detection and prevention of HPV-associated pathologies, and cervical cancer in particular, aiming to summarize and analyze the latest international literature data on this issue.

As a result of this study, it was shown that for countries implementing the National program of vaccination against HPV of high carcinogenic risk, a decrease in the incidence of both cervical pathologies of varying severity and other cancers associated with the HPV carriage was registered.

While effective implementation of actual experience and future advances in human papillomavirus vaccine prophylaxis may make it possible for all countries to move to the high levels of vaccination coverage required to eliminate HPV-associated pathologies, the results also suggest that the path to complete cervical cancer elimination as a global public health problem can be extremely difficult due to a number of existing limitations.

Key words: *cervical pathology, cervical cancer, HPV, screening, prevention, vaccination*

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ВПЧ-АССОЦИИРОВАННЫЙ РАК ШЕЙКИ МАТКИ: СОВРЕМЕННОЕ СОСТОЯНИЕ И ПЕРСПЕКТИВЫ

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РЕЗЮМЕ

Ежегодно в мире диагностируется 570 000 новых случаев рака шейки матки (РШМ), и 311 000 человек погибают от этого заболевания. РШМ – четвёртый по распространённости вид рака и, соответственно, четвёртая по распространённости причина смерти от рака у женщин во всём мире. Многочисленные данные о возникновении и развитии РШМ свидетельствуют об ассоциации в большинстве случаев (до 90 %) с вирусами папилломы человека (ВПЧ) высокого канцерогенного риска (ВКР).

В свою очередь стратегии профилактики РШМ основаны на скрининге, а смертельные исходы от данной онкопатологии представляется возможным предотвратить путём проведения вакцинопрофилактики и лечения при раннем обнаружении заболевания.

В представленном обзоре большое внимание уделяется актуальным в настоящее время вопросам выявления и профилактики ВПЧ-ассоциированных патологий, в частности рака шейки матки, с целью обобщения и анализа последних международных литературных данных по данной проблематике. В результате проведения настоящего исследования показано, что для стран, реализующих Национальную программу вакцинации против ВПЧ ВКР, зарегистрировано снижение заболеваемости как патологиями шейки матки различной степени тяжести, так и другими онкозаболеваниями, ассоциированными с носительством данного вируса.

Несмотря на то, что эффективное использование накопленного на сегодняшний день опыта и будущих достижений вакцинопрофилактики папилломавирусной инфекции может позволить всем странам перейти к высокому уровню охвата, который необходим для окончательной ликвидации ВПЧ-ассоциированной патологии, полученные результаты также свидетельствуют о том, что путь к полной ликвидации рака шейки матки как глобальной проблемы общественного здравоохранения может оказаться чрезвычайно трудным в силу ряда существующих ограничений.

Ключевые слова: *патология шейки матки, рак шейки матки, ВПЧ, скрининг, профилактика, вакцинация*

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1. INTRODUCTION

According to the World Health Organisation (WHO), malignant neoplasms are one of the most common causes of death worldwide [1].

According to GLOBOCAN, there were 604,127 new cases and 341,831 deaths from cervical cancer in 2020 [2].

Currently, high incidence and mortality rates from cervical cancer (CC) remain an urgent problem, the solution to which is of great importance in improving the demographic situation both in the Russian Federation and worldwide. We used the joint WHO and IARC-GLOBOCAN project, which provides annual data on cancer incidence worldwide, in order to estimate CC incidence for this literature review (Fig. 1).

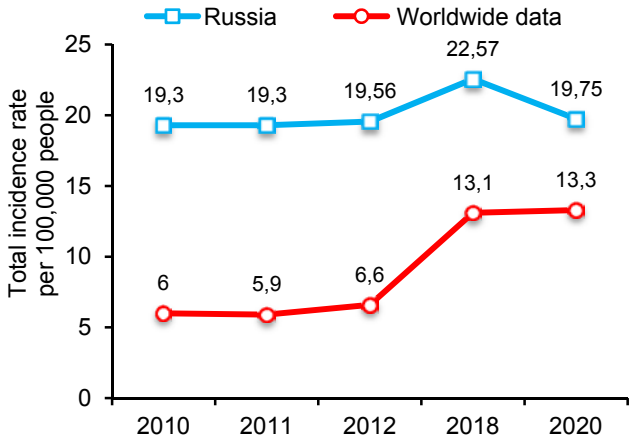

FIG. 1.

Dynamics of standardized worldwide incidence rates of cervical cancer through 2010 to 2020

Over the past two decades, it has become evident that viruses are significant for the development of human malignant neoplasms [3]. Human papillomavirus (HPV) with high carcinogenic risk (HCR) is considered a major cause of CC [4]. CC precursors are premalignant diseases (cervical dysplastic changes of varying severity), as well as various inflammatory conditions and damage to the squamous epithelium of the cervical mucous membrane – cervicitis and cervical erosion, respectively. In this case, human papillomavirus contributes to the acceleration of malignant transformation of the damaged epithelium into malignant pathology. Thus, it is important to perform testing for HPV infection when performing secondary prevention.

As a result of timely detection and treatment of cervical pathology from the moment of HPV initiation, it is possible to prevent the occurrence of both the premalignancy (dysplastic changes of varying severity) and further progression to the development of malignant neoplasm.

The aim of this study is to review the course of HPV infection from infection of female patients without morphological changes in the cervical epithelium to progression to malignancy and to discuss surveillance approaches for monitoring and implementation of public health programs (in particular HPV vaccination for high carcinogenic risk HPV and CC prevention programs) to achieve control and eventual elimination of this cancer.

Methods. A literature search was conducted in PubMed and Google Scholar databases using different variations of the following keywords: cervical pathology, cervical cancer, human papillomavirus, HPV, prevention, vaccination, screening. Full-text articles from 2014 to 2022 were included in the study. The language of the studies was not an obstacle to inclusion in this literature review. A total of 81 references are included in the review.

2. HPV INFECTION

A large body of research in the world literature is devoted to the influence of HPV on the emergence and development of premalignant and malignant processes in various morphological changes of the cervical epithelium. This section summarizes literature data on HPV in female patient groups ranging from healthy patients to CC patients.

The healthy group includes female patients without morphological changes in the cervical epithelium. Worldwide, 10.4 % of women with normal cervical cytological findings are HPV carriers [4]. Higher prevalence was found in less developed regions (22.1 % in Africa, 20.4 % in Central America and Mexico) compared to North America (11.3 %), Europe (8.1 %), Asia (8.0 %) [4]. A study of women with normal cytology published in 2010 showed the highest prevalence of HPV (23.2 %) among women under 25 years of age [4]. HPV 16 was the most common type [5]. For example, the prevalence of this virus in a study of high-risk HPV infection among healthy women in Tehran, Iran, was 5.1 %. HPV 16 was also the most frequently detected genotype. However, it should be noted that in recent years, HPV 31, 33, 45 and 58 have been most commonly detected in many East Asian countries in a cohort of healthy women [6].

Background pathologies of the cervix include diseases such as cervicitis, cervical erosion and endocervical polyps.

Cervicitis is an extremely common gynecological disease among women (30–40 % of cases) aged 20–40 years, characterized by inflammation of the cervix, which increases the risk of sexually transmitted infections [7]. This background cervical disease can cause a number of reproductive system disorders such as endometritis, salpingitis, pelvic inflammatory disease, chorioamnionitis and other complications. Chronic cervicitis was reported to be associated with various stages of cervical cancer progression, including cellular transformation and stimulation of proliferation, invasion, angiogenesis, and metastasis [7].

Depending on the nature of the course of inflammation, viral cervicitis caused by the human papillomavirus is distinguished. In Beijing, China, the prevalence of HPV

among patients with cervicitis was reported to be 75.8 %, among which HPV 16, 18, and 52, three most common HPV types, were detected with an incidence rate of 19 %, 11.6 %, and 15.2 %, respectively [8].

According to epidemiological data, 5–15 % of HPV patients are diagnosed with *chronic* HPV-associated cervicitis. The most frequent unfavorable course and outcome of the disease is noted among women with viral-bacterial or viral-fungal infection of the lower genital tract. In addition, these associations lead to a protracted course of the disease with numerous recurrences. Thus, chronic exo- and endocervicitis, high prevalence of HPV create prerequisites for the formation of premalignant cervical diseases [9]. The International Papillomavirus Society (IPVS; http://ipvsoc.org/) considers HPV-associated cervicitis as a primary marker of cervical premalignancy.

Cervical erosion is a chronic inflammation with the highest incidence of cervical lesions in recent years [10] and is one of the frequent gynecological problems (15–30 % of cases). Erosion occurs as a consequence of cervical infection and represents the basis for cancer, as there is a high risk of HPV contact with basal cells. Currently, data on HPV incidence for this pathology are not presented in the world literature [10, 11].

Endocervical polyps. The etiology of cervical polyps remains unknown, but many theories have been presented. One theory suggests that they can be the result of cervical vascular thrombosis and can disrupt blood flow resulting in the formation of a polyp. Other theories describe that they occur due to infection or chronic inflammation of the cervix [12]. One case study on the association of HPV with endocervical polyps has been mentioned in the literature. It was found that among HPV-positive women, endocervical polyps were present in 6.9 % of cases. However, the most frequent genotypes of HPV-positive samples are HPV 16 and 18 [13].

Cervical dysplasia is divided into two subtypes: low and high malignancy – which are associated with HPV infections to varying degrees.

LSIL (CIN1, cervical intraepithelial neoplasia I) is a low grade squamous intraepithelial lesion [14]. A diagnosis of CIN is not a reason for screening and initiation of treatment, as CIN1 suggests a low risk for the development of severe dysplastic changes [15]. The association between CIN1 and HPV remains controversial. However, there are studies suggesting that CIN1 is mainly caused by HPV of low carcinogenic risk. There is also evidence that HPV of high carcinogenic risk is closely associated with CIN1 [16]. HPV 16 and 18 cause 25 % of CIN1 cases [17]. According to a study conducted in China, HPV 16, 52 and 18 are the most common HPV genotypes in CIN1 [18].

Differences in HPV infection risk and CIN1 outcomes may be related to regional differences in populations [16].

HSIL (CIN2 + (CIN3 + *In situ*)) is a high grade squamous intraepithelial lesion [14].

Preinvasive cervical cancer is a stage of malignant neoplasm in which malignant changes are localized only in the cervical epithelium. In this case, the malignant cells have not yet broken through the basement membrane and therefore have not penetrated even into the subepithelial tissue. Therefore, this stage of the disease is referred to as intraepithelial, null or carcinoma *in situ* [19].

HPV 16 and HPV 18 are known to cause up to 50 % of CIN3, but oncogenic HPV types such as HPV 31, 33, 45, 52 and 58 pose a risk of developing CIN3 equivalent to the risk of HPV 18 [19, 20]. Moreover, a mixed infection of HPV 31 and 33 among women over 30 years of age exceeds the oncogenic risk of HPV 16 [20, 21]. According to a study conducted by Chinese scientists, CIN2 and CIN3 are characterized by the presence of HPV 52, 16, 58, 33 and 18 [18]. It should be noted that genotypes HPV 39, 56, 58 and 68 are associated with a lower risk of progression to CIN3 [21]. HPV 16 is known to have the strongest impact on the development of cervical cancer *in situ* [22].

It is worth noting that **cervical cancer** is a preventable disease. However, it is limited by social and economic status and education level. Most women are poorly informed about the causes, risk factors, prevention and treatment of CC. CC incidence in developed countries remains lower than in less developed countries. In fact, approximately 95 % of deaths from cervical cancer occur in low-income countries. HPV infection is the most significant predominant factor in the development of cervical cancer [23]. According to the literature, HPV 16, 18, 45, 31, 33, 52, 58, and 35 are the most common HPV types among women with CC in descending order of frequency. They account for 91 % of invasive CC cases [9]. However, it is worth noting that worldwide, the percentage of cervical cancer cases caused by HPV 16 and 18 is about 70 % [19]. HPV 68, 26, 66, 67, 73 and 82 are rarely detected in women with CC, but the carcinogen classification system is constantly being updated [24].

3. ONCOGENESIS OF CERVICAL CANCER

3.1. Infection

The cervix is formed by a simple columnar secretory epithelium. The vaginal cavity is lined by a multilayered non-keratinizing squamous epithelium. The epithelium is the area where more than 90 % of lower genital tract malignancies are initiated; it is also particularly vulnerable to high-risk HPV [25].

The process of carcinogenesis, starting with cellular changes caused by HPV infection and ending with CC, can take from 10 to 40 years (but in rare cases CC can also develop in 1–2 years), which provides a window for clinical prevention, diagnosis and treatment [25].

Cervical cancer is a result of a continuous process: normal cervical epithelium after infection with HPV progresses to CIN, then transforms into invasive squamous cell carcinoma (ISC) [26].

A viral infection caused by HPV begins with the virus entering the cervical epithelium through microcracks. HPV genomes then migrate into vesicular nuclei together with L2 protein, where they become low-copy episomes. Once in the host cell nucleus, HPV genomes rapidly replicate to 10–200 copies per cell, marking the initial phase of

amplification and leading to the establishment of infection. During this initial phase, only the early viral promoter is transcriptionally active, leading to the expression of early HPV proteins: E1, E2, E6 and E7.

The expression of E1 and E2 proteins then leads to the regulation of virus replication in infected cells, which leads to the expression of other early-stage proteins. Further, the expression of E5, E6 and E7 oncoproteins starts, which promotes cell survival and uncontrolled proliferation [27].

The presence of E5 leads to inappropriate activation of the epidermal growth factor receptor (EGFR), triggering a series of events followed by the production of vascular endothelial growth factor (VEGF), thereby promoting angiogenesis, which is one of the hallmarks of cancer progression. E6 production leads to repression of several signaling systems. E6 and E6AP can form a complex capable of tagging the p53 protein for its degradation, thereby reducing levels of this tumor suppressor protein. p53 degradation leads to dysregulation of Bcl-2 (a regulator of apoptosis) expression and inhibition of Bak. Moreover, E6 can mark the degradation of Bak, an important pro-apoptotic protein, and bind to p300, thus inhibiting its p53 activation mechanism. In addition, E6 can bind to FAS ("death receptor" whose activation leads to apoptosis) and accelerate its degradation. All these mechanisms triggered by E6 result in apoptosis defense. E7 has the ability to bind to pRB (retinoblastoma protein) of tumor suppressors p107 and p130, which are E2F regulators (transcription factor). This binding releases E2F, which in turn can continuously induce cell cycle progression. In general, interference of HPV proteins leads to uncontrolled cell overgrowth [27].

3.2. Viral persistence and integration

During persistent high-risk HPV infection, HPV integration events known to cause genome instability can often be detected. There is a growing number of studies suggesting that integration of high-risk HPV DNA may be a prerequisite and/or driving force for HPV carcinogenesis, maintenance of a malignant phenotype, and development of cervical cancer [28]. Ongoing studies have established the association of HPV integration with cervical CIN levels, which could potentially be used as a marker to assess the risk of cervical cancer among patients with HPV infection [28]. Numerous studies have shown that HPV integration typically involves disruption of the open reading frames of the viral E1 and E2 regions, leading to activation of the E6 and E7 oncogenes. The E6 and E7 oncogenes have several cellular targets that promote malignant transformation with the mechanism described above, accompanied by increased ubiquitin degradation of p53, Bak, inhibition of pRb and activation of cyclin-dependent kinases [1, 29, 30].

During the infectious process, the virus may be present in episomal, integrated or combined (mixed) form in the host cell genome. In its integrated form, the virus can cause changes in cellular functions that promote replication of viral particles and malignant cell transformation. Currently, there are several hypotheses regarding the correlation between viral status and lesion stage. It has been suggested that the virus is fully integrated into the genome in advanced malignant lesions. Other studies have failed to establish a definite stage of full integration [31]. HPV genome integration has been found in two types: as a single integrated genome and as multiple tandem repeats of the viral genome into the cellular genome [32].

The correlation between structural variations in the host genome and HPV integration is poorly studied. In particular, it is unclear whether tandem integration is preceded by chromosome aberrations that facilitate HPV integration or whether HPV integration causes more extensive chromosomal changes. However, there is information in the literature that the integration of HPV DNA into the human genome causes various genetic alterations such as amplification of oncogenes, inactivation of tumor suppressor genes, inter- or intrachromosomal rearrangements, and genetic instability [33]. While host cell genome instability and HPV genome integration are more common in invasive tumor diseases compared to CIN lesions, it is unknown whether genome instability differs between integrated and non-integrated forms of HPV [34].

3.3. Elimination

Most HPV infections do not cause symptoms and pass spontaneously within 1–2 years due to rapid immune clearance. The immune system eliminates the virus within six months in 50 % of infected women and in 90 % of women within two years of persistence [35]. Approximately 90 % of patients with HPV infection have innate and humoral immune-mediated virus clearance within a few months after viral infection [36].

Only if the infection persists, it can lead to the development of a premalignant CIN lesion (1–2 years) and progression to CC can take up to 10–15 years [37, 38]. Many factors contributing to HPV persistence and triggering carcinogenic pathways remain elusive [39].

During the progression of a premalignant lesion, the host immune system detects infiltration of $CD4^+$-, $CD8^+$-lymphocytes (a type of T-lymphocytes) and macrophages, increase in pro-inflammatory cytokines and induction of neutralizing antibodies [40]. A slow immune response to infection causes a decrease in antibody titers. Neutralizing antibodies (Nab) are triggered after viral infection and target only viral particles and not virus-infected cells, which thus cannot cure the infection. In addition, the role of macrophages and natural killers (NK) involved in the immune response is unclear. Langerhans cells, major antigen presenting cells (APC) in the epithelium, play an important role in the recognition of HPV infection and induction of the cellular immune response [36].

A combination of innate and adaptive immunity prevents HPV infection. Effector T cells targeting early viral proteins can eliminate virus-infected cells. However, the immune response that can protect against re-infection with the same or even a different type of HPV is dis-

puted. Studies attempting to investigate whether antibodies developed after natural HPV infection provide protection against re-infection have provided conflicting results [36]. A US study showed that there was no evidence of homologous immunity against any of the HPV types studied, suggesting that intratype competition is weak or absent [41]. At the same time, according to previous results obtained by these scientists, it has been shown that HPV 16 viral particles can induce low levels of neutralizing antibodies against HPV 31. Clinical trial data have shown that vaccines targeting HPV 16 provide partial protection against HPV 31 [41].

During the HPV persistence phase, during which the host immune system cannot eliminate the virus, expression of E6 and E7 proteins may contribute to lesion progression, which usually results from E2 promoter methylation and viral integration, contributing to immune abnormalities [36].

The development of cervical cancer depends not only on negative regulation of cell cycle control and accumulation of genetic damage by viral oncoproteins, but also on immune evasion [25]. Mechanisms of immune system evasion by HPV include:

• suppression of the antigen presentation mechanism (antigen presentation is the process of presenting a fragment of antigen to a T lymphocyte in order to trigger a T cell response);

• resistance to cytotoxicity mediated by cytotoxic T lymphocytes (CTL);

• recruitment of immune cells that inhibit the immune response, such as immature dendritic cells (DC), tolerogenic DCs, T regulatory cells (Treg), tumor-associated macrophages (TAM) and myeloid-derived suppressor cells (MDSC).

In addition, overexpression of E6 and E7 proteins impairs cellular DNA repair, resulting in genome instability and immune escape [42].

4. POTENTIAL BIOMARKERS OF CERVICAL CANCER

The results of a study published in 2021 involving more than 40,000 women showed that E6/E7 mRNA detection has the highest sensitivity compared to conventional cytological examination and p16/Ki-67 testing [43]. Considering these results, the E6/E7 mRNA assay seems to be a very good candidate for ultrasensitive screening. Scientific consensus on the optimal sensitivity of tests used in cervical cancer screening remains open [43].

TABLE 1

POTENTIAL BIOMARKERS OF CERVICAL CANCER

CC Biomarkers	Sensitivity / specificity, %	Conclusion	Source
miR-9	67.3 % / 80 %		
miR-21	82.7 % / 72 %	miR-9, miR-21 and miR-155 may be prospective biomarkers for the diagnosis of HPV-associated cervical cancer	Park S. et al. (2017) [45]
miR-155	65.4 % / 96 %		
SIM1	38.5 % / 100 %	*SIM1* methylation status may be a potential diagnostic biomarker of cervical cancer	Kim H.J. et al (2018) [46]
SEPT9	89.5 % / 63.3 %	*SEPT9* promoter methylation is a potential biomarker for early detection of cervical cancer and its overexpression may determine radioresistance	Jiao X. et al. (2019) [47]
ZNF582	71 % / 81 %	*ZNF582* can be used as a potential biomarker for CIN3 prognosis	Li N. et al. (2019) [48]
PAX1	86 % / 85 %	Diagnosis of *PAX1* methylation can be included in the cervical cancer screening diagram	Fang C. et al. (2019) [49]
SOX1	96 % / 99 %	*SOX1* sensitivity and specificity make it suitable for use in cervical cancer early detection programs	Zhang L. et al. (2020) [50]

Currently, potential highly sensitive biomarkers for cervical cancer also include *SOX14*. It belongs to a group of genes involved in the binding of high mobility group domains to DNA, which stimulates the differentiation process in the cell cycle. Regarding cervical cancer, *SOX14* potentiates cell proliferation and invasiveness. Detection of *SOX14* allowed differential diagnosis of precancerous lesions and cervical cancer with a sensitivity of 94.12 % and specificity of 86.46 % [44].

In addition to the examples described above, potential biomarkers of premalignant lesions and CC presented in the references are summarized in Table 1.

The JAK/STAT pathway, with which the E6/E7 oncoproteins interact, has been highlighted among the numerous cells signaling pathways involved in cervical cancer carcinogenesis. Signal transmission in this pathway contributes to the tumor progression and the development of metastases. Currently, JAK/STAT pathway inhibitors are known to be of interest in ongoing clinical trials. However, it is important to focus on the evaluation of the recurrence-free period and overall survival of CC patients [51]. Notch is another pathway which is important for cervical cancer progression. This signaling pathway is associated with differentiation of epithelial cells with HPV. Most invasive cases of cervical cancer show cytoplasmic localization of Notch1, with Notch1 in the cell nucleus correlating with worse treatment outcomes [52].

The role of HPV in the development of CC is undeniable. However, the *HLA* (Human Leukocyte Antigen) gene, probably responsible for genetic predisposition to cervical cancer, has attracted the attention of researchers. Several studies in recent years have found independent risk variants associated with the 6p21.3 locus of the *HLA* gene. The estimated inherited susceptibility to cervical cancer infection ranges up to 7 %. However, the authors conclude that studies on larger populations of HPV-negative cases of cervical cancer, which are diagnosed statistically later than HPV-positive cases and correlate with poorer survival, are needed to verify these findings [53].

5. HPV VACCINATION: RESULTS AND PROSPECTS

By understanding the role of HPV in the development of cervical cancer, researchers have focused on developing suitable strategies to detect and prevent this disease [27]. The ultimate goal of HPV vaccination is to reduce the occurrence of cervical premalignancies and inflammatory diseases by preventing infection with the major oncogenic HPV types [54].

Currently, there are three commercially available preventive vaccines that vary in the number of HPV types and target (some are not available in certain countries). Cervarix is a bivalent vaccine targeting HPV 16 and 18; Gardasil is a quadrivalent vaccine against HPV 6, 11, 16, 18; Gardasil 9 is a 9-valent vaccine targeting the same HPV types as the quadrivalent vaccine (6, 11, 16, 18) as well as types 31, 33, 45, 52 and 58 [55, 56]. Quadri- and bivalent first-generation HPV vaccines have been available since 2006 and 2007, respectively [57]. The target group for vaccination recommended by WHO is girls aged 9 to 14 years who are not sexually active because they have a better immune response to the vaccine than adolescents [56].

Large international clinical trials have shown that HPV vaccines are safe and highly effective against persistent vaccine-type infection and premalignant cervical lesions in women (vaccine efficacy ≥ 93 %) [55].

Data demonstrating the high efficacy of HPV vaccination have recently been published for Australia [58]. Due to the success of the HPV vaccination programme, the introduction of vaccination for both sexes, the recent switch to a two-dose vaccination schedule, and the 2017 changes to the National Cervical Screening Programme, estimates suggest that an incidence rate of less than 4 CC cases per 1,000,000 women is likely to be achieved by 2035 if current vaccination coverage rates can be maintained. Therefore, it is likely that Australia will be the first country in the world to eliminate cervical cancer as a public health problem. However, other countries are expected to follow suit within the next decade or two [58].

In addition, HPV vaccination is also effective in preventing cervical diseases [56]. For example, Scotland researchers show a reduction in low and high CIN associated with high utilization of bivalent HPV vaccine at the population level [59]. Results from a recent Japanese study showed that women aged 20–24 years who received HPV vaccine had significantly lower rates of abnormal cervical cytological examination results compared to those who did not receive the vaccine [60]. An Australian study showed that vaccination with quadrivalent HPV vaccine also helps to reduce the incidence of HSIL and LSIL among women [61]. Results of Canadian studies show that HPV vaccination was moderately effective in preventing HSIL among adolescents, but much less effective in older age groups, especially among those with a history of abnormal cytological findings [62]. These two vaccines also protect against HPV 6 and 11, which cause anogenital warts [56].

In 2014, estimated HPV vaccination coverage rates for young and adolescent girls were more than 30 % in developed countries but less than 3 % in less developed regions [58].

In 2018, WHO called for action to achieve global elimination of cervical cancer, and to develop a strategic plan that includes goals and targets for scaling up HPV vaccination, cervical screening, and treatment of premalignant and malignant diseases [58].

Worldwide, at least one dose has been administered to nearly 118 million women [63]. Global vaccination coverage is estimated at 15 % and 40 % in high-income countries. In Europe, full vaccination coverage is estimated at 35 % [64]. 90 % of global CC deaths occur in low- and middle-income countries, which struggle to implement effective prevention programs due to lack of financial resources and low public awareness. However, it is important to note that the situation in these countries should

improve annually. As of mid-2020, 41 % of all low- and middle-income countries have initiated national HPV vaccination programs [65]. The dissemination of vaccination faces a number of challenges such as the cost of the vaccine (in the absence of a national vaccination programme), the lack of information on HPV vaccine, and the difficulty of completing vaccination [66]. The COVID-19 pandemic is expected to increase the above-mentioned challenges to HPV vaccination [67].

Thus, discussions about cervical cancer elimination have largely focused on the opportunities and challenges of vaccination programme scale-up. These disparities in HPV vaccination coverage may explain differences in the incidence, prevalence, and mortality associated with CC around the world.

In countries that have achieved high vaccination coverage, a 73–85 % reduction in vaccine-type HPV prevalence has been noted [55]. It is worth noting that as of December 2019, 124 countries and territories have implemented national immunization programs for HPV vaccination [54].

It has been estimated that current achieved vaccination coverage could potentially prevent up to 12.5–13.4 million cases of cervical cancer by 2069 and could achieve an average cervical cancer incidence of approximately 4 per 100,000 women per year [58].

TABLE 2

IMPLEMENTATION OF CERVICAL CANCER PREVENTION PROGRAMS IN SOME COUNTRIES

Country	National cervical cancer screening programme	National HPV vaccination programme	Source
Russian Federation	No As part of the medical check-up it is mandatory to perform the Pap (Papanicolaou) test that involves collecting cervical cells from the group of women 21–29 years old once every 3 years; group of women 30–65 years old – the Pap test + HPV testing every 5 years.	No	Cervical intraepithelial neoplasia, erosion and ectropion (2022) [70]
Austria	The Pap test (after the age of 18)	Yes, for girls and boys	Sroczynski G. et al. (2020) [71]
Belgium	The Pap test every 3 years, possibility of HPV DNA test	Yes	Jolidon V. et al. (2020) [72]
Czech Republic	Girls over the age of 15 should have the Pap test every year	Yes	Altova A. et al. (2021) [73]
Denmark	Women aged 23 to 49 should have the Pap test every 3 years, women aged 50 to 59 years – every 5 years. HPV test for women aged 60 to 64 – once.	Yes, girls ≥ 12 years old	Pedersen K. et al. (2018) [74]
Estonia	Women aged 30 to 55 should have the Pap test every 5 years	Yes, girls aged 12 to 14	Ojamaa K. et al. (2018) [75]
France	Women aged 25 to 65 should have the Pap test every 3 years	Yes, girls aged 11 to 14. Additional vaccination option for girls aged 15 to 19	de Rycke Y. et al. (2020) [76]
Netherlands	Women aged 30 to 60 should have HPV test every 5 years	Yes, vaccination for girls at the age of 12	de Munter A.C. et al. (2021) [77]
Germany	The Pap test – before the age of 35, co-testing – at ages 35 to 65	Yes, for boys and girls aged 9 to 14	Osowiecka K. et al. (2021) [78]
Australia	The Pap test every 2 years, HPV DNA test every 5 years	Yes, girls aged 12 to 17	Kramer J. (2021) [79]
Portugal	Determined in certain regions of the country (HPV test, the Pap test), performed every 3 years or every 5 years for the age groups of 25–60, 25–64 and 30–65	Yes, girls under the age of 13	Fernandes C. et al. (2022) [80]

6. CERVICAL CANCER PREVENTION PROGRAMS

In 1993, the European Guidelines for Quality Assurance and Principles for this screening were published. This year was the key date for the introduction of cervical cancer screening in Europe [68].

After 2015, the criteria for the screening programme were clearly defined: the age of the target group; the time intervals for the screening test; the algorithm for further management of the patient depending on the results [69]. Table 2 presents current information on the implementation of cervical cancer prevention programs in some countries.

Both screening and HPV vaccination programs do not yet have a unified regime. Most of the countries listed above base CC screening on the Pap tests every 3 years. HPV testing is used in Belgium, Denmark and parts of Portugal. The Netherlands is the only country in Europe where cytology has been completely replaced by HPV testing. A programme based on HPV testing was launched in the country in 2017 [81].

TABLE 3

AGE-STANDARDIZED CERVICAL CANCER INCIDENCE AND MORTALITY RATES PER 100,000 FEMALE POPULATION IN EUROPEAN COUNTRIES IN 2020

Country	Incidence	Mortality
Russian Federation	20.5	8.8
Austria	7.7	2.6
Belgium	11.1	2.9
Czech Republic	13.5	5.2
Denmark	14.8	3.2
Estonia	26.9	6.3
France	10.1	3.2
Netherlands	10.0	2.1
Germany	11.1	3.2
Portugal	15.6	4.6
Australia	6.0	1.7

Many countries have HPV vaccination programs. In Austria and Germany, boys are also vaccinated. Differences in approaches to CC prevention are reflected in the incidence and mortality rates presented in each country. The incidence and mortality rates for cervical cancer, based on data published by the International Agency for Research on Cancer, are presented in Table 3.

The lack of a national HPV vaccination programme speaks volumes. The lowest risk of death among the countries included in this review is noted in Australia, where cytological scrape screening is used in addition to the HPV vaccination programme.

Based on the above, it seems reasonable to standardize recommendations for CC prevention programs. There is both an urgent need to fund HPV vaccination in countries where it is not available and to include HPV HCR vaccination in mandatory National Preventive Vaccination Calendars.

8. SUMMARY AND CONCLUSIONS

In recent years, scientific publications have increasingly drawn conclusions about the need for timely screening measures for the most accurate and effective detection of women with premalignant cervical lesions for early intervention and prevention of malignant neoplasm. It is believed that cervical screening, differentiated management of patients with HPV-associated cervical disease and HPV vaccination will be complementary synergistic strategies for CC prevention in the coming decades.

However, the above arguments suggest that the complete elimination of cervical cancer as a global public health problem may be extremely difficult to achieve due to the following reasons: lack of uniform standards for CC prevention, lack of implementation of a publicly funded HPV vaccination programme in some countries (e. g., the Russian Federation).

In addition to the above, in order to improve the implementation of CC prevention, it is important to highlight the following areas: accelerated evaluation of the clinical efficacy of new diagnostic tools, development of HPV vaccines with wider genotype coverage and shorter dosing regimens.

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Conflict of interest

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

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BIOCHEMISTRY

MARKERS OF Th1 POLARIZED Th17 CELLS (LITERATURE REVIEW)

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ABSTRACT

T helpers (Th) producing IL-17 (Th17) have high plasticity and under the influence of external conditions are able to redifferentiate into cells with a different phenotype, primarily in Th1-lymphocytes, forming a population that combines the characteristics of both Th17 and Th1 and has a high pro-inflammatory potential, as well as a unique ability to overcome histohematic barriers. These cells are currently assigned a key role in the pathogenesis of many inflammatory diseases, including autoimmune ones: they account for up to half of the lymphocytes present in infiltrates of inflamed tissues. The paper discusses the reasons for the increased plasticity of Th17 cells in comparison with the main T helper populations (Th1 and Th2) and considers in detail the mechanisms of formation of IFNγ producing Th17, taking into account not only the redifferentiation of mature Th17, but also possible alternative pathways, in particular, Th1 cell redifferentiation or naive $CD4^+$ T lymphocytes direct differentiation into cells with an intermediate Th1/Th17 phenotype. The main inducers of differentiation of IFNγ producing Th17 cells and the reversibility of this process are also discussed. Particular attention is paid to the methods for identifying Th1 polarized Th17 cells: this population is heterogeneous, and its size significantly depends on the type of markers used to characterize these cells – Th1/Th17-associated transcription factors, key cytokines, as well as chemokine receptors and other membrane molecules. As a result, the data in the works on this problem are poorly comparable with each other. The unification of approaches to identifying a population of Th1 like Th17 cells will solve this problem and make it possible to use an assessment of the size and activity of such a population as diagnostic or prognostic markers.

Key words: *Th17, Th1, plasticity, redifferentiation, Th17.1, ex-Th17*

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МАРКЕРЫ Th1-ПОЛЯРИЗОВАННЫХ КЛЕТОК Th17 (ОБЗОР ЛИТЕРАТУРЫ)

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РЕЗЮМЕ

Т-хелперы (Th, T helpers), продуцирующие IL-17 (Th17), обладают высокой пластичностью и под влиянием внешних условий способны редифференцироваться в клетки с другим фенотипом, прежде всего в Th1-лимфоциты, формируя популяцию, сочетающую в себе характеристики как Th17, так и Th1 и обладающую высоким провоспалительным потенциалом, а также уникальной способностью преодолевать гистогематические барьеры. Именно этим клеткам в настоящее время отводится ключевая роль в патогенезе многих воспалительных заболеваний, включая и аутоиммунные: в инфильтратах воспалённых тканей на их долю приходится до половины присутствующих там лимфоцитов. В работе обсуждаются причины повышенной пластичности клеток Th17 в сравнении с основными Т-хелперными популяциями (Th1 и Th2) и подробно рассматриваются механизмы формирования IFNγ-продуцирующих Th17 с учётом не только редифференцировки зрелых Th17, но и возможных альтернативных путей, в частности редифференцировки клеток Th1 или непосредственной дифференцировки наивных $CD4^+$Т-лимфоцитов в клетки с промежуточным Th1/Th17-фенотипом. Также обсуждаются основные индукторы дифференцировки IFNγ-продуцирующих клеток Th17 и обратимость этого процесса. Особое внимание в обзоре уделено способам идентификации Th1-поляризованных клеток Th17: эта популяция неоднородна, и её размер существенно зависит от типа маркеров, используемых для характеристики данных клеток – Th1/Th17-ассоциированных транскрипционных факторов, ключевых цитокинов, а также хемокиновых рецепторов и других мембранных молекул. Как следствие, данные в работах по этой проблеме плохо сопоставимы друг с другом. Унификация подходов к выявлению популяции Th1-подобных Th17 позволит решить эту проблему и даст возможность использовать оценку размера и активности такой популяции в качестве диагностических или прогностических маркеров.

Ключевые слова: *Th17, Th1, пластичность, редифференцировка, Th17.1, ex-Th17*

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The population of T-helpers producing interleukin (IL) 17 (Th17) shows considerable heterogeneity and plasticity. The cytokine milieu is capable not only of adjusting the program of differentiation of naive $CD4^+$ T lymphocytes into Th17, but also of inducing redifferentiation of mature Th17, and first of all, we are talking about the acquisition by these cells of a Th1-like phenotype associated with high production of interferon (IFN) γ [1–4]. The nomenclature of such Th1 polarized Th17s is not uniform, although it reflects their transformed state: in different papers these cells are designated as Th17/Th1 [5, 6], Th1/Th17 [7, 8], Th1/17 [9], Th17/1 [10], Th17-1 [11], Th17.1 [12–14], as well as Th1* [15], "non-classical Th1s" [6] or simply as "IFNγ-producing Th17". However, it is not only a matter of nomenclature – the analysis of these works shows that the population of Th1-like Th17 is heterogeneous, and the above-mentioned works often refer to phenotypically and functionally different subpopulations.

Despite the small size of IFNγ-producing Th17 subpopulation in peripheral blood, its content in infiltrates of inflamed tissues reaches 60 % [13], and it is these cells that are currently assigned a key role in the pathogenesis of many inflammatory diseases, including autoimmune ones [5, 7, 8, 10, 13, 14]. Unification of data on non-classical Th17 cell variants is therefore of high relevance and is the subject of this review.

CHARACTERIZATION OF Th1-LIKE Th17 CELLS

The fact that the Th17 population includes a fraction of lymphocytes producing, along with IL-17, the Th1-associated cytokine IFNγ was noted in one of the first studies of Th17 cells in humans [16]. At that time, membrane markers to differentiate such a non-classical population from traditional Th17 were also identified – chemokine receptors CCR6, CCR4 and CXCR3 [16]. In particular, it has been shown that peripheral blood memory T cells expressing IL-17A in response to *ex vivo* stimulation carry the CCR6 receptor on the membrane [5, 16] and are divided into two subpopulations with different cytokine profiles depending on the co-expression of CCR4 and CXCR3 molecules: CCR6/CCR4 co-expression on the membrane marks memory T cells that selectively produce IL-17A but not IFNγ, whereas $CCR6^+CXCR3^+$ T cells produce IL-17A and IFNγ or IFNγ alone [16]. Although the concept of IFNγ-producing Th17 cells was later corrected, the combination of the above-mentioned chemokine receptors ($CCR6^+CCR4^-CXCR3^+$) in combination with markers of memory T cells is still widely used to identify this T-helper subpopulation, especially in clinical studies that do not involve prolonged cultivation and evaluation of intracellular factor expression [7, 12, 13, 15].

Mechanisms of formation of T lymphocytes co-expressing Th17 and Th1 markers

Traditionally, Th17 cell differentiation is initiated in the presence of IL-6, transforming growth factor β (TGFβ), IL-1β and IL-23, sequentially activating transcription factors STAT3 and RORC (in mice, RORγt) [17]. The primary inducers of differentiation are IL-6/TGFβ or IL-6/IL-1β, and IL-23 is included in the process later, when the IL-23R receptor appears on the membrane of activated T lymphocytes: signaling through this receptor stimulates cell expansion and is necessary to maintain cell function, in particular, to synthesize IL-17 [18]. Classical Th17 express the key transcription factor RORC, carry specific markers such as the lectin-like killer cell receptor CD161 and the chemokine receptor CCR6 on the membrane, and are able to produce the characteristic cytokines IL-17A, IL-17F, and IL-22 [17, 19].

However, the Th17 population is unstable – differentiated Th17 lymphocytes are capable of transforming into cells of a different phenotype in the local cytokine milieu upon restimulation, and the Th17 shift towards Th1 is most easily realized. An effective inducer of this shift is expectedly IL-12, a major cytokine in Th1 differentiation. Elevated levels of IL-12 induce the development of a subpopulation of Th1-like Th17 cells, in which the expression of Th1-associated transcription factors T-bet/STAT4 and the chemokine receptor CXCR3 is initiated, as well as the synthesis of the key Th1 cytokine IFNγ [3, 10]. As a consequence, the newly formed subpopulation has phenotypic features common to Th17 and Th1 lineages ($CD4^+CD161^+CCR6^+CXCR3^+IL\text{-}17^+IFN\gamma^+$ T cells) [6, 10]. It is variously identified and labeled in the current literature – in this paper we will use the most common name Th17.1 [12–14]. A part of Th17.1 cells ($IL\text{-}17^+IFN\gamma^+$Th17 cells) may completely lose IL-17 production and differentiate into so-called "ex-Th17" cells, which produce only IFNγ, but retain the expression of Th17-associated transcription factor RORC, membrane molecules CD161 and CCR6 ($CD4^+CD161^+CCR6^+CXCR3^+IL\text{-}17^-IFN\gamma^+$T cells) [5, 10, 20, 21], as well as the ability to effectively respond to IL-23 [10]. In addition, some authors have recorded additional transitional forms, for example, with different variants of expression of membrane molecules CD161 and CCR6: $CD4^+CD161^+CCR6^-CXCR3^+IL\text{-}17^-IFN\gamma^+$ T lymphocytes or $CD4^+CD161^-CCR6^+CXCR3^+IL\text{-}17^-IFN\gamma^+$ T cells [22]. Normally, the content of Th1-like Th17 in the peripheral blood of healthy donors is extremely low, but these cells account for the bulk of $CD4^+IL\text{-}17^+$ T lymphocytes in the site of inflammation, including autoimmune one [10], so another informal name for them is "pathogenic Th17", as opposed to classical Th17, which are not pathogenic in various models of autoimmunity and produce substantial amounts of the anti-inflammatory cytokine IL-10 [23].

It should be noted that IL-12 is not the only inducer of Th17 redifferentiation into Th1: it has been shown that IL-23, a typical for the Th17 lineage cytokine, can also induce the appearance of cells with a Th1-like phenotype upon restimulation [23–25]; therefore, it is currently attributed not only to Th17 expansion, but also to their pathogenicity [3, 23]. Moreover, the high level of IL-23R expression by memory T cells is restricted to non-classical Th17 cells co-expressing IFNγ and CXCR3 [12].

An important factor regulating the Th17-Th1 transition is TGFβ, a cytokine that, in combination with IL-6, is consi-

dered a classic stimulator of the primary differentiation of naive CD4$^+$T lymphocytes into Th17. Although TGFβ has now been shown to be dispensable for primary stimulation, it plays an important role in the fate of Th17 by inhibiting the expression of the Th1-associated transcription factor T-bet [10, 23]. This stabilizes the phenotype of classical Th17 and prevents the formation of pathogenic Th1-like variants of this population [10].

Another important point is related to the origin of T lymphocytes co-expressing Th17 and Th1 markers – all the above considerations assume that these cells are formed from mature differentiated Th17, and this has indeed been demonstrated in many studies [3, 10, 26], although at least two other mechanisms of their origin are theoretically possible: first, they may develop directly from naive CD4$^+$T lymphocytes during primary differentiation; second, they may transform from classical Th1. There are no data on the first option yet, but its probability is low, since one of the main inducers of Th1-like Th17 development – IL-23 – has no receptor on naive T lymphocytes [24], and the second inducer – IL-12 – will initiate the development of classical Th1 in this case. Regarding the origin of IFNγ-producing Th17 from Th1, this option has been evaluated in many studies and has not been confirmed to date. In contrast, it has been shown that fractionated Th1 is unable to transform into Th17 or Th17.1 in response to the Th17-polarizing cytokines IL-1β, IL-23, IL-6, and IL-21 *in vitro* [10], and the clonal structure of T cell receptors (TCR) in the Th17.1 population is closer to that of Th17 than Th1 [10]. Therefore, polarized Th17 cells are considered as a major source of IFNγ-producing Th17 cells. The reversibility of Th17 redifferentiation into Th1 is controversial: some authors report the impossibility of "non-classical Th1" (ex-Th17) returning to Th17 due to epigenetic mechanisms [27], while others have shown *ex vivo* transdifferentiation of "non-classical Th1" into Th17 and Th1/Th17 under Th17-polarizing conditions [22].

Causes of Th17 cell plasticity toward Th1 cells

As for the plasticity of the Th17 population, it is important to note that regardless of the direction of redifferentiation, it is generally less stable than the main T-helper populations, Th1 and Th2: unlike the latter, Th17 cells do not form a positive feedback loop during differentiation, the so-called autoactivation, in which the produced cytokine (IFNγ for Th1 or IL-4 for Th2), acting as an auto- or paracrine factor, binds to receptors on the cell membrane and enhances its own production, which stabilizes the corresponding phenotype. In addition, the second important component of the phenotype stabilization process – alternative T-helper lineage differentiation suppression – does not work in Th17 cells, whereas in Th1 and Th2 cells this mechanism is well established, including in relation to Th17. Thus, the Th1-associated regulator T-bet binds to the transcription factor RUNX1 and blocks its interaction with RORγt, eventually suppressing Th17 differentiation [28]. The major Th2 response inducer GATA3 [29], the cytokines IL-2 (via STAT5) [30], IFNγ (via STAT1) and IL-12 (via STAT4) also have inhibitory effects against RORγt. Moreover, unlike the key regulators of Th1/Th2 lineages, T-bet and GATA3, RORγt regulates the transcription of a significantly smaller number of loci in Th17 cells [31], which, in the absence of reliable mechanisms to stabilize its expression, does not allow us to consider this factor as a full-fledged "master regulator" capable of providing an effective program of Th17 differentiation, which determines the high instability of the population.

Regarding the preferential plasticity of Th17 towards Th1, several reasons are noted. One is that a key cytokine in Th17 cell differentiation, IL-23, shares a common subunit with IL-12, a major Th1 inducer, and the IL-23R receptor also shares one of the two subunits with IL-12R [32]. As a consequence, the signals initiated in Th17 upon IL-23 binding to the corresponding receptor activate not only STAT3 (a key transcription factor in Th17 differentiation) but also STAT4 (a differentiation factor for Th1), albeit to a significantly lesser extent [33]. That is, the stabilizing IL-23-dependent signal for the Th17 lineage, implemented through STAT3, simultaneously promotes IFNγ co-expression and a shift towards the Th1 phenotype through STAT4 activation [34]. At the molecular level, Th17-Th1 plasticity is associated with permissive epigenetic modifications in Th1-associated loci of Th17 cells [35], in particular in the *IFNG* locus: while in Th2 cells the *IFNG* locus has no noticeable traces of remodeling, in Th17 cells the chromatin structure at this locus bears high similarity to that in Th1 cells [20]. In the work of A. Mazzoni et al. the transformation of *in vitro* generated Th17 into "non-classical" Th1 (ex-Th17) was accompanied by DNA demethylation at *TBX21* (encodes T-bet) and *IFNG* loci with simultaneous DNA methylation at *IL-17A/RORC2* [36], although in an earlier study using memory Th17 clones under similar conditions no epigenetic modifications were detected at the *IFNG* locus [37]. Whether epigenetic priming of Th1-associated loci of Th17 cells is associated with the above-described cross-activation of the transcription factor STAT4 in response to IL-23/IL23R-dependent signaling remains an open question.

MARKERS OF Th1-LIKE Th17 CELLS

To date, two major variants have been identified in the Th1-polarized Th17 cell population. The first is Th17-lymphocytes, producing along with classical IL-17 the major Th1 cytokine IFNγ, as well as co-expressing the transcription factor T-bet, the chemokine receptor CXCR3 and a number of other Th1-associated molecules [3, 10]. This subpopulation combines phenotypic and functional characteristics of both Th17 and Th1 lineages and is most generally represented in the literature as CD4$^+$CD161$^+$CCR6$^+$CXCR3$^+$IL-17$^+$IFNγ$^+$T cells [6, 10]. In some cases, when these cells are identified using membrane molecules only, without assessment of cytokine synthesis, two more chemokine receptors, CCR4 and CCR10, are added to the line of markers to separate the populations of classical Th17 and Th22, which, when identified using these membrane markers, have the same phenotype (CCR6$^+$CXCR3$^{-/low}$), but can be dif-

ferentiated by the CCR4/CCR10 combination: CCR4 is represented in both populations, whereas CCR10 is highly expressed on Th22 cells but absent in Th17 cells [12]. As a result, the interested subpopulation of Th17 cells co-producing IL-17/IFNγ will have a $CD4^+CD161^+CCR6^+CCR4^{-/low}$ $CCR10^-CXCR3^+$ phenotype, in contrast to classical Th17 with a $CD4^+CD161^+CCR6^+CCR4^+$ $CCR10^-CXCR3^-$ phenotype [12]. In addition, in *ex vivo* studies, Th17.1 and ex-Th17 cells are typically isolated from pre-fractionated memory T cells, either central ($CCR7^+CD45RA^-$) or effector ($CCR7^-$ $CD45RA^-/CD45R0^+$). Obviously, this is not the limit of detail: there are studies in which, along with membrane molecules, a wide range of intracellular molecules are evaluated, both at the mRNA and protein levels, but in most studies, especially clinical ones, this subpopulation is identified by key cytokines (IL-17/IFNγ), chemokine receptors (CCR6/CXCR3) or a combination of both. These cells do not have a single name, they have many designations in the literature (to be discussed below), in this paper we use the most common one – Th17.1 [12–14].

The second variant of Th1-polarized Th17 cells is actually the result of further redifferentiation of Th17.1 towards Th1, in which the cells lose IL-17 synthesis, producing only IFNγ, but retain the other "attributes" of the initial population – expression of Th17-associated transcription factor RORC and membrane molecules CD161/CCR6 ($CD4^+CD161^+CCR6^+CXCR3^+IL\text{-}17^-IFN\gamma^+$T cells) [2, 5, 10, 20]. These are the so-called "ex" Th17 (ex-Th17), aka "non-classical Th1" [6] or Th1* [15]. It should be noted that the ex-Th17 subpopulation cannot be identified only by cytokine production or only by the expression of membrane markers: in the first case it overlaps with classical Th1 and in the second case with Th17.1 cells. This is why they are often mistakenly "underestimated" or "overestimated".

However, identification problems apply not only to ex-Th17 cells but also to the Th1-polarized Th17 population as a whole. As noted above, the nomenclature of such cells is not uniform; different studies have labeled these cells as Th17/Th1, Th1/Th17, Th1/17, Th17/1, Th17-1, Th17.1, and so on. Although it is clear from the name that these are products of Th17 to Th1 transformation, careful analysis of such papers shows that they often refer to different subpopulations.

Let's look at a few examples. The work of R. Ramesh et al. [12] is one of the few in which a subpopulation of Th17-lymphocytes co-producing IL-17/IFNγ has been identified as accurately as possible to date: in it, memory T cells – central ($CD4^+CCR7^+CD45RO^+$) and effector ($CD4^+CCR7^-CD45RO^+$) – were sequentially isolated from human peripheral blood cells, which were further fractionated based on the expression of CCR6/CCR4/CXCR3 chemokine receptors and IL-17/IFNγ intracellular cytokines ($CD4^+CCR6^+CCR4^{lo}CXCR3^{hi}IL\text{-}17^+IFN\gamma^+$T lymphocytes). The subpopulation was first designated in the paper as Th17.1, which is the most common variant of its name to date.

However, other works using the same designation, Th17.1 [13, 14] do not refer to the same population – or rather, not to it alone. So, in a study by J. Ramstein et al. [13], Th17.1 cells were identified in peripheral blood and bronchoalveolar lavage of sarcoidosis patients by co-expression of CCR6/CXCR3 ($CD4^+CCR6^+CCR4^-$ $CXCR3^+$T lymphocytes). Similarly, the Th17.1 subpopulation in blood memory cells of healthy donors and rheumatoid arthritis patients ($CD4^+CD45R0^+CCR6^+CCR4^-CXCR3^+$T lymphocytes) was determined by co-expression of CCR6/CXCR3 in the study of W. Dankers et al. [14]. Clearly, when only CCR6/CXCR3 chemokine receptors are used as markers, a general Th1-like Th17 population including Th17.1 and ex-Th17 is identified. The subpopulation denoted in the literature as Th1/Th17 [7, 8], was also identified by co-expression of CCR6/CXCR3 chemokine receptors – in circulating $CD70^+$ T lymphocytes of multiple sclerosis patients ($CD4^+CD70^+CCR6^+CXCR3^+$T lymphocytes) [8] and in central memory cells from peripheral blood of such patients CCR6/CXCR3 ($CD4^+CCR7^+CD45RA^-$ $CCR6^+CXCR3^+$T lymphocytes) [7]. There were no cytokines determined here, so we are talking about a general Th1-like Th17 population including both Th17.1 and ex-Th17. This also applies to the subpopulation of circulating Th1* cells in the study by N. Nishihara et al. ($CD4^+CD45RO^+CXCR3^+CCR4^-CCR6^+$T lymphocytes) [15] or to Th1/17 cells from the blood of healthy donors in the study by T. Duhen et al. ($CD4^+CD45RO^+CD25^-$ $CD127^+CCR6^+CXCR3^+$T lymphocytes) [9]. Remarkably, in a number of studies described above, subsequent evaluation of cytokines in T lymphocytes co-expressing CCR6/CXCR3 showed that only a small fraction of these cells co-produce IL-17/IFNγ, whereas the major part (in bronchoalveolar lavage – up to 60 %) – IFNγ alone [9, 13].

On the other hand, there are many studies in the literature, in which Th1-like Th17 cells are identified by co-expression of IL-17/IFNγ cytokines, such as the Th17-1 subpopulation identified in the blood of healthy donors ($CD4^+IL\text{-}17^+IFN\gamma^+$T-lymphocytes) [11], Th17/Th1 cells from the blood and intestinal mucosa of patients with Crohn's disease ($CD4^+IL\text{-}17^+IFN\gamma^+$T lymphocytes) [5], and Th17/Th1 cells identified in the blood and synovial fluid of patients with juvenile idiopathic arthritis ($CD4^+IL\text{-}17^+IFN\gamma^+$T lymphocytes) [10]. All these papers obviously refer to a subpopulation, which is named Th17.1 in our review. In a study by L. Maggi et al. [6], the Th17-specific membrane molecule CD161 was used as markers along with IL-17/IFNγ cytokines to identify Th17/Th1 cells in blood: its use allowed to identify not only the subpopulation of IL-17/IFNγ-co-producing Th17, i. e. Th17.1 ($CD4^+CD161^+IL\text{-}17^+IFN\gamma^+$T-lymphocytes), but also the second major subpopulation of Th1-like Th17 – ex-Th17, or "non-classical" Th1 ($CD4^+CD161^+IL\text{-}17^-$ $IFN\gamma^+$ cells), differentiating it with CD161 from classical Th1 ($CD4^+CD161^-IL\text{-}17^-IFN\gamma^+$T cells) [6].

To summarize: the use of membrane markers CCR6/CXCR3 allows the identification of both major subpopulations of Th1-like Th17, Th17.1 and ex-Th17, but does not differentiate them from each other. Evaluation of IL-17/IFNγ cytokine co-expression only detects Th17.1 but not ex-Th17. Only a combined approach using membrane molecules as markers along with cytokines makes it possible to separate these subpopulations.

Transcription factors deserve special attention as potential markers in such studies: although the evaluation of T-bet and RORC expression is a standard in defining classical Th1 and Th17 populations, their use to identify subpopulations of Th17.1 and ex-Th17 seem to be inefficient: few studies show that if RORC expression in Th17.1 and ex-Th17 subpopulations is similar and comparable to that in classical Th17, T-bet has low expression in Th17.1, although its level in ex-Th17 is comparable to that in classical Th1 [13].

CONCLUSION

Despite the fact that Th1-polarized Th17 are also detected in healthy donors, the interest in this population is primarily due to the presence (and significant prevalence) of these cells in foci of inflammation in multiple sclerosis [7, 8, 24], sarcoidosis [13], rheumatoid arthritis [10, 14], and inflammatory bowel disease [5, 38], and their contribution to the development of these diseases has been proven. Moreover, the process of Th17 redifferentiation into Th1 is currently considered as a promising target for therapy. In this regard, the issue of method unification for isolation of these cells is very relevant, especially considering that the population of Th1-like Th17 cells is heterogeneous and includes at least two variants – Th17.1 and ex-Th17 – apparently reflecting different stages of Th17 transformation. The role of each subpopulation in pathogenesis and their unique properties are still poorly understood: there are only a few studies in which Th17.1 and ex-Th17 cells have been isolated and evaluated individually. Most researchers use one of two methods to identify Th1-like Th17 cells – by co-expression of CCR6/CXCR3 chemokine receptors or IL-17/IFNγ cytokines – and obtain results that are not always comparable with each other. The reason for such contradictions seems to be that the ex-Th17 subpopulation, overlooked in the determination of IL-17/IFNγ-co-producing cells, varies greatly depending on localization and milieu: while in peripheral blood its size is small (~5 %), as in short-term culture during Th17-to-Th1 transformation *in vitro*, in sites of inflammation the share of ex-Th17 reaches 60 % [9, 13]. In this regard, the use of CCR6/CXCR3 chemokine receptors as markers provides a more accurate representation of the size of the Th1-like Th17 population than the assessment of cytokine synthesis, but the preferred strategy for the identification of these cells seems to be the simultaneous assessment of the cell expression of IL-17/IFNγ cytokines and Th17-associated membrane markers (CCR6 and/or CD161): it allows not only to identify both subpopulations, Th17.1 and ex-Th17, but also to separate them and to differentiate ex-Th17 from the classical Th1 population.

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Conflict of interest

There are no conflicts of interest, financial or otherwise.

Compliance with Ethical Standards

This article does not describe studies performed by the authors involving humans or using animals as subjects.

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INTERNAL DISEASES

INFLUENCE OF HEALTH RESORT TREATMENT ON THE LEVEL OF SYSTEMIC INFLAMMATION IN PATIENTS AFTER NEW CORONAVIRUS INFECTION

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ABSTRACT

Background. *Low-grade inflammation is currently one of the main risk factors for the development of adverse events in the cardiovascular system, including death from cardiovascular diseases or their complications that cause mortality in the post-COVID period.*

The aim of the study. *To assess the impact of health resort treatment in the conditions of the Southern Coast of Crimea on clinical and functional parameters, as well as on the level of systemic inflammation in patients after a new coronavirus infection.*

Materials and methods. *The study included 67 patients (54.9 ± 9.05 years) in the post-COVID period who underwent health resort treatment at the I.M. Sechenov Academic Research Institute of Physical Treatment Methods, Medical Climatology and Rehabilitation. Methods of health resort treatment included climatic treatment on the Southern Coast of Crimea, therapeutic breathing exercises, terrainkur, and various methods of respiratory therapy. In all patients, the study of the C-reactive protein (CRP) in peripheral blood upon admission and at discharge was carried out.*

Results. *Despite a statistically significant improvement in most clinical and functional parameters, the level of CRP and the number of leukocytes and their subpopulations in peripheral blood in patients who underwent health resort treatment did not differ significantly ($p > 0.05$) from the baseline values obtained upon admission at the I.M. Sechenov Academic Research Institute of Physical Treatment Methods, Medical Climatology and Rehabilitation. The CRP index upon admission and at discharge corresponded to the lower limit of the interval specific for low-grade inflammation (from 3 to 10 mg/l).*

Conclusion. *Our results indicate the lack of effectiveness of the presented plan of health resort treatment in the correction of low-intensity inflammation, as well as the necessity for deeper scientific research in the direction of studying the mechanisms of low-grade inflammation development and the methods of its management.*

Key words: *SARS-CoV-2, inflammation, health resort treatment, post-COVID, CRP*

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ВЛИЯНИЕ САНАТОРНО-КУРОРТНОГО ЛЕЧЕНИЯ НА УРОВЕНЬ СИСТЕМНОГО ВОСПАЛЕНИЯ У ПАЦИЕНТОВ, ПЕРЕНЁСШИХ НОВУЮ КОРОНАВИРУСНУЮ ИНФЕКЦИЮ

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РЕЗЮМЕ

Обоснование. *Низкоинтенсивное воспаление (НИВ) на данный момент является одним из главных факторов риска развития неблагоприятных событий со стороны сердечно-сосудистой системы, включая смерть от сердечно-сосудистых заболеваний или их осложнений, обуславливающих смертность в постковидном периоде.*

Цель исследования. *Оценить влияние санаторно-курортного лечения в условиях Южного берега Крыма на клинические и функциональные показатели, а также на уровень системного воспаления у пациентов, перенёсших новую коронавирусную инфекцию.*

Материалы и методы. *Обследовано 67 пациентов (возраст – 54,9 ± 9,05 года) в постковидном периоде, проходивших санаторно-курортное лечение в ГБУЗ РК «Академический научно-исследовательский институт физических методов лечения, медицинской климатологии и реабилитации имени И.М. Сеченова». Методы санаторно-курортного лечения включали климатотерапию на Южном берегу Крыма, лечебную дыхательную гимнастику, терренкуры, различные методы респираторной терапии. Всем пациентам было проведено исследование уровня С-реактивного белка (СРБ) в периферической крови при поступлении и на момент выписки.*

Результаты. *Несмотря на статистически значимое улучшение большинства клинических и функциональных показателей, уровень СРБ и количество лейкоцитов и их субпопуляций в периферической крови у пациентов, прошедших санаторно-курортное лечение, статистически значимо не отличались ($p > 0,05$) от исходных показателей, полученных в день поступления в ГБУЗ РК «Академический научно-исследовательский институт физических методов лечения, медицинской климатологии и реабилитации имени И.М. Сеченова». Показатель СРБ при поступлении и выписке соответствовал нижней границе интервала, характерного для НИВ (от 3 до 10 мг/л).*

Заключение. *Полученные нами результаты свидетельствуют о отсутствии эффективности представленного плана санаторно-курортного лечения в коррекции НИВ, а также о необходимости более глубоких научных изысканий в направлении изучения механизмов формирования НИВ и методов борьбы с данным состоянием.*

Ключевые слова: *SARS-CoV-2, воспаление, санаторно-курортное лечение, постковид, СРБ*

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The manifestations of the new coronavirus infection (NCI) acute period are only the tip of the iceberg that modern public health is already facing. The number of people with SARS-CoV-2 as of January 20, 2023 is estimated to be more than 500 million, and this number is increasing every day [1]. The diversity and unpredictability of distant manifestations of NCI makes the post-COVID period an extremely dangerous interval of time. Complications from various organs and systems can lead to both disability and fatal outcome due to the development of life-threatening conditions [2].

Despite the polymorphic manifestations of post-COVID syndrome, systemic inflammation and disturbance of the hemostasis system are undoubtedly an important part of all disorders [2]. The role of low-grade systemic inflammation is often completely underestimated.

The main indicator characterizing the status of low-grade inflammation (LGI) is the level of C-reactive protein (CRP) in peripheral blood ranging from 3 to 10 mg/L [3, 4]. Currently, LGI status is a risk factor for cardiovascular adverse events (including death from cardiovascular disease (CVD) or complications) [5]. Cardiovascular events are one of the most common manifestations of post-COVID syndrome, accounting for post-COVID mortality [6].

In connection with the above, it is of utmost importance to search for the most effective methods of combating low-grade inflammation at the stage of health resort treatment of the post-COVID patient.

The aim of our study was to evaluate the effect of health resort treatment in the conditions of the Southern coast of Crimea on clinical and functional parameters, as well as the level of systemic inflammation in patients who had a new coronavirus infection.

MATERIALS AND METHODS

The study included 67 patients admitted to the Pulmonology Department of the I.M. Sechenov Academic Research Institute of Physical Treatment Methods, Medical Climatology and Rehabilitation for health resort treatment after a new coronavirus infection.

Women accounted for 58.2 % ($n = 39$) of the study population, men – 41.7 % ($n = 28$); mean age – 54.9 ± 9.05 years.

An average of 160 ± 18 days passed from the onset of the first symptoms to the patient's admission to the Pulmonology Department.

The inclusion criteria for the study were: new coronavirus infection and referral to health resort treatment within more than 14 days after discharge from the Infectious Disease Hospital or recovery, as well as the absence of concomitant pathologies.

Exclusion criteria were: presence of complicated forms of previous viral pneumonias with expressed functional pulmonary and extrapulmonary disorders; age more than 75 years; general contraindications for health resort treatment. Patients were included in the study after signing informed consent.

All patients on admission were clinically examined and medical history data were collected. On admission and at discharge, the patients underwent laboratory examination of peripheral blood, and clinical and functional parameters were assessed. Interpretation of clinical symptoms (cough and dyspnea) was performed using a three-point scale, according to which 1 point – moderate severity; 2 points – medium severity; 3 points – pronounced clinical symptom. Function tests included electrocardiogram, spirogram with determination of forced vital capacity (FVC), forced expiratory volume in 1 s (FEV1) and inspiratory capacity (IC). The mMRC (Modified Medical Research Council) Dyspnea Scale, OCD (Oxygen Cost Diagram) and VAS (Visual Analogue Scale) were used.

Methods of health resort treatment: nebulizer therapy with bronchodilators and mucolytics as needed; halo-inhalation therapy using Galoneb apparatus; exercises on Coach 2 breathing simulators with inspiratory load; high frequency chest wall oscillation; diaphragmatic breathing training; hypercapnic-hypoxic training; chest massage; therapeutic exercises (breathing assembly); physiotherapy methods (magnetic therapy on the chest); Terrainkur; climatotherapy (round-the-clock or dosed aerotherapy); air and sun baths; sea bathing.

Data were analyzed using licensed Statistica 12 statistical software (StatSoft Inc., USA). Initially, all studied indicators were tested for normality of distribution using the Shapiro-Wilk W-test; samples in which the test was $p \geq 0.1$ were taken as normal distribution, while the W test value of $p < 0.1$ was taken as non-normal distribution. When processing non-parametric data, the Wilcoxon T-test for related samples was used to compare groups. Indicators were considered statistically significant at $p < 0.05$. Under normal distribution, the paired Student's T-test for related samples was used to handle non-parametric data to compare groups. Indicators were considered statistically significant at $p < 0.05$.

RESULTS

As can be seen from the data presented in Table 1, patients who had a new coronavirus infection and underwent health resort treatment showed a statistically significant decrease in the frequency and severity of cough, severity of dyspnea, feeling of heaviness in the chest and fatigue ($p < 0.001$). A statistically significant increase in distance in the 6-minute walk test ($p < 0.001$), a decrease in the mMRC Dyspnea Scale and a positive trend in OCD ($p < 0.001$) and VAS ($p < 0.05$) were recorded. There was also a statistically significant improvement in FVC and IC ($p < 0.05$).

Despite the statistically significant improvement of most clinical and functional parameters, the level of CRP and the number of leukocytes and their subpopulations in the peripheral blood of patients who underwent health resort treatment, statistically significantly did not differ ($p > 0.05$) from the baseline values obtained on the day of admission to the I.M. Sechenov Academic Research Institute of Physical Treatment Methods, Medical Climatology and Rehabilitation. The CRP index upon admission and at discharge corresponded to the lower limit of the in-

TABLE 1

CLINICAL AND FUNCTIONAL PARAMETERS, M ± σ

Parameters	Before treatment	After treatment	Statistical significance of differences, *p*
Cough – frequency, points	0.731 ± 0.962	0.257 ± 0.532	$p < 0.001$
Cough – severity, points	0.492 ± 0.704	0.208 ± 0.409	$p < 0.001$
Dyspnea – severity, points	1.208 ± 0.879	0.507 ± 0.587	$p < 0.001$
Feeling of heaviness in the chest, points	0.477 ± 0.704	0.044 ± 0.208	$p < 0.001$
Fatigue, score	1.059 ± 0.850	0.268 ± 0.479	$p < 0.001$
Diastolic BP, mm Hg	79.720 ± 0.842	79.121 ± 0.566	$p > 0.05$
FVC, %	102.402 ± 2.188	105.310 ± 2.339	$p < 0.05$
FEV1, %	97.372 ± 2.425	98.212 ± 2.182	$p > 0.1$
IC, %	96.536 ± 3.879	102.356 ± 3.115	$p < 0.05$
6-Minute Walk Test, m	502.742 ± 10.883	532.136 ± 8.847	$p < 0.001$
mMRC Dyspnea Scale, points	1.380 ± 0.084	1.174 ± 0.090	$p < 0.05$
Oxygen Price Chart, points	6.891 ± 0.208	7.835 ± 0.300	$p < 0.001$

Note. The table shows the quantitative (M ± σ) attributes. Statistical significance of differences between the values of indicators before and after health resort treatment was calculated using Student's T-test for related samples. BP - blood pressure.

terval specific for low-grade inflammation (3 to 10 mg/L) (Table 2). The values of the main biochemical markers were also not statistically significantly different ($p > 0.05$) from the baseline values obtained on the day of admission.

DISCUSSION

In contrast to acute inflammation caused by injury or acute infection and accompanied by marked dysfunction and symptoms and signs from organs and organ systems, chronic low-grade systemic inflammation may go undetected for years and be detected only by routine laboratory tests. However, despite the paucity of symptoms, gradually disrupting metabolic and repair processes in intact tissues, chronic inflammation leads to the development of numerous age-related diseases, functional and morphological reorganization of organs and organ systems and increased risk of life-threatening conditions [7].

The role of inflammation in the pathophysiology of the early stages of diseases associated with atherothrombotic complications has been recognized for more than 25 years. A number of inflammatory mediators produced by leukocytes attracted to the damaged subendothelial compartment of arteries contribute to the progression of atherosclerosis. This effect is multifactorial and is determined by the increased migration of new leukocytes into the lesion area, promoting the formation of altered macrophages – foam cells containing lipids [8], increased expression of endothelial adhesion molecules [9] and stimulation of smooth muscle cell proliferation [10], which ultimately causes plaque instability [11] and rupture [12, 13].

In addition, inflammation is an important determinant of the onset and development of dysmetabolic disorders, increasing the risk of type 2 diabetes mellitus, non-alcoholic fatty liver disease (NAFLD), and CVD [14].

The state of LGI itself is a polyethiologic problem. The current literature explains the occurrence of LGI, on the one hand, by uncorrectable genetic traits of an individual, namely, the presence of single-nucleotide polymorphisms of genes of the main proinflammatory molecules [15], and, on the other hand, by conditions that are amenable to therapeutic intervention, such as increased intestinal permeability to certain bacterial proinflammatory com-

TABLE 2

LABORATORY INDICATORS, ME [Q1; Q3]

Indicators	Before treatment	After treatment	Statistical significance of differences, *p*
Cholesterol, mmol/L	5.7 [5.0; 6.8]	5.8 [4.8; 6.5]	$p > 0.05$
Glucose, mmol/L	5.4 [4.8; 6.0]	5.2 [4.9; 5.7]	$p > 0.05$
ALT, u/l	22.5 [19.0; 28.6]	22.2 [17.1; 27.2]	$p > 0.05$
AST, u/l	25.05 [21.7; 30.9]	24.6 [19.8; 29.7]	$p > 0.05$
LDH, u/l	311.0 [263.0; 351.0]	329.0 [297.0; 379.0]	$p > 0.05$
ALP, u/l	142.0 [110.0; 165.0]	149.0 [113.0; 182.5]	$p > 0.05$
Creatinine, μmol/l	88.5 [81.5; 96.0]	87.0 [79.0; 97.0]	$p > 0.05$
Urea, mmol/l	5.1 [4.4; 6.0]	5.0 [4.2; 5.9]	$p > 0.05$
Total protein, g/l	78.0 [72.0; 80.0]	77.0 [71.5; 80.0]	$p > 0.05$
Albumin, g/l	37.0 [36.0; 39.0]	39.0 [36.0; 40.0]	$p > 0.05$
CRP, mg/l	3.0 [3.0; 4.0]	3.0 [3.0; 4.0]	$p > 0.05$
Absolute Leukocyte Count, 10^9/l	6.0 [5.1; 7.4]	6.2 [5.3; 7.5]	$p > 0.05$
Absolute Neutrophil Count, 10^9/l	3.5 [2.7; 4.5]	3.4 [2,6; 4.3]	$p > 0.05$
Absolute Lymphocyte Count, 10^9/l	1.8 [1.5; 2.2]	1.8 [1.4; 2.3]	$p > 0.05$
Absolute Monocyte Count, 10^9/l	0.4 [0.2; 0.5]	0.4 [0.2; 0.6]	$p > 0.05$
Absolute Band Neutrophil Count, 10^9/l	0.2 [0.1; 0.3]	0.1 [0.1; 0.3]	$p > 0.05$
Absolute Segmented Neutrophils Count, 10^9/l	3.2 [2.5; 4.1]	3.2 [2.5; 4.0]	$p > 0.05$

Note. The table presents quantitative (Me [Q1; Q3]) attributes. Differences in quantitative attributes were identified using the Wilcoxon test. ALT — alanine aminotransferase; AST – aspartate aminotransferase; LDH – lactate dehydrogenase; ALP – alkaline phosphatase.

ponents [16] and the presence of chronic infection [17], on the other hand, conditions that are amenable to therapeutic intervention, such as increased intestinal permeability to certain bacterial proinflammatory components [16], the presence of chronic infection [17], and dysmetabolic disorders, in particular obesity and type 2 diabetes mellitus [18].

Low-grade inflammation certainly aggravates the course of the acute period of SARS-CoV-2 infection, and in this case, the already existing chronic dysregulation of the immune system is complicated by the accession of acute infection, which is rather complicated in pathogenetic terms [19]. This combination of chronic and acute processes leads to certain consequences on the part of organs and systems and in the post-COVID period, primarily changing the homeostasis of the cardiovascular system and hemocoagulation system, increasing the risk of fatal complications [5, 6].

In this regard, the control of LGI in post-COVID patients is, in our opinion, one of the highest priorities to ensure the health of the population now and in the postpandemic period. To address this problem, it is necessary to eval-

uate the impact of already available and applied methods of health resort treatment used in the post-COVID period and, if necessary, to make adjustments to existing protocols for the management of post-COVID patients.

In our study, the patients underwent health resort treatment in the conditions of the Southern Coast of Crimea, received therapeutic diets, therapeutic breathing exercises and various methods of respiratory therapy.

Despite the significant clinical effect and improvement of functional parameters, the main marker of systemic inflammation in these patients did not undergo statistically significant changes and remained at the level corresponding to the lower limit of the interval specific for LGI (from 3 to 10 mg/l) (Table 2).

A number of other biochemical indicators also did not change statistically significantly, but most of the indicators were within the reference range (Table 2).

The mechanism of anti-inflammatory effect of exercise affecting the ratio of proinflammatory and anti-inflammatory cytokines has been described in the literature [20, 21]; however, in our study, a course of therapeutic exercise (24 days) had no significant effect on LGI status in post-COVID patients.

Based on the possible causes of LGI listed above, such methods can be pharmacological and non-pharmacological interventions aimed at regulating the permeability of the intestinal barrier, improving the liver, sanitation of chronic foci of infection and correction of dysmetabolic processes.

CONCLUSIONS

Complex health resort treatment of patients in the post-COVID period is accompanied by improvement of clinical and functional parameters and general condition. Health resort treatment has practically no significant effect on the change of the main laboratory biochemical indicators. Currently available methods of health resort treatment do not affect the level of low-grade systemic inflammation in patients in the post-COVID period. All this determines the need for more profound scientific research to study the mechanisms of LGI formation and methods of combating this condition.

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Conflict of interest

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

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CARDIOLOGY

CLINICAL SYMPTOMS AND ECG DATA IN WOMEN WITH ACUTE CORONARY SYNDROME

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ABSTRACT

Background. *There are many differences in chest pain symptoms between men and women in terms of location, nature, and additional symptoms. The issue of describing the differences in chest pain in men and women with acute coronary syndrome (ACS), as well as their correlation with changes in the electrocardiogram (ECG) and coronary angiography (CAG) remains relevant.*
Methods. *The study included 588 patients of the cardiology department of the Novokuznetsk City Clinical Hospital No. 1 from 2013 to 2017 with a diagnosis of ACS. Depending on the gender, the subjects were divided into two groups: Group I – 330 men; Group II – 258 women.*
Results. *ACS with ST elevation was more common in men (45.8 %) than in women (33.3 %; p = 0.002). There were no pathological ECG changes in women in 58.1 % of cases, in men – in 45.5 % (p < 0.001). ECG type Q/ST elevation was detected more often in men (45.8 %) than in women (33.3 %; p = 0.002). The absence of coronary artery lesions was observed in 27.9 % of men and 44.2 % of women (p < 0.001). Hemodynamically significant coronary artery stenosis was more common in men (57.6 %) than in women (38.7 %; p < 0.001). In a typical angina clinic, hemodynamically significant coronary artery disease in patients with Q/without ST elevation ACS was detected in 40.2 % of men and in 58.5 % of women (p = 0.002). In the atypical angina clinic, hemodynamically significant lesions of coronary artery were more common in men (40.6 %) than in women (34.1 %; p = 0.02).*
Conclusion. *In women atypical chest pains and intact coronary arteries were detected more often than in men, and hemodynamically significant coronary artery stenosis were found less often than in men. In men, a more pronounced pathology of the coronary arteries in ACS was revealed, in women – great difficulties in diagnosing ACS.*

Key words: *acute coronary syndrome, gender differences, diagnosis of coronary symptoms*

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ОСОБЕННОСТИ КЛИНИЧЕСКОЙ СИМПТОМАТИКИ И ДАННЫХ ЭКГ У ЖЕНЩИН С ОСТРЫМ КОРОНАРНЫМ СИНДРОМОМ

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РЕЗЮМЕ

Существует много различий в симптомах боли в груди между мужчинами и женщинами в отношении локализации, характера и дополнительных симптомов. Остаётся актуальным вопрос описания различий болевого синдрома в груди у мужчин и женщин с острым коронарным синдром (ОКС), а также соотнесение их с изменениями электрокардиограммы (ЭКГ) и данными коронароангигорафии (КАГ).

Методы. *В исследование были включены 588 пациентов кардиологического отделения ГБУЗО КО «Новокузнецкая городская клиническая больница № 1» с 2013 по 2017 г. с диагнозом ОКС. В зависимости от половой принадлежности исследуемых разделили на две группы: I группа – 330 мужчин; II группа – 258 женщин.*

Результаты. *ОКС с подъёмом сегмента ST (ОКС nST) чаще наблюдался у мужчин (45,8 %), чем у женщин (33,3 %; p = 0,002). Патологических изменений на ЭКГ у женщин не было в 58,1 % случаев, у мужчин – в 45,5 % (p < 0,001). Тип ЭКГ Q/nST чаще выявлялся у мужчин (45,8 %), чем у женщин (33,3 %; p = 0,002). Отсутствие поражения коронарных артерий (КА) отмечено у 27,9 % мужчин и у 44,2 % женщин (p < 0,001). Гемодинамически значимый стеноз КА чаще встречался у мужчин (57,6 %), чем у женщин (38,7 %; p < 0,001). При типичной клинике стенокардии гемодинамически значимое поражение КА у больных ОКС Q/бnST (без подъёма сегмента ST) выявлено в 40,2 % случаев у мужчин и в 58,5 % – у женщин (p = 0,002). При атипичной клинике стенокардии гемодинамически значимые поражения КА встречались чаще у мужчин (40,6 %), чем у женщин (34,1 %; p = 0,02).*

Заключение. *У женщин чаще, чем у мужчин, выявляли атипичные боли в грудной клетке, интактные КА и реже – гемодинамически значимые стенозы КА. У мужчин выявлена более выраженная патология коронарных артерий при ОКС, у женщин – большие трудности диагностики ОКС.*

Ключевые слова: *острый коронарный синдром, гендерные различия, диагностика коронарных симптомов*

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INTRODUCTION

Cardiovascular disease is the major reason for disability and mortality among men and women worldwide. Acute coronary syndrome (ACS) requires immediate diagnosis and treatment to prevent complications and death [1, 2]. It is generally believed that difficulties and errors in diagnosis are characteristic of a rare pathology. Coronary heart disease (CHD) is a frequent and leading cause of death worldwide [3], and yet its diagnosis in medical practice is a complex problem. The study of Y.A. Prilutskaya et al. [4] showed that more than half of hospitalized patients (51 %) with a referral diagnosis of "unstable angina pectoris" had no ACS. Patients with "non-OCS" were diagnosed with various variants of chronic CHD or non-cardiac pathology. Acute coronary pathology was confirmed in only 38 % in the group with "possible ACS" (21 %); less than half (45 %) of this group were men. The difficulties in diagnosing this syndrome are due to clinical heterogeneity of ACS with and without ST segment elevation, ambiguity of pathogenesis associated with the development of atherosclerotic plaques in epicardial arteries of the heart or their functional stenosis and, often, microvascular dysfunction. The nature of chest pain among patients with ACS is of crucial diagnostic importance in some situations, but in other cases it only indicates the direction of examination. It is known that women develop CHD 5–10 years later than men [3, 5], i. e. at an older age and with a different specificity of symptoms [6, 7]. In addition, studies show that physicians use less aggressive diagnostic and therapeutic approaches to the treatment of women with CHD than men [8]. Symptoms of CHD in women often resemble other non-cardiac conditions [9, 10], especially when diabetes mellitus or other diseases are present. All this interferes with the recognition of ACS and may be associated with late treatment or delayed hospitalization [8, 11, 12]. There are many differences in chest pain symptoms between men and women in terms of its nature, localization and additional symptoms [6, 13, 14]. For example, it has previously been shown that women with ACS were less likely to report chest pain as a chief complaint and more likely to report nausea, shoulder and upper back pain. Also, women with ACS had more symptoms compared to men [15]. On the other hand, on the contrary, there is evidence that typical symptoms are more common and have greater prognostic value among women than men with myocardial infarction, regardless of whether it is diagnosed using gender-specific criteria [16]. Other information is provided by O.T. Steiro et al. [17]: differences in the manifestation of MI symptoms without ST elevation between gender and age groups were small. This may be due to known regional gender differences in clinical presentation, comorbidities, access to care and invasive treatments. Therefore, more data are needed to determine the prevalence of gender differences in ACS, as well as the factors responsible for these differences, especially cultural, socio-economic, educational and psychosocial factors [18].

The aim of this study was to describe the differences of chest pain among men and women, especially when combined with ECG and coronary angiography findings.

MATERIAL AND METHODS

Within the framework of a prospective study from 2013 to 2017, data on 724 patients consecutively admitted to the base of the cardiology department of the Novokuznetsk City Clinical Hospital No. 1 with a diagnosis of ACS. Inclusion criteria were: ACS at the time of admission with subsequent diagnosis verification in the hospital; age > 18 years; hemodynamic stability; consent to participate in the study. Exclusion criteria were critical conditions (shock, pulmonary edema, presence of medical ventilator). A sample size of 588 patients was obtained based on inclusion and exclusion criteria. The study was performed in accordance with the principles of the Declaration of Helsinki, and all patients gave informed consent. The study record was approved by the local ethical committee of Research Institute for Complex Issues of Cardiovascular Diseases (Minutes No. 8 dated 10.10.2021).

The clinical status of the patient was analyzed, in particular the leading syndromes: typical, atypical, non-anginal chest pain, dyspnea [3]. Taking into account that dyspnea can be both a leading syndrome and combined with chest pain, all patients with dyspnea were grouped under the term "actual dyspnea" (A-dyspnea). Cerebral complaints were assessed if the patient had a history of dizziness, syncope, or concussion. Pulmonary pathology was established on the basis of fluorosis, silicosis, bronchial asthma, chronic obstructive pulmonary disease, previous lung surgeries; thyroid pathology – on the basis of thyroidectomy, thyroid resection, ultrasound data and endocrinologist's thyroid treatment; pathology of the musculoskeletal system – on the basis of residual changes after a lower limb trauma, arthrosis of the hip and knee joints, arthritis, herniated discs and spinal implants. ECG data in 12 leads (6 standard leads, 6 chest leads) were analyzed. The following ECG types were identified: "normal", "Q/ST elevation" and "other ECG pathology". Echocardiography was considered for differential diagnosis in case of complications. According to the CAG data (the study was performed via transradial approach on the Allura CV20 angiography unit (Philips, the Netherlands), the degree of stenosis was determined using computer quantitative image processing.

Depending on the gender, the subjects were divided into two groups: Group I – 330 men; Group II – 258 women.

Statistical processing was performed using the SPSS Statistics 19.0 software package (IBM Corp., USA). The distribution of quantitative variables was checked for normality using the Kolmogorov-Smirnov criterion. If the distribution is normal, data are presented as mean values (M) and mean errors (m); if the distribution is non-normal, data are presented as median (Me) and quartiles (25^{th} and 75^{th} percentiles). Student's t-test, Mann – Whitney U test and chi-squared test were used to compare the two groups.

RESULTS

All patients with acute coronary syndrome

Clinical and anamnestic characteristics of the examined patients with ACS are presented in Table 1. Patients admit-

TABLE 1

CLINICAL AND ANAMNESTIC CHARACTERISTICS OF PATIENTS WITH ACUTE CORONARY SYNDROME (*n* = 588)

Parameters	Men (*n* = 330)	Women (*n* = 258)	*p*
Age, M ± SD	56.5 ± 0.6	61.8 ± 0.59	< 0.001
Age of patients with AH, M ± SD	57.4 ± 9.56	62.03 ± 9.35	< 0.001
Medical history			
Typical pain, *n* (%)	98 (29.7)	91 (35.3)	0.151
Atypical pain, *n* (%)	180 (54.5)	139 (53.9)	0.871
Non-anginal pain, *n* (%)	21 (6.4)	9 (3.5)	0.115
Dyspnea – leading syndrome, *n* (%)	31 (9.4)	19 (7.4)	0.062
A-dyspnea, *n* (%)	128 (38.8)	142 (55.0)	< 0.001
AH, *n* (%)	193 (58.5)	196 (76.0)	< 0.001
Cardiac arrhythmia, *n* (%)	66 (20.0)	60 (23.3)	0.393
Atrial fibrillation, *n* (%)	26 (7.9)	14 (5.4)	0.24
Diabetes mellitus, *n* (%)	5 (1.5)	25 (9.7)	< 0.001
Prior stroke, *n* (%)	12 (3.6)	13 (5.0)	0.403
Cerebral complaints, *n* (%)	25 (7.6)	38 (14.7)	0.008
Pulmonary pathology, *n* (%)	18 (5.5)	10 (3.9)	0.411
Thyroid pathology, *n* (%)	3 (0.9)	14 (5.4)	< 0.001
MSS pathology, *n* (%)	27 (8.2)	23 (8.9)	0.867
ECG data			
Normal, *n* (%)	150 (45.5)	150 (58.1)	< 0.001
Without Q/ST elevation, *n* (%)	29 (8.8)	22 (8.5)	0.911
Q/ST elevation, *n* (%)	151 (45.8)	86 (33.3)	0.002
Types of ACS			
ACS without ST elevation, *n* (%)	179 (54.2)	172 (66.7)	0.002
ST elevation ACS, *n* (%)	151 (45.8)	86 (33.3)	0.002
CAG data			
Intact coronary arteries, *n* (%)	92 (27.9)	114 (44.2)	< 0.001
Coronary artery stenosis < 70 %, *n* (%)	48 (14.5)	44 (17.1)	0.473
Coronary artery stenosis ≥ 70 %, *n* (%)	190 (57.6)	100 (38.8)	< 0.001

Note. AH – arterial hypertension; MSS – musculoskeletal system.

ted to the hospital after the 1st day from the onset of symptoms prevailed (456 patients – 77.6 %); the proportion of patients admitted on the 1st day from the onset of symptoms was lower (132 patients – 22.4 %). There were no statistically significant differences in the duration of hospitalization between men and women. At the prehospital stage all patients with ST elevation ACS received narcotic analgesics; only one patient received thrombolytic therapy.

Female patients were statistically significantly older ($p < 0.001$) than males. Anamnestically, arterial hypertension (AH) was detected in 389 (66.2 %) patients. High blood pressure was statistically significantly more frequent

in the female group (196 cases (76.0 %)) than in the male group (193 cases (58.5 %); $p < 0.001$). Women with hypertension were older than men: 62.03 ± 9.35 and 57.04 ± 9.56 years, respectively ($p < 0.001$). Atrial fibrillation was detected in 40 patients (6.8 %) with no significant differences between men and women (7.9 % and 5.4 %, respectively; $p = 0.24$).

When assessing the gender peculiarities of the prevalence of ECG changes, no pathological ECG changes were found in women at the time of examination in 58.1 % of cases, in men – in 45.5 % of cases ($p < 0.001$). At the moment of hospitalization 237 patients (40.3 %) were diagnosed with ST elevation ACS. ST elevation ACS was statistically significantly less frequent in the female group (86 (33.3 %)) than in the male group (151 (45.8 %); $p = 0.002$). ACS without ST elevation was statistically significantly more frequent among women (172 patients (66.7 %)) than among men (179 patients (54.2 %)) ($p = 0.002$). All patients underwent coronary angiography. 44.5 % of women and 28.2 % of men had no coronary artery lesions ($p < 0.001$). Hemodynamically significant coronary artery stenosis (≥ 70 %) was statistically significantly less frequent among women (38.4 %) compared with men (57.3 %; $p < 0.001$). According to the results of CAG, PCI was performed in 97.8 % of patients with hemodynamically significant stenosis (≥ 70 %), and no gender differences were found.

When analyzing concomitant pathology, women were statistically significantly more likely to have type 2 diabetes mellitus, thyroid pathology and cerebral complaints (more often – dizziness, less often – history of syncope, without previous stroke) ($p < 0.05$).

Next, the prevalence of ECG changes depending on the significance of the coronary artery lesion was assessed (Fig. 1).

The comparison of ECG and CAG revealed that among the patients with Q/ST elevation ECG type there were statistically significantly more cases of significant coronary artery lesions (55.6 %; $p < 0.001$). At the same time, in patients with normal ECG, coronary arteries were more often intact in 65.4 % of cases ($p < 0.001$).

Acute coronary syndrome with ST elevation

No gender differences in pain patterns and CAG data were found when evaluating symptoms and clinical data of patients with ST elevation ACS. A-dyspnea was observed in 96 (40.5 %) patients when evaluating clinical symptoms of the patients. It was found that A-dyspnea was noted more frequently in women (51.2 %) than in men (34.4%; $p = 0.012$). ECG comparison revealed that Q/ST elevation ECG type was statistically significantly more frequent in men (63.7 % in men, 36.3 % in women; $p = 0.001$). Type 2 diabetes mellitus, thyroid pathology and cerebral complaints were statistically significantly more frequent in women with ST elevation ACS ($p < 0.001$) (Table 2).

Patients with ACS with ST elevation had predominantly typical (29.1 % of cases) and atypical chest pain (57.4 % of cases). In case of intact coronary arteries atypical pain clinic was more frequent (32 patients (23.5 %)), and in case of hemodynamically significant coronary artery stenosis – typical anginal pain (60 patients (87.0 %)) ($p < 0.001$) (Table 3).

Comparison of clinical symptoms at different CAG changes in patients with ST elevation ACS did not reveal gender differences (Fig. 2).

Acute coronary syndrome without ST elevation

The clinical and medical history characteristics of patients with ACS without ST elevation are presented in Table 4.

At the time of inclusion, men had a younger age compared to women ($p < 0.001$). Arterial hypertension was prevalent in women with ACS without ST elevation ($p < 0.001$). The groups did not differ in terms of pain nature. When assessing the clinical symptoms of the studied patients, dyspnea was detected in 174 (50.1 %) patients. It was found that A-dyspnea was noted more frequently in women (98 (57.0 %) patients) than in men (76 (42.5 %) patients; $p = 0.009$). No gender differences were found in the comparison of ECGs in patients with ACS without ST elevation. CAG analysis revealed that hemodynamically significant CA stenosis was statistically significantly more frequent in men (82 (45.8 %) patients). Whereas in women, coronary arteries were more often intact (94 (54.7 %) patients; $p < 0.001$). A con-

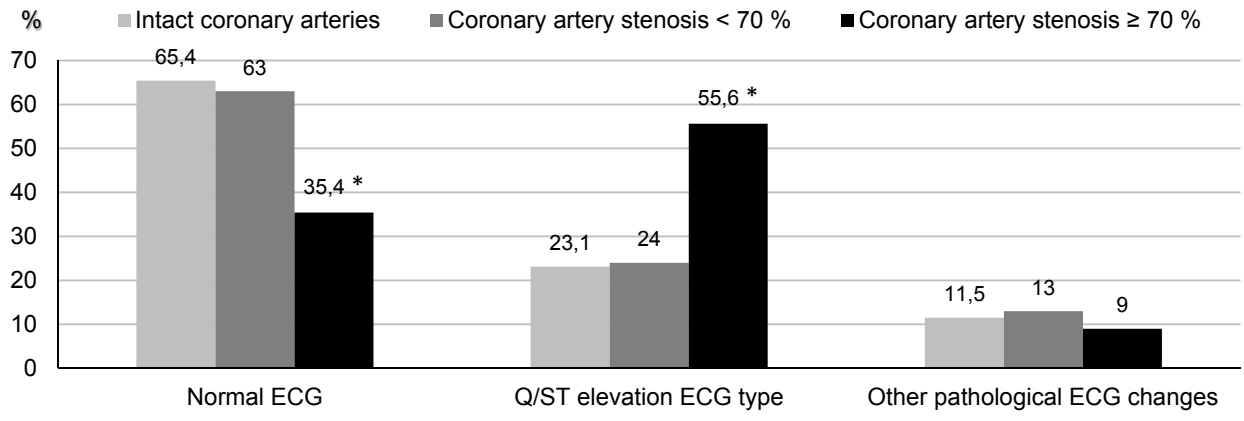

FIG. 1.

*ECG characteristics in comparison with coronary angiography results in patients with acute coronary syndrome: * – p < 0.05*

TABLE 2

CLINICAL AND ANAMNESTIC CHARACTERISTICS OF PATIENTS WITH ACUTE CORONARY SYNDROME WITH ST ELEVATION (*n* = 237)

Parameters	Men (*n* = 151)	Women (*n* = 86)	*p*
Age, M ± SD	56.0 ± 11.48	62.7 ± 9.76	0.001
Age of patients with AH, M ± SD	58.2 ± 9.48	63.5 ± 9.59	0.001
Medical history			
Typical pain, *n* (%)	45 (29.8)	24 (27.9)	0.872
Atypical pain, *n* (%)	85 (56.3)	51 (59.3)	0.753
Non-anginal pain, *n* (%)	9 (6.0)	3 (3.5)	0.598
Dyspnea – leading syndrome, *n* (%)	12 (7.9)	8 (9.3)	0.947
A-dyspnea, *n* (%)	52 (34.4)	44 (51.2)	0.012
AH, *n* (%)	89 (58.9)	65 (75.6)	0.01
Cardiac arrhythmia, *n* (%)	15 (9.9)	18 (20.9)	0.019
Diabetes mellitus, *n* (%)	5 (3.3)	13 (15.1)	< 0.001
Prior stroke, *n* (%)	6 (4.0)	3 (3.5)	0.868
Cerebral complaints, *n* (%)	4 (2.6)	12 (14.0)	0.001
Pulmonary pathology, *n* (%)	6 (4.0)	3 (3.5)	0.868
Thyroid pathology, *n* (%)	0	18 (20.9)	< 0.001
MSS pathology, *n* (%)	12 (7.9)	10 (11.6)	0.262
ECG data			
Q/ST elevation, *n* (%)	96 (63.7)	31 (36.1)	0.001
CAG data			
Intact coronary arteries, *n* (%)	27 (17.9)	20 (23.3)	0.450
Coronary artery stenosis < 70 %, *n* (%)	16 (10.6)	6 (7.0)	0.446
Coronary artery stenosis ≥ 70 %, *n* (%)	108 (71.5)	60 (69.8)	0.512

Note. AH – arterial hypertension; MSS – musculoskeletal system.

TABLE 3

CHARACTERISTICS OF THE LEADING ANAMNESTIC SIGNS IN COMPARISON WITH THE RESULTS OF CORONARY ANGIOGRAPHY IN PATIENTS WITH ACUTE CORONARY SYNDROME WITH ST ELEVATION (*n* = 237)

Parameters	Typical pain (*n* = 69)	Atypical pain (*n* = 136)	Non-anginal pain (*n* = 12)	Dyspnea (n = 20)	*p*
Intact coronary arteries, *n* (%)	5 (7.2)	32 (23.5)	5 (41.7)	5 (25.0)	< 0.001
Stenosis < 70 %, *n* (%)	4 (5.8)	13 (9.6)	4 (33.3)	1 (5.0)	< 0.001
Stenosis ≥ 70 %, *n* (%)	60 (87.0)	90 (66.2)	3 (25.0)	14 (70.0)	< 0.001

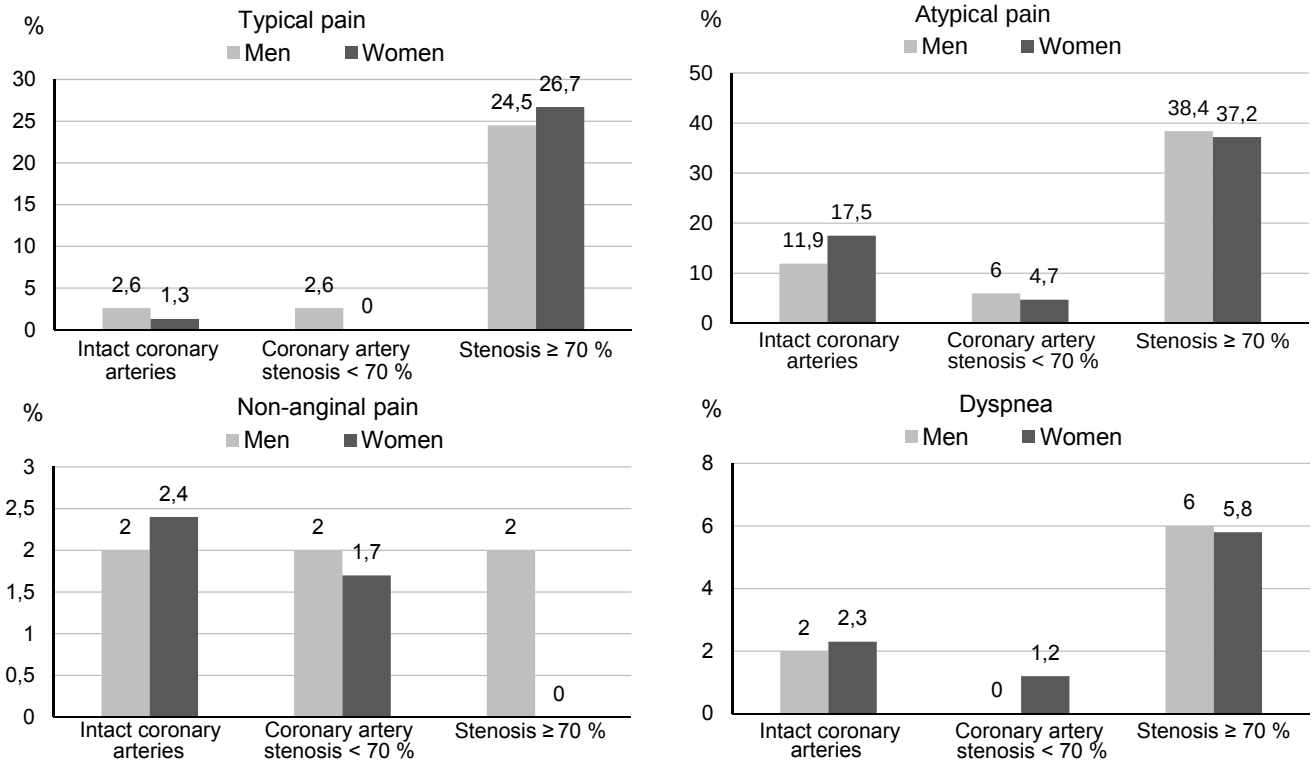

FIG. 2.

Clinical symptoms at various changes in coronary angiography in men and women with acute coronary syndrome with ST elevation (n = 237), p > 0.05

comitant diagnosis of type 2 diabetes mellitus and thyroid pathology was statistically significantly more frequently detected in women with ACS without ST elevation ($p < 0.001$).

Patients with ACS without ST elevation had typical, atypical, and non-anginal chest pain. In case of intact coronary arteries, atypical pain clinic was more common (99 (54.1 %) patients), while typical anginal pain was more common in case of hemodynamically significant coronary artery stenosis (60 (50.0 %) patients; $p < 0.001$) (Table 5).

The results of correlation of clinical symptoms and coronary artery changes detected through CAG in men and women with ACS without ST elevation are presented in Figure 3.

With the predominance of typical angina clinic in case of ACS without ST elevation, intact coronary arteries were statistically significantly less frequently detected in men than in women (15.4 % vs. 27.4 %; $p = 0.002$). The same trend was noted for hemodynamically significant coronary artery lesions (40.2 % in men, 58.5 % in women; $p = 0.002$). In cases with atypical pain, intact coronary arteries were diagnosed in more than half of the cases among both men (61.5 %) and women (62.5 %), while hemodynamically significant coronary artery lesions were statistically significantly more common for men (40.6 % (men) vs 34.1 % (women); $p = 0.02$).

DISCUSSION

Our study shows that women with acute coronary syndrome more often than men were diagnosed with atypical chest pain and less often with typical chest pain. Intact coronary arteries more often revealed in women during CAG, while hemodynamically significant coronary artery stenoses were found less often (70 % and more). Also, type 2

TABLE 4

CLINICAL AND ANAMNESTIC CHARACTERISTICS OF PATIENTS WITH ACUTE CORONARY SYNDROME WITHOUT ST ELEVATION (*n* = 351)

Parameters	Men (*n* = 179)	Women (*n* = 172)	*p*
Age, M ± SD	56.9 ± 10.4	61.4 ± 9.3	< 0.001
Age of patients with AH, M ± SD	56.2 ± 9.57	61.3 ± 9.19	< 0.001
	Medical history		
Typical pain, *n* (%)	98 (29.7)	91 (35.3)	0.811
Atypical pain, *n* (%)	97 (54.1)	92 (53.4)	0.981
Non-anginal pain, *n* (%)	21 (6.4)	9 (3.5)	0.056
Dyspnea – leading syndrome, *n* (%)	31 (10.6)	19 (7.4)	0.126
A-dyspnea, *n* (%)	76 (42.5)	98 (57.0)	0.009
AH, *n* (%)	104 (58.1)	131 (76.2)	< 0.001
Cardiac arrhythmia, *n* (%)	51 (28.5)	42 (24.4)	0.457
Diabetes mellitus, *n* (%)	0	12 (7.0)	< 0.001
Prior stroke, *n* (%)	6 (3.4)	10 (5.8)	0.354
Cerebral complaints, *n* (%)	21 (11.7)	26(15.1)	0.362
Pulmonary pathology, *n* (%)	12 (6.7)	7 (4.1)	0.475
Thyroid pathology, *n* (%)	3 (1.7)	9 (5.2)	< 0.001
MSS pathology, *n* (%)	15 (8.4)	13 (7.6)	0.832
	ECG data		
Normal, *n* (%)	150 (83.8)	150 (87.2)	0.451
Without Q/ST elevation, *n* (%)	29 (16.2)	22 (12.8)	0.451
	CAG data		
Intact coronary arteries, *n* (%)	65 (36.3)	94 (54.7)	< 0.001
Coronary artery stenosis < 70%, *n* (%)	32 (17.9)	38 (22.1)	0.392
Coronary artery stenosis ≥ 70%, *n* (%)	82 (45.8)	40 (23.3)	< 0.001

Note. AH – arterial hypertension; MSS – musculoskeletal system.

diabetes mellitus, thyroid pathology and cerebral complaints were statistically significantly more common among women. When comparing clinical symptoms, ECG changes and CAG data, further gender differences were found.

Traditionally, it is believed that among women, atypical symptoms are more common among patients with ACS. However, L.H. Ruane et al. [19] showed that complaints of severe or pressing pain, irradiation to the throat and back, isolated upper back pain, nausea or vomiting were more common among women compared to men. Similar characteristics of pain syndrome among women were found in the study by M.G. van der Meer et al. [20]. They were more often found to have subdural localization, irradiation to the jaw, neck, shoulder, palpitations, nausea, and dizziness. It should be rec-

TABLE 5

CHARACTERISTICS OF THE MAIN ANAMNESTIC SIGNS IN COMPARISON WITH THE RESULTS OF CORONARY ANGIOGRAPHY IN PATIENTS WITH ACUTE CORONARY SYNDROME WITHOUT ST ELEVATION (*n* = 351)

Parameters	Typical pain (*n* = 120)	Atypical pain (*n* = 183)	Non-anginal pain (*n* = 18)	Dyspnea (*n* = 30)	*p*
Intact coronary arteries, *n* (%)	35 (28.2)	99 (54.1)	13 (72.2)	12 (40.0)	< 0.001
Stenosis < 70 %, *n* (%)	25 (20.8)	36 (19.7)	2 (11.1)	7 (23.3)	< 0.001
Stenosis ≥ 70 %, *n* (%)	60 (50.0)	48 (26.2)	3 (16.7)	11 (36.7)	< 0.001

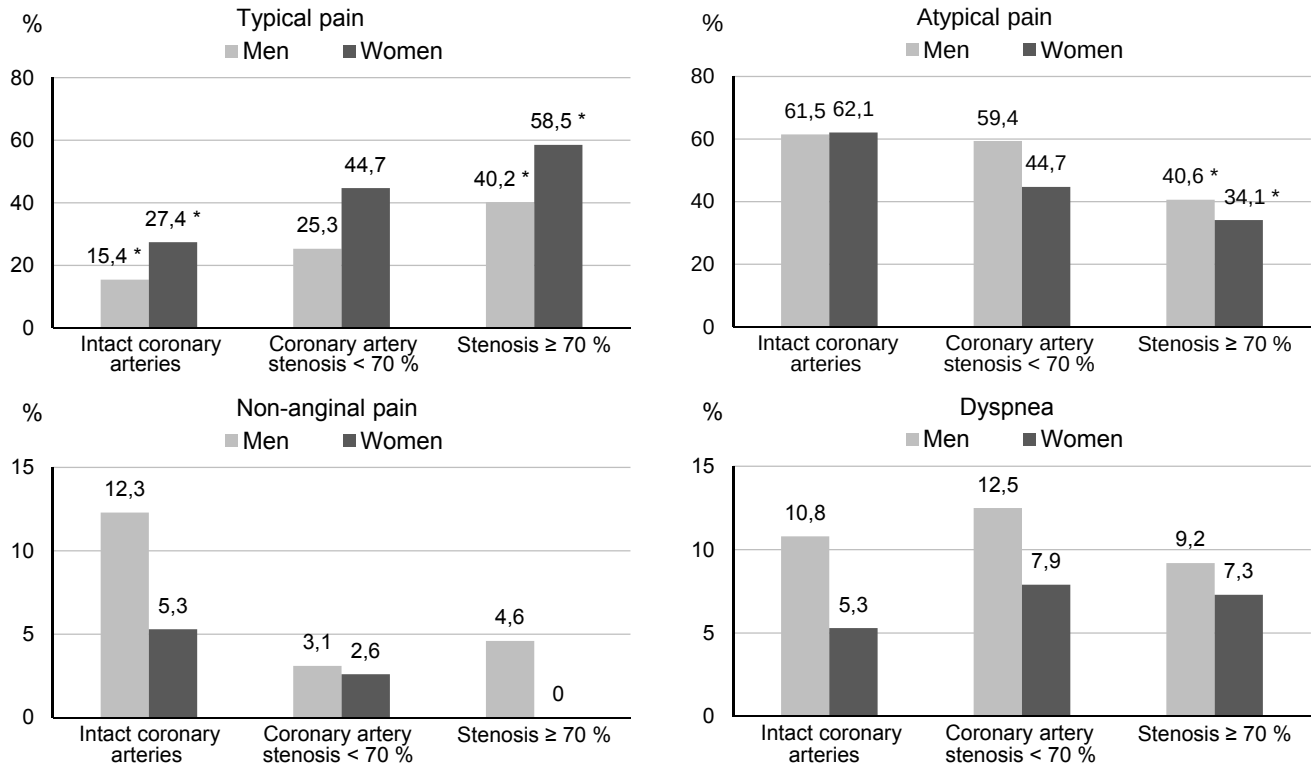

FIG. 3.

*Clinical symptoms at various changes in coronary angiography in men and women with acute coronary syndrome without ST elevation: * – p < 0.05*

ognized that these symptoms are fairly typical of anginous pain. Also, the diagnostic value of clinical symptoms and risk factors for predicting CHD in patients with chest pain admitted to the emergency department was good and did not differ between women and men [20]. Similarly, when comparing only patients with a confirmed diagnosis of ACS, no significant differences in symptom presentation were noted [19]. Indeed, typical angina pains were detected equally often in male and female subjects of our work in the subgroup of patients with ACS without ST elevation and hemodynamically significant coronary artery lesions. Another recent work showed that male patients are more likely to feel left or mid-chest pain, with duration ranging from < 20 min to > 20 min, pain quality – moderate with a tendency to become severe. Women are more likely to feel chest pain that irradiates to the neck and chin, duration usually > 20 min, pain syndrome – mild to moderate [14]. The authors emphasize that a significant difference was found in the characteristics of chest pain in men and women with ACS. Regarding the localization, duration and nature of chest pain, men with ACS mostly have more typical symptoms, whereas women have atypical symptoms. The results of this study are consistent with our findings overall for the entire cohort of study subjects. P.G. Reuter et al. showed in their study [21] that predictors of the final diagnosis of ACS of patients admitted with chest pain or discomfort differed depending on gender. For men, these predictors were age, smoking, severe and persistent pain, nonrespiratory retrosternal localization, irradiating pain and additional symptoms. For women, the following factors were included in the predictive model: age ≥ 60 years, prior CHD, nonrespiratory and irradiating pain. Also, the discriminatory performance of the model was poor for females and good for males [21].

The presence of a more pronounced coronary artery lesion in men is quite consistent with the generally ac-

cepted ideas [3]. Consequently, the absence of coronary artery lesions is less common in men than in women [22]. Both of these facts are explained by the higher incidence of microvascular lesions in women [3, 23]. Indeed, current knowledge shows that angina is not necessarily associated with obstructive coronary artery disease. There are several other pathophysiological variants (vasospastic angina, microvascular angina, endothelial dysfunction, etc.). [3]. Our study also shows that more than a quarter of women with intact coronary arteries have typical anginal pain on admission. Compared to other similar studies, our study showed a very high percentage of patients without coronary artery changes during invasive CAG, which seems to be explained by the inclusion and exclusion criteria of the study.

When considering comorbidity, such comorbidities as diabetes mellitus, arterial hypertension, thyroid disease, varicose vein disease and bronchial asthma were more common among women with stable CHD compared to men [24], which is consistent with the data from our sample. If we consider epidemiological studies, the structure of comorbidity differs markedly between men and women: osteoporosis, varicose vein disease and arthritis are more common among women, while prostate disease, alcohol abuse and endocrine/metabolic disorders are more common among men [25]. When considering risk factors of patients with ACS, it was found that hypercholesterolemia and smoking were predominant among men, and unfavorable heredity among women. There were no differences in the detection of diabetes mellitus [19]. Perhaps younger mean age of the patients in this study was the reason for the difference in our data.

A limitation of the study is that when studying ACS, the male-to-female frequency ratio, the predictive role of the symptoms identified, and comorbidities were examined in patients who had already been selected for CAG, rather than at initial contact with patients in the emergency and outpatient settings. Another limitation of the study is that short-term outcomes (during hospitalization) and drug therapy during hospitalization were not studied. It is clear that this information could additionally highlight the peculiarities of the course of ACS depending on the gender of patients, but this was not our aim. The aim was to compare the clinical symptomatology of men and women with ACS on admission to hospital. We also did not perform multivariate analyses of the presenting symptoms, as such analyses were not originally foreseen in the study design.

CONCLUSION

When assessing symptoms and clinical data of ACS patients, gender differences were revealed: atypical chest pain was detected in women more often than in men, typical chest pain – less often; CAG in women more often revealed intact coronary arteries, less often hemodynamically significant coronary artery stenoses (70 % and more). Also, type 2 diabetes mellitus, thyroid pathology and cerebral complaints were statistically significantly more common among women. Comparison of clinical symptoms, ECG changes and CAG data also revealed further gender differences, identifying symptoms in favor of significant coronary artery lesion and symptoms suggestive of possible absence of coronary artery lesion. In addition, a more pronounced pathology of CA in ACS in men and greater difficulties in diagnosing ACS in women were found.

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Conflict of interest

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

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CLINICAL LABORATORY DIAGNOSTICS

MYOCARDIAL DAMAGE BIOMARKERS AND THE FIRST CASE OF MACROTROPONIN I DETECTION IN ENDURANCE ATHLETES

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ABSTRACT

Background. *High levels of cardiac troponin in the blood indicates myocardial injury, including those caused by intense exercises. Recent studies have shown that an elevation in the troponin concentration in the patients can be caused by the macrotroponin circulation. There is no data in the scientific literature describing this problem in athletes.*

The aim. *To identify the cases and possible causes of high blood levels of cardiac markers in athletes before and after endurance exercises.*

Materials and methods. *The study was conducted on 11 male cross-country skiers aged 15–21 years. The study included two stages: the first was conducted at the end of the preparatory period (November, 2020–2021), the second – in the middle (March) of the 2020–2021 competitive period. At each stage, two blood samples were taken from a vein: the first – in the morning on an empty stomach after a day of rest, the second – 12–14 hours after a high-intensity exercise. In the blood serum, the activity of total creatine kinase (CK), weight concentration of the cardiac isoenzyme of creatine kinase (CK-MB), and concentration of high sensitive troponin I (Tn) were measured.*

Results. *The activity of CK in athletes exceeded the upper limit threshold, and decreased from the preparatory (November) to the competitive (March) period. The concentration of CK-MB in response to exercise increased by 2 times and was not accompanied by the signs of myocardial injury. The most sensitive indicator, responsive to physical activity, was troponin I. However, the highest elevation of Tn in blood of one athlete, both before and after the exercise, was associated with the presence of macrotroponin without signs of myocardial injury.*

Conclusions. *An increase of muscle tissue injury biomarkers in blood, including the heart muscle (CK, CK-MB, and Tn), by 2–4 times is a typical reaction for the body of a cross-country skier to an intense exercise. High levels of troponin in the blood, both before and after training or competition, may be associated with the presence of macrotroponin.*

Key words: *cross-country skiing, troponin I, macrotroponin, creatine kinase, myocardial injury*

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БИОМАРКЕРЫ ПОВРЕЖДЕНИЯ МИОКАРДА И ПЕРВЫЙ СЛУЧАЙ ВЫЯВЛЕНИЯ МАКРОТРОПОНИНА I У АТЛЕТОВ, ТРЕНИРУЮЩИХСЯ НА ВЫНОСЛИВОСТЬ

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РЕЗЮМЕ

Обоснование. *Высокий уровень тропонина свидетельствует о повреждении миокарда, в том числе вызванного интенсивными тренировками. Исследования последних лет показали, что у больных увеличение концентрации тропонина может быть вызвано циркуляцией макроформ. В научной литературе нет данных, описывающих данную проблему у атлетов.*

Цель исследования. *Выявить случаи и возможные причины высокого уровня кардиомаркеров в крови у атлетов до и после физической нагрузки на выносливость.*

Материалы и методы исследования. *Обследовано 11 лыжников-гонщиков мужского пола (15–21 лет). Эксперимент включал 2 этапа: первый – в ноябре 2020–2021 гг.; второй – в марте 2020–2021 гг. На каждом этапе проводили два забора крови из вены: первый – после дня отдыха, второй – через 12–14 ч после высокоинтенсивной тренировки. В сыворотке определяли активность общей креатинфосфокиназы (СК), концентрацию сердечной изоформы креатинфосфокиназы (СК-МВ) по массе и тропонина I (Тн), определённого высокочувствительным методом.*

Результаты исследования. *Активность СК превышала верхнюю границу нормы и снижалась от ноября к марту. Концентрация СК-МВ в ответ на тренировку повышалась в 2 раза и не сопровождалась признаками повреждения миокарда. Наиболее чувствительным показателем, реагирующим на тренировку, оказался Тн. Однако самое высокое повышение тропонина в крови у одного атлета было связано с наличием макротропонина без наличия признаков миокардиального повреждения.*

Заключение. *Повышение в крови уровня биомаркеров повреждения мышечной ткани, в том числе миокарда, в 2–4 раза является характерной реакцией для организма лыжника-гонщика в ответ на интенсивную тренировку. Высокий уровень тропонина в крови как до, так и после тренировки может быть связан с наличием макроформ протеина.*

Ключевые слова: *лыжные гонки, тропонин I, макротропонин, креатинкиназа, повреждение миокарда*

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OBJECTIVES

Moderate physical activity has a positive effect on human health, which is not the case for professional sports, in which physical activity is extreme. The athlete's body works at its limits during competition. Changes in athletes' biochemical indicators are often beyond normal values for ordinary people. First of all, the levels of muscle tissue biomarkers – creatine kinase and cardiac troponin – are increased.

High sensitive troponin I (Tn) has been the "gold standard" in the diagnosis of acute myocardial infarction in all developed countries since 2018, as it is a completely specific marker for heart muscle. The 99th percentile of this marker being repeatedly exceeded in the blood usually indicates the development of an acute myocardial infarction or other myocardial injury. Blood levels of Tn can remain increased up to 7 days after damage [1]. In recent years, a growing amount of data has been accumulating that suggest that Tn is increased in various diseases accompanied by myocardial injury [2]. An increase in cardiac troponin levels can be induced by intense and prolonged exercise [3, 4]. The increase of Tn level in blood is associated with its release from cardiomyocytes as a result of increased permeability of the sarcolemma [5]. As well, exceeding reference values of Tn in athletes indicates myocardial injury due to insufficient myocardial adaptation to a certain level of physical activity [6]. Determination of Tn after exercise in recreational long-distance runners or sport walkers may have predictive significance in the development of cardiovascular complications, especially after the age of 50 years [7].

Increased Tn levels caused by a novel coronavirus infection (COVID-19), including in athletes, have recently been reported in international forums [8–10]. High blood Tn concentration is prevalent in hospitalized patients with COVID-19 and is associated with decreased survival and development of complications [11]. Tn elevation mechanism in COVID-19 patients is associated with myocardial injury due to thromboembolism, acute respiratory distress syndrome (ARDS) combined with systemic inflammatory reaction [12]. The increase in marker levels during COVID-19 disease may be the result of direct myocardial injury by the virus [11].

However, there are a certain number of false-positive Tn assays due to the presence of so-called «macrotroponins» in the bloodstream rather than cardiac damage [13]. Macrotroponin is a complex formed by endogenous autoantibodies to Tn and circulating cardiac troponin I in the blood [14]. The molecular mass of these complexes varies from 340 to 900 kDa and indicates the participation of immunoglobulins A or G in their formation [15]. Accumulation of macrotroponins may lead to macrotroponinemia by analogy with macroprolactinemia (macroprolactin is an immune complex of the pituitary hormone molecule prolactin with autoantibodies) [16]. Detection of high blood troponin levels associated with macrotroponinemia can lead to false-positive diagnosis of acute myocardial infarction, hospitalization and unnecessary, dangerous and expensive manipulations in a medical institution, such as contrast-enhanced coronary angiography [17]. We did not find a description of this problem in athletes in the scientific literature.

THE AIM OF THE STUDY

To identify the cases and possible causes of high blood levels of cardiac markers in athletes before and after endurance exercises.

MATERIALS AND METHODS

The study was conducted as part of the research work "Development of full assessment of performance and damage to vital organs in competitive sportsmen based on innovative laboratory-biochemical automated methods", approved by the local ethics committee of the Lesgaft National State University of Physical Education, Sport and Health, St. Petersburg (protocol No. 4, registration number No. 0089 dated 30.12.2017). The study included 11 male cross-country skiers of St. Petersburg aged 15–21 years with different sports qualifications (First-Class Sportsmen, Candidates for Master of Sport, Masters of Sport), trained by a coach in one group and following the same training and competition plan, which was the reason for their allocation into one group (Table 1).

TABLE 1

ANTHROPOMETRIC CHARACTERISTICS OF CROSS-COUNTRY SKIERS (*n* = 11)

Parameters	Me	min	max
Age, years	17	15	21
Height, cm	179.0	164.0	185.0
Weight, kg	72	61.0	80.0
BMI, kg/m^2	22.4	19.4	25.0

The experimental part of the study included two stages: the first (I) – at the end of the preparatory period, the second (II) – in the middle of the competitive period of cross-country skiers 2020–2021. At each stage, after signing informed voluntary consent (for minors – by their legal representatives) for medical intervention, two blood samples were taken from a vein into vacuum systems. The first blood sampling was performed in the morning on an empty stomach after a day of rest. At stage I, the second blood sampling was performed 12–14 hours after a high-intensity exercise (10 km cross-country skiing) in the evening, as close as possible to the competitive load. At stage II, the second

blood collection was performed after a 10 km competitive ski race at the same time.

Biochemical and immunochemical analyzers of Architect line were used to perform the analyses using reagents and control materials of the equipment manufacturer (Abbott, USA). The activity of total creatine kinase (CK), weight concentration of the cardiac isoenzyme of creatine kinase (CK-MB) and concentration of high sensitive troponin I (Tn) were measured (the 99th percentile for the indicated method of analysis in men is 34 ng/L). Important to note that immunochemical methods for CK-MB and Tn determination are method-dependent, and absolute values expressed in ng/L may differ by several times when using equipment or reagents from another manufacturer.

The obtained data were processed using Statgraphics 19 program (Statgraphics Technologies, Inc., USA). The sample was assessed for conformity to a normal distribution using standardized skewness and standardized kurtosis. For normal distribution of traits, the mean ± standard deviation (mean ± SD) was used; for distributions not conforming to normal, the mean and 25th and 75th percentiles (mean [Q_1; Q_3]) were used. The statistical significance of differences between blood sampling results was assessed using Student's t-test for related samples with normal distribution of variables or using the Wilcoxon signed-rank test for related samples. Statistical significance of differences in the values of the studied parameters was established at $p \leq 0.05$.

RESULTS AND DISCUSSION

Total CK activity at the end of the preparatory period of cross-country skiers after a day of rest was 290.1 [192.9; 330.4] u/l; it increased to 393 ± 164.8 u/l 12 hours after a high-intensity exercise ($p < 0.05$). In clinical practice, 190 u/l is considered the upper limit of normal for men, but this threshold value is not recommended for professional athletes [18]. The increase in total CK activity in blood is mainly due to an increase in myocyte cell membrane permeability, which is explained by acidosis and accumulation of strong organic acids in cells during intense physical activity [19]. It is believed that the increase in enzyme activity in the blood of endurance training athletes can be up to 800 u/l without serious consequences [18]. There are also scientific data showing multiple excesses of normal CK activity in individual athletes associated with mechanical damage to skeletal muscle [20]. The intensity of CK release from muscles into the vascular bed is an individual characteristic, and the dynamics of the level of this enzyme in blood serves as an integral indicator of the delayed effect of physical activity [21] and depends on the type of sport [22].

Before competition, athletes' CK activity significantly decreased (219.7 ± 129.6 u/l) compared to the preparatory period ($p < 0.01$). After the race, enzyme activity still decreased slightly in most athletes (204.6 ± 47.8 u/l); data are shown in Figure 1. We can assume that athletes recovered faster due to the change in the nature of physical activity. We hypothesize that competitive activities of cross-country skiers have a less damaging effect on skeletal muscles than intense/long training loads of 2 workouts per day.

Any physical activity affects the cardiovascular system of the human body. The effect of exercise on cardiac muscle can be assessed by changes in the concentration of cardiac-specific markers in the blood. Weight concentration of CK-MB during the preparatory period in cross-country skiers before and after training were 5.4 [3.2; 6.4] and 6.4 ± 2.7 ng/ml, respectively ($p < 0.01$). Changes in blood troponin I levels during the same period showed a higher sensitivity in response to exercise: 8.6 [4.5; 37.9] ng/L before exercise and 18.3 [7.6; 29.0] ng/L after exercise. It is reasonable to conclude from these results that assessing the effect of exercise on the cardiac muscle by changes in Tn concentration is preferable to using weight concentration of CK-MB. However, the Tn results were more variable than CK-MB, which supports the assumption of an individual response in each athlete (Table 2).

The data presented in Table 2 shows that 6 athletes had an increase in Tn level and this increase was statistically significant. In other cross-country skiers the change was insignificant: for example, in athlete #7 the marker concentration decreased from 3.2 to 1.4 ng/L, but these figures are at the limit of method sensitivity and when using the laboratory method of the previous generation even of the same manufacturer (before 2016) corresponded to the digital value 0 (the indicator in the blood is not determined). Two athletes exceeded the upper reference level threshold (34 ng/L) at both blood collection points.

In the competitive period, the blood Tn level remained at the level of the preparatory period after exercise. Pre-race CK-MB and Tn values were 7.7 ± 3.8 ng/ml and 18.8 [11.0; 99.3] ng/L, respectively; post-race values were 5.8 ± 2.3 ng/ml and 14.1 [9.7; 33.1] ng/L. CK-MB concentration after the competition decreased to the level of the preparatory period before exercise. CK-MB levels exceeded the upper reference level at all-time points of blood sampling (Fig. 2).

Blood Tn levels increased in individual cross-country skiers throughout the study. We hypothesized that competitive load influences the increase in Tn concentration to a greater extent than exercise load. However, given the high variation of results within the group, no statistically significant differences between the indicators were found (Table 3).

An increase in Tn concentration after the race was detected in the majority of athletes, as in the preparatory period. Unfortunately, not all athletes were able to participate in stage II of the study: 2 athletes (blood samples #6 and #9) finished their sports career, and one athlete was ill for a long time (sample #7) and showed the worst race time. It should be noted that a significant increase in the level of the marker was recorded in two athletes both before and after the competition. We also noted a high level of Tn in these athletes during the preparatory period. There was a multiple increase in Tn levels in ath-

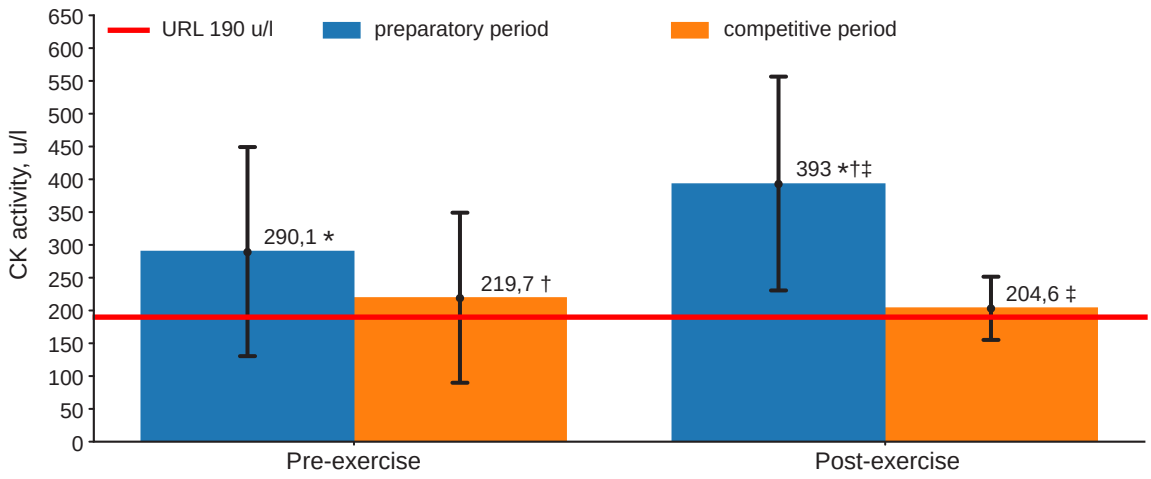

FIG. 1.

*Creatine kinase activity in cross-country male skiers before and after exercise in different training periods (mean ± SD): URL – upper reference level for men over 17 years old – 190 U/l (Abbott, USA); * – p < 0.05 compared to pre-exercise values in the preparatory period; † – p < 0.01 compared to post-exercise values in the preparatory period; ‡ – p < 0.05 – compared to post-exercise values in the preparatory period*

TABLE 2

INDIVIDUAL CHANGES IN BLOOD TROPONIN CONCENTRATION IN CROSS-COUNTRY SKIERS AT STAGE I OF THE STUDY (*n* = 10)

Sample #	Tn level before exercise, ng/L	Tn level after exercise, ng/L	ΔTn, ng/L	Level change, in % of baseline
1	98.9	118.4	+19.5	+19.7
2	5.0	17.9	+12.9	+258.0
3	4.5	4.1	–0.4	–8.9
4	8.6	18.7	+10.1	+117.4
5	81.5	94.1	+12.6	+15.5
6	3.9	7.6	+3.7	+94.9
7	3.2	1.4	–1.8	–56.3
8	37.9	29.0	–8.9	–23.5
9	7.3	–*	–*	–*
10	9.3	11.5	+2.2	+23.7
11	30.4	27.4	–3.0	–9.9

Note. * – Athlete #9 results were excluded due to the presence of hemolysis in the post-exercise sample.

lete #1 that did not fit the overall picture of what was going on. Any increased biomarker value, in our opinion, should be analyzed in combination with other indicators of the athlete's health status. Thus, the increase in troponin I in athlete #1 was not associated with the other laboratory parameters studied (Fig. 3).

Analyzing these graphs, it can be noted that the increase in CK as well as CK-MB is within the normal range

of changes during sports training and does not exceed 3-fold of the upper limit of normal. The state of skeletal muscles is normal for this athlete. An increase in Tn level by more than 10 times (such changes are interpreted in clinical cardiology as an indicator of mass death of cardiomyocytes) may mislead a sports physician in diagnosing myocardial injury. In this study, an electrocardiogram (ECG) was performed to rule out myocardial pathology, which showed no pathologic changes. On ECG, only slight sinus bradycardia (heart rate – 50 bpm) was registered, which in most cases is typical for athletes of this sport [23].

According to the latest scientific data, it is not possible to identify the initial pathological characteristics of myocardial injury in athletes based on ECG or echocardiography findings alone. During further examination in the Almazov National Medical Research Center, delayed contrast-enhanced MRI of the heart was performed in athlete #1 [24]. The study results showed no myocardial integrity abnormalities, no thickened pericardium, no pathologic effusion in the pericardial cavity, no ventricular dilatation, no dilated atria, no signs of myocardial edema. Thus, the repeated increase in troponin I in this athlete is not associat-

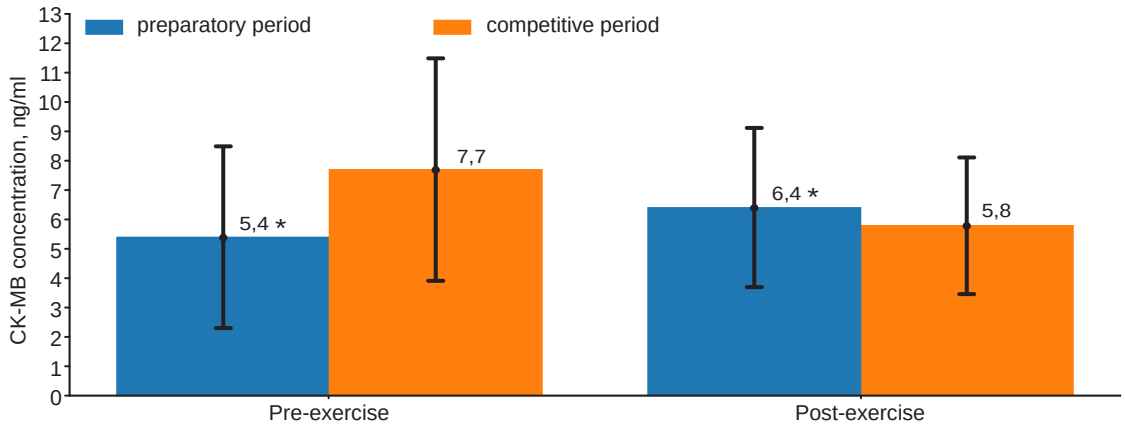

FIG. 2.

*Concentration of cardiac isoenzyme of creatine kinase in cross-country male skiers before and after exercise in different training periods (mean ± SD): * – $p < 0.05$ compared to pre-exercise values in the preparatory period*

TABLE 3

INDIVIDUAL CHANGES IN TROPONIN CONCENTRATION IN CROSS-COUNTRY MALE SKIERS BEFORE AND AFTER THE RACE (*n* = 9)

Sample #	Tn level before the race, ng/L	Tn level after the race, ng/L	ΔTn, ng/L	Level change, in % of baseline
1	613.4	772.6	+159.2	+26.0
2	35.8	9.7	–26.1	–72.9
3	11.9	18.7	+6.8	+57.1
4	10.0	33.1	+23.1	+231.0
5	162.7	243.2	+80.5	+49.5
7	–*	5.4	–*	–*
8	24.6	13.3	–11.3	–45.9
10	9.5	8.4	–1.1	–11.6
11	12.9	14.1	+1.2	+9.3

Note. * – Athlete #7's sample was excluded before the race for technical reasons.

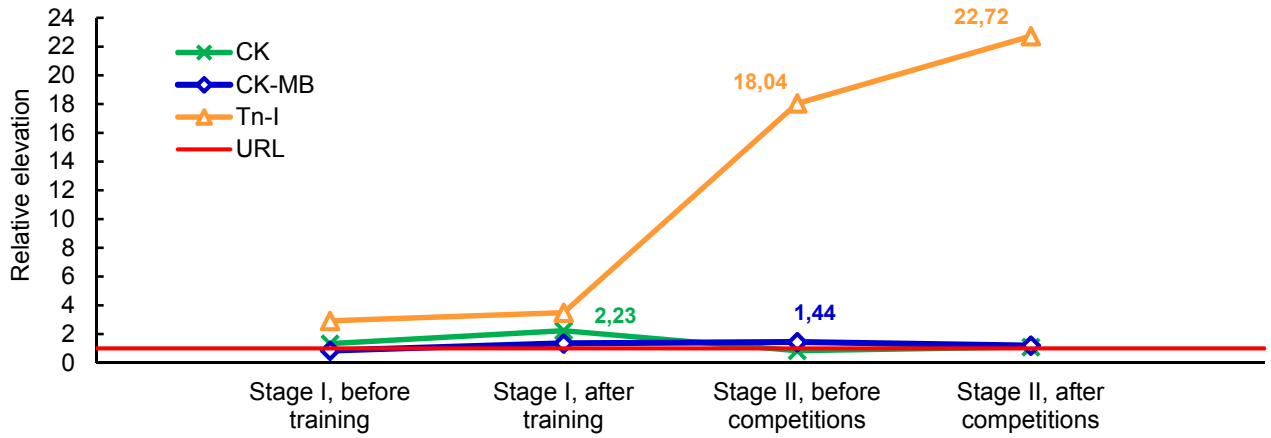

FIG. 3.

Relative increase in injury markers as related to the upper normal level in sportsman No. 1: URL – upper reference level

ed with the development of abnormal myocardial changes. Further strategy of our study was aimed at establishing the cause of such a significant increase of Tn in blood. In the course of reviewing the scientific literature of recent years, we hypothesized the presence of macrotroponin in this athlete. In a study by J.V. Warner et al. it was shown that in 5 % of patients with increased high sensitive Tn levels, high molecular weight complexes containing immunoreactive troponin I and immunoglobulins (macrotroponin) are detected in the blood. The researchers also noted that patients with myocarditis and Tn macrocomplexes have higher and more prolonged marker increases compared to patients without the presence of macrotroponin. In most subjects with macrotroponins, high sensitive Tn levels did not exceed 100 ng/L [15]. In a study by P.A. Kavsak et al. it was shown that increased blood levels of Tn are associated with the presence of troponin I macrocomplexes, which can be determined by polyethylene glycol precipitation. The results of assays using Beckman (USA) equipment and reagents are slightly less susceptible to changes in the presence of macrotroponins I in the blood [25]. L. Lam et al. found the differences between samples with and without macrotroponins to be statistically significant ($p < 0.001$) using gel permeation chromatography (GPC). The authors also noted the effect of autoantibodies to Tn on troponin immunoassay results [26]. Autoantibodies to Tn may possibly potentially influence cardiac remodeling [27]. Animal experiments have shown that decreased levels of autoantibodies to troponin and other cardiac-specific proteins (alpha-actin 1 and beta-myosin 7B) may be an indicator of cardiomyocyte adequacy and adaptation to exercise stress [28].

To determine the presence or absence of Tn macrocomplexes in the serum of athlete #1, we used the proven method of precipitation of high molecular weight protein complexes using a 25 % aqueous solution of polyethylene glycol 6000 (Sigma, Germany). After incubation of serum and polyethylene glycol in a 1:1 ratio, the mixture was centrifuged at 5000 g for 20 min. Tn immunochemical determination was performed in the supernatant, taking into account the dilution of the sample. Assay results confirmed precipitation of macrotroponin complexes; less than 10 % of the original Tn remained in the supernatant. After precipitation of macrotroponin complexes in other athletes with polyethylene glycol, which was performed in the II (competitive) stage of our study, less than 20 % of macrotroponin of the baseline amount of troponin I were detected in the sera of all skiers except athlete #1. Thus, it can be assumed that the high blood Tn level in the athlete is a false positive, not related to myocardial injury, but is due to the development of an autoimmune process. In addition, we can assume that troponin I levels in athletes with autoantibodies to Tn will be significantly higher than in ordinary people with low physical activity and the presence of such pathology. We believe that the situation with macrotroponins will be much the same as the laboratory phenomenon of "macroprolactinemia" and the development of the phenomenon of high levels of thyroperoxidase antibodies. Unfortunately, such patients do not have clinical manifestations of immune pathology for lots of months, which leads to diagnostic errors in the work of endocrinologists, oncologists and medical officers of other specializations. High-intensity and prolonged myocardial exercise in professional athletes leads to increased cardiomyocyte permeability and increased entry of intracellular proteins into the blood, which may induce an immune response. The effect of COVID-19 can be especially dangerous to the athlete who continues to train, as muscle cells are quickly affected by the coronavirus. For example, athlete #1 had the highest level of immunoglobulin G to the virus in the second stage of the study compared to the other athletes after the December 2020 illness.

CONCLUSION

A 2–4-fold increase in blood levels of biomarkers of muscle tissue damage, including cardiac muscle (CK, CK-MB and Tn), is a typical reaction for a cross-country skier in response to intense exercise. The most sensitive indicator responsive to physical activity of a cross-country ski-

er is high sensitive troponin I. Significant increase of troponin I concentration in blood (5 times and higher in relation to the upper reference interval), in our opinion, requires in-depth laboratory analysis and deeper medical examination to exclude possible myocardial pathology. In some cases, multiple troponin increases may be associated with the presence of macrotroponin in the blood. The phenomenon of blood macrotroponin detection in athletes requires further study to better understand its clinical and predictive significance.

Conflict of interest

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

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MICROBIOLOGY AND VIROLOGY

COMPARISON OF THE EFFECTIVENESS OF SOLID NUTRIENT MEDIUM IN THE *IN VITRO* CULTIVATION OF *NEISSERIA GONORRHOEAE* ISOLATES

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ABSTRACT

Background. *Neisseria gonorrhoeae is a facultatively anaerobic microorganism which is extremely demanding to the composition of a nutrient media and cultivation conditions. In a situation of the increasing shortage and cost of foreign components for the preparation of solid nutrient media, it is important to study the possibility of growing hard-to-cultivate microorganisms on domestically produced nutrient media.*

The aim of the study. *To evaluate the growth of gonococcus colonies on two types of solid nutrient media – chocolate agar with growth and selective additives prepared using imported reagents and chocolate agar with growth additives manufactured by "Gem LTD" (Moscow, Russian Federation).*

Materials and methods. *A reference strain of N. gonorrhoeae NCTC 12700/ ATCC 49226 and two types of chocolate agar (the first one – prepared in the State Scientific Center of Dermatovenerology and Cosmetology using imported components and the other one – from the domestic manufacturer "Gem LTD") were used in the research.*

Results. *The equivalence of the growth properties of both studied types of nutrient media when cultivating pure gonococcus was revealed.*

Conclusions. *Ready-to-use chocolate agar with growth additives produced by "Gem LTD" can be successfully used in the laboratory for the cultivation of N. gonorrhoeae pure culture. Primary isolation of N. gonorrhoeae strains from clinical material is more appropriate to carry out on a medium that suppresses the growth of foreign microflora due to the inclusion of antibiotic additive. The organization of production of domestic bacteriological media for microorganisms with high nutrient requirements reduces the dependence of domestic microbiology on import and ensures their rapid delivery to laboratories.*

Key words: *Neisseria gonorrhoeae, nutrient medium, laboratory diagnostics, chocolate agar, selective supplement, Isovitalex, VCAT, Martin and Lewis inhibitor*

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СРАВНЕНИЕ ЭФФЕКТИВНОСТИ ИСПОЛЬЗОВАНИЯ ТВЁРДЫХ ПИТАТЕЛЬНЫХ СРЕД ПРИ КУЛЬТИВИРОВАНИИ ИЗОЛЯТОВ *NEISSERIA GONORRHOEAE* В ЛАБОРАТОРНЫХ УСЛОВИЯХ

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РЕЗЮМЕ

Обоснование. *Neisseria gonorrhoeae – факультативно анаэробный микроорганизм, крайне требовательный к составу питательной среды и условиям культивации. В ситуации нарастающего дефицита и увеличения стоимости зарубежных компонентов для приготовления твёрдых питательных сред актуальным является исследование возможности выращивать труднокультивируемые микроорганизмы на питательных средах отечественного производства.*

Цель исследования. *Проведение оценки роста колоний возбудителя гонококковой инфекции на двух типах твёрдых питательных сред – шоколадном агаре с ростовыми и селективными добавками, приготовленном с использованием импортных реагентов, и шоколадном агаре с ростовыми добавками производства российской компании ООО «Гем» (Москва).*

Материалы и методы. *В исследовании был использован контрольный штамм N. gonorrhoeae NCTC 12700/ATCC 49226 и два вида шоколадного агара: приготовленный в ФГБУ «Государственный научный центр дерматовенерологии и косметологии» Минздрава России с использованием импортных компонентов и от отечественного производителя ООО «Гем».*

Результаты. *Была выявлена равноценность ростовых свойств исследованных питательных сред при культивировании чистой культуры гонококка.*

Заключение. *Готовый к использованию шоколадный агар с ростовыми добавками производства ООО «Гем» может успешно использоваться в лаборатории для культивирования чистой культуры N. gonorrhoeae. Первичное выделение штаммов N. gonorrhoeae из клинического материала более целесообразно проводить на среде, обеспечивающей подавление роста посторонней микрофлоры за счёт включения антибиотикосодержащей добавки. Организация производства отечественных бактериологических сред для микроорганизмов с высокими питательными потребностями снижает зависимость отечественной микробиологии от импорта и обеспечивает быструю их доставку в лаборатории.*

Ключевые слова: *Neisseria gonorrhoeae, питательная среда, лабораторная диагностика, шоколадный агар, селективная добавка, Isovitalex, VCAT, ингибитор Мартина и Льюиса*

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INTRODUCTION

Neisseria gonorrhoeae is a facultatively anaerobic microorganism which is demanding to the composition of a nutrient media and cultivation conditions. The WHO Global Health Sector Strategy on Sexually Transmitted Infections (2016–2021) [1] identifies Neisseria gonorrhoeae as one of the three most important sexually transmitted infections (STIs) that require urgent and coordinated actions at the international level. The gonococcal pathogen has also been included in the WHO's list of priority pathogens to guide and promote research and development (R&D) of new antibiotics with a high priority level [2]. The relevance of Neisseria gonorrhoeae infection is determined by its ability to cause high morbidity among people of reproductive age with a pronounced negative impact on fertility and increase the risk of co-infection with other STIs. Moreover, it is important to note the lack of specific prophylaxis and the progressive resistance of *N. gonorrhoeae* to antimicrobial agents [3].

The isolation of a pure culture of gonococcus is the classical culture method, which is still the "gold standard" for the diagnosis of gonorrhea. The principal advantage of the method is the isolation of a viable culture of *Neisseria gonorrhoeae*, which is used for further molecular-biological and genetic studies, as well as for assessing the sensitivity of the microorganism to antimicrobial agents, conducted by bacteriological method [4, 5].

The culture medium for isolation of *N. gonorrhoeae* includes an agar base providing nutrients in the form of casein and peptones, phosphate buffer to maintain pH and corn starch to neutralize toxic fatty acids that may be found in the agar. Ox-blood hemoglobin provides delivery of X-factor (hemin). Isovitalex enrichment supplement is a source of vitamins, amino acids, coenzymes, glucose, iron ions and other factors that improve Neisseria growth. Selective medium differs from conventional culture medium in that it contains antimicrobial agents (e. g., vancomycin, colistin, and nystatin or other antifungal agent) that inhibit the growth of other bacteria and fungi without inhibiting the growth of gonococci. The use of selective media favors isolation of a pure culture of Neisseria since the anatomical source of the specimen also usually contains other bacterial species, although it has been shown that in rare cases some gonococcal strains may be sensitive to the concentrations of vancomycin used [6]. The medium for the isolation of *N. gonorrhoeae* should be inexpensive but characterized by specificity and sensitivity.

In 2008, in connection with the practical implementation of the international GASP (Gonococcal Antimicrobial Surveillance Program) [7] program in Russia, the State Scientific Center of Dermatovenerology and Cosmetology of the Russian Ministry of Health developed a package of "Standard Operating Procedures" (SOPs), including "Standard Operating Procedures for Species Identification of the Gonorrhea Pathogen" [8]. The paper proposes media for the initial isolation of *N. gonorrhoeae* from clinical specimens and for subsequent culturing to determine antibiotic resistance. To isolate *N. gonorrhoeae*, chocolate agar based on GC Medium Agar Base is used. According to the SOP, bovine hemoglobin and Isovitalex growth supplement are added to the GC Medium Agar Base when preparing non-selective culture medium. VCAT (Martin and Lewis inhibitor) supplement containing vancomycin, colistin, anisomycin and trimethoprim is additionally added to obtain selective agar.

Due to the introduction of economic sanctions against the Russian Federation by the United States, the European Union and a number of other countries, the purchase of products for bacteriological high-tech nutrient media is limited, so it seems relevant to search for domestic import-substituting nutrient media. One of the national companies engaged in industrial production of ready-made nutrient media with a composition suitable for cultivation of cultures of gonococcal pathogen is "Gem LTD" (Moscow).

The aim of this study was to evaluate the efficiency of *N. gonorrhoeae* colony growth on two types of chocolate agar – produced by "Gem LTD" (specialized agar for *N. gonorrhoeae* cultivation) and medium prepared from imported reagents in accordance with the SOP.

MATERIALS AND METHODS

Two types of chocolate agar were used for growing the culture of *N. gonorrhoeae* – produced by "Gem LTD" and prepared according to the instructions in the SOP for species identification of the gonorrhea pathogen developed by the State Scientific Center of Dermatovenerology and Cosmetology of the Russian Ministry of Health.

Chocolate agar produced by "Gem LTD" is a dense nutrient medium prepared in accordance with the requirements of TU 9385-003-16665457-2013 [9]. The agar contains defibrinated sheep blood, which enriches the medium with the iron-containing pigment hemin (growth factor X). Thermostable hemin is released from erythrocytes when blood is added to the chocolate agar base at about 80 °C. To improve the growth properties of the nutrient medium, thermolabile factor V (NAD, Nicotinamide adenine dinucleotide), which is involved in oxidation-reduction (redox) reactions, is additionally added to chocolate agar cooled to 45–50 °C. The ready-to-use medium is poured into 90 mm diameter Petri dishes. The dishes with the medium are hermetically packed in polyethylene bags and stored in a dry place protected from light at a temperature of 2–8 °C for 2 months.

When preparing chocolate agar at the State Scientific Center of Dermatovenerology and Cosmetol-

ogy of the Russian Ministry of Health in accordance with the SOP, components of imported origin are used: GC Medium Agar Base is produced by Indian or American companies (Pronadisa, Thermo Fisher Scientific, Becton Dickinson); lyophilized bovine hemoglobin (Hemoglobin Bovine), Isovitalex and VCAT are purchased mainly from the American supplier, Becton Dickinson. Preparation of test portions and autoclaving of agar base and hemoglobin are carried out at the laboratory center of the State Scientific Center of Dermatovenerology and Cosmetology of the Russian Ministry of Health; additives are added immediately before pouring the dishes. 90 mm diameter dishes are used, which are packed in polyethylene bags after pouring and drying and stored at 2–8 °C for up to 3 weeks.

To control the quality of media, the reference strain of *N. gonorrhoeae* NCTC 12700/ATCC 49226, recommended for antibiotic sensitivity testing [10], was used. The strain was stored in cryo-medium at –80 °C and was seeded from the storage medium into Petri dishes with medium prepared according to the SOP one day before the study.

A saline suspension equivalent to the McFarland standard of 0.5–10^8 colony-forming units per ml (CFU/ml) was prepared from an overnight culture of the reference strain, and test inoculates of 10^4, 10^3 and 10^2 CFU/ml were prepared from it. Each inoculum was seeded on 3 dishes of chocolate agar from each manufacturer in a volume of 0.1 ml, corresponding to 10^3, 10^2 and 10 CFU. For sterility control, 3 chocolate agar dishes of each manufacturer were incubated without seeding.

The growth pattern and the number of colonies grown on the agar surface were evaluated after 24 and 48 h of incubation at 37 °C and increased (3–5 %) CO_2 content. During the study, qualitative and quantitative control of the growth of *N. gonorrhoeae* on nutrient media was performed. Qualitative control is based on the evaluation of the growth pattern of the test culture. An indicator of quantitative control was the yield of microbial cells when test inoculums were sown.

RESULTS AND DISCUSSION

After 24 h of incubation, growth of colonies up to 0.5 mm in diameter, opaque, rounded in shape was observed on all three dishes with chocolate agar produced by "Gem LTD". The number of colonies grown was directly proportional to the number sown (Fig. 1), namely: an average of 750 colonies of *N. gonorrhoeae* per dish grew on dishes with 10^3 CFU/mL seeding, 90 colonies with 10^2 CFU/ml seeding, and 7 colonies with 10 CFU/ml seeding. There was no colony growth 24 h after sowing on all the dishes with medium prepared according to the SOP, but after 48 h Neisseria colonies appeared in the following numbers: on dishes with 10^3 CFU/ml sowing the number of colonies increased to an average of 950 colonies per dish; on dishes with 10^2 CFU/ml sowing – up to 80 colonies; on dishes with 10 CFU/ml sowing – up to 7 colonies. Regardless of the culture medium, all colonies were gray, with a shiny surface, opaque, convex, rounded, 1.5–2 mm in diameter. Belonging of microorganisms to the genus *Neisseria* was confirmed by oxidase test: one drop of Tetramethyl-p-phenylenediamine dihydrochloride reagent was applied to the grown colonies; after 10 s the colonies acquired blue coloring (Fig. 2).

FIG. 1.
N. gonorrhoeae colonies on chocolate agar manufactured by "Gem LTD" 24 hours after inoculation with 10 CFU per plate

No growth of foreign microflora was observed on control dishes, which were not sown, after 24 and 48 h of incubation, which confirms the fact of absence of contamination of nutrient media at all stages of preparation.

Thus, chocolate agar – both prepared according to the SOP and produced by "Gem LTD" – provides growth and manifestation of typical culture and morphological properties of gonococcus within 24–48 hours. The later appearance of *N. gonorrhoeae* colonies on the medium prepared according to the SOP is explained by the presence of the selective VCAT supplement containing antimicrobial agents (vancomycin, colistin, anisomycin and trimethoprim), which inhibit, among other things, the growth of gonococcus.

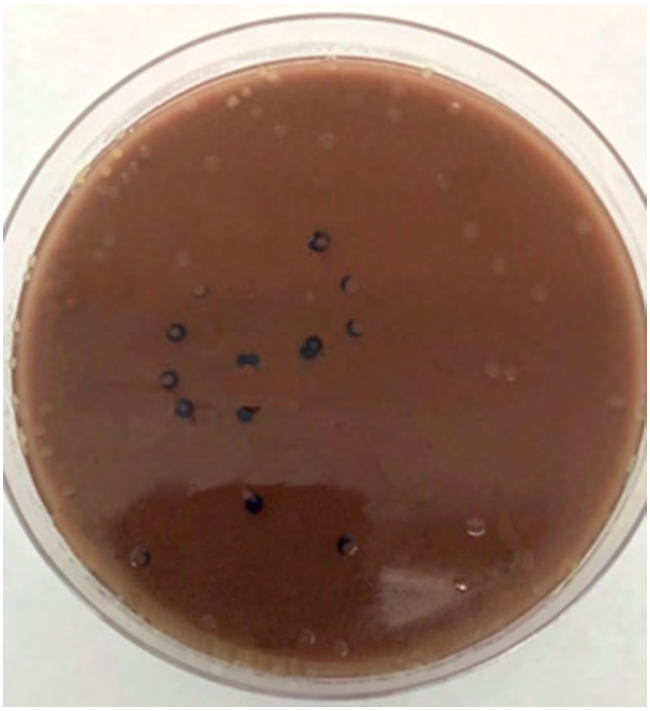

FIG. 2.
N. gonorrhoeae colonies on chocolate agar manufactured by "Gem LTD" 48 hours after inoculation with 100 CFU per plate: oxidase test

CONCLUSION

The study conducted showed the equivalence of growth properties of chocolate agar prepared according to both SOP and "Gem LTD". Both types of culture medium support the growth of the test strain of gonococcus even at as low a test inoculum level as 10^2 CFU/ml, which is important when isolating a pure culture of gonococcus from clinical material.

Ready-to-use chocolate agar with growth additives produced by "Gem LTD" can be successfully used in the laboratory for cultivation of a pure culture of *N. gonorrhoeae*, in particular for routine colony passage as part of collection or other laboratory and experimental work. When selective components are added to the agar, it can be tested for the isolation of pathogenic *Neisseria* spp. from clinical specimens as well. Thus, under the conditions of economic and other sanctions, leading to restrictions on the import of reagents for the preparation of nutrient media of foreign production, the availability of domestic media of high quality for work with difficult-to-cultivate microorganisms, which includes *N. gonorrhoeae*, is particularly important for the continuous implementation of work on monitoring antimicrobial resistance.

Conflict of interest

The authors of this article declare no conflicts of interest.

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THE IMPACT OF OLFACTORY AND GUSTATORY PERCEPTION ON METABOLIC HOMEOSTASIS IN OBESE PATIENTS

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ABSTRACT

Obesity is currently a major global public health problem. As a result, in recent decades there has been a growing interest in studying the impact of this disease on the functioning of the central nervous system. One of the least understood aspects is the impact that obesity has on sensory systems.
The olfactory and gustatory systems are closely related to various vital functions, such as the nocifensors activation, the stimulation of digestive reflexes. In addition, these sensory systems are known to play an important role in the mechanisms of food consumption through the regulation of appetite and satiety, influencing food choice and, therefore, they are involved in the development of obesity. A number of clinical studies have shown that obese patients are more likely to suffer from hyposmia compared to lean people of the same age.
The reasons why this relationship exists remain largely unclear. The aim of this review is to assess the available data on this topic and to identify new promising areas for further research. The review was conducted in the PubMed databases for 2017–2023.

Key words: *obesity, olfaction, taste, sensory systems*

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ВЛИЯНИЕ ОБОНЯТЕЛЬНОГО И ВКУСОВОГО ВОСПРИЯТИЯ НА МЕТАБОЛИЧЕСКИЙ ГОМЕОСТАЗ У ПАЦИЕНТОВ С ОЖИРЕНИЕМ

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РЕЗЮМЕ

В настоящее время ожирение представляет собой серьёзную глобальную проблему общественного здравоохранения. В результате в последние десятилетия наблюдается рост интереса к изучению влияния этого заболевания на функционирование центральной нервной системы. К одному из наименее изученных аспектов можно отнести влияние, которое ожирение оказывает на сенсорные системы.
Системы обоняния и вкуса тесно связаны с различными жизненно важными функциями, такими как активация защитных механизмов организма, стимуляция пищеварительных рефлексов. Кроме того, известно, что данные сенсорные системы играют важную роль в механизмах потребления пищи за счёт регуляции аппетита и насыщения, влияния на выбор продуктов и, следовательно, участвуют в развитии ожирения. Ряд клинических исследований продемонстрировали, что пациенты с ожирением чаще страдают от гипосмии по сравнению с худыми людьми того же возраста.
Причины, по которым существует эта взаимосвязь, во многом остаются неясными. Целью данного обзора является оценка имеющихся данных по этой тематике и определение новых перспективных областей для дальнейших исследований. Обзор проведён в базах PubMed за 2017–2023 гг.

Ключевые слова: *ожирение, обоняние, вкус, сенсорные системы*

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Obesity is a complex multifactorial disease defined by excess fat mass that poses a health risk [1]. As a serious global public health problem and a major determinant of disability and mortality, obesity increases the risk of developing chronic non-communicable diseases such as type 2 diabetes mellitus, metabolic syndrome, arterial hypertension and many others.

According to the World Health Organization (WHO), more than 1 billion people worldwide suffer from obesity: 650 million adults, 340 million adolescents and 39 million children. This number continues to grow and experts estimate that by 2025, approximately 167 million people (adults and children) will have obesity complications.[1]

For a long time, the effects on the central nervous system caused by metabolic disorders were ignored. In the 1950s, the effects of diabetes on brain function began to be studied and it was noted that this organ was also affected by hyperglycemia, leading to the development of behavioral and cognitive changes. More recently, its effects on sensory systems have been studied [2].

The brain is in charge of food odor processing and has receptors for most of the hormones, neuropeptides and nutrients responsible for eating behavior. The consequences of modifications in homeostasis, nutrient overload and sensory system changes in the development and maintenance of obesity have not yet been studied.

CURRENT STATE OF THE PROBLEM

Gustation and olfaction are polymodal sensory systems that provide communication with many brain structures that regulate essential visceral functions, including metabolism, as well as the endocrine, cardiovascular, respiratory, and immune systems.

In everyday life, gustation and olfaction are considered relatively unimportant to many people. In fact, disorders in this area may not even be recognized by the patients themselves [3].

The ongoing COVID-19 pandemic has sparked interest in the study of the gustatory and olfactory senses, but even so, anosmia and dysgeusia are considered to be symptoms that may contribute to the early differential diagnosis of benign respiratory tract infections [4]. They may also be a likely sign of subsequent central nervous system (CNS) involvement associated with so-called Long-COVID, being a manifestation of cognitive impairment [5].

Studies have reported that anosmia, i. e., loss of sense of smell, during COVID-19 occurred in a range of 11 to 84 % of cases. This variation is due to the use of different diagnostic techniques [6]. Psychophysical testing is more reliable than results obtained through subjective assessment [7, 8].

Therefore, the actual prevalence of these disorders is unlikely to be studied because patients may not report being asymptomatic or aware of these abnormalities [8].

The majority of patients with COVID-19 have impaired olfaction and gustation, representing only a small part of diffuse chemosensory disorder. Dysregulation of chemosensory systems may underlie the much higher mortality rate of acute respiratory distress syndrome COVID-19 in comparison with acute respiratory syndrome of various origins.

CORRELATION BETWEEN GUSTATORY, OLFACTORY DYSFUNCTIONS AND SOMATIC DISEASES

Although olfactory and/or gustatory disorders have become widely studied due to the COVID-19 pandemic, they are also detected in various physiological and pathological conditions such as aging [9], neurodegenerative diseases [10, 11], autoimmune diseases [12, 13], cancer [14], and dysmetabolic disorders [15–18].

It should be noted that some of the aforementioned conditions that may correlate with gustatory/olfactory dysfunction are risk factors for fatal SARS-CoV-2 virus infection, such as obesity [19], advanced age, cardiovascular disease, dementia, diabetes mellitus, and chronic liver or kidney disease [20, 21].

Deterioration of gustation and/or olfaction has been consistently reported by people with chronic non-communicable diseases such as arterial hypertension, diabetes mellitus or cancer. This may be an indicator of diffuse chemosensory disorder, possibly aggravating the prognosis of these patients.

Under normal conditions, chemoreceptor dysfunction of one system does not seem to lead to the progression of chronic non-communicable diseases. This impairment is presumably largely compensated for by other chemosensory mechanisms. However, under conditions of stress, such as during COVID-19 pneumonia and metabolic imbalance [22, 23], this system may become vital for the brain to organize an effective functional homeostatic response that can significantly increase life expectancy. This means that dysfunction of several chemosensory systems can lead to severe consequences during a number of diseases [23].

INFLUENCE OF CHEMOSENSORY SYSTEMS ON METABOLIC PROCESSES IN THE BODY

Olfactory or gustatory dysfunctions can be quantitative or qualitative [24] and have been suggested to influence eating and social behavior, mood, quality of life and performance [25].

[1] https://www.who.int/news/item/04-03-2022-world-obesity-day-2022-accelerating-action-to-stop-obesity

In fact, all cells in the human body can detect the presence of various molecules in the environment, but only a few can use this ability to inform the CNS to organize adaptive neural or neuroendocrine responses that can affect the whole system.

In addition to olfaction and gustation, a wide range of chemicals in our body are monitored by other cells: carotid corpuscles, single chemoreceptor cells, pulmonary neuroendocrine cells and enterochromaffin cells [26].

The chemosensory system of olfaction is probably the most studied. Olfactory perception can occur through the nasal mucosa (orthonasal perception) or through the oral cavity (retronasal perception) [27] via seven-transmembrane G-protein-coupled receptors (GPCRs).

Notably, similar receptors are expressed in many other tissues [28] and are deeply involved in angiogenesis and modulation of vascular tone [29], as well as in the regulation of lipid and glucose metabolism [30].

In addition, receptors for many hormones are present in the olfactory mucosa, including receptors for insulin, leptin, orexin, cholecystokinin, adiponectin, neuropeptide Y(NPY) and ghrelin in addition to glucose transporters [31]

Carotid corpuscle/glossopharyngeal nerve dysfunction has also been postulated in the potential pathogenesis of nonrespiratory diseases such as metabolic syndrome, type 2 diabetes mellitus and arterial hypertension [23, 32, 33].

Studies in recent years have hypothesized that vagal nerve dysfunction is significant for the development of obesity and metabolic syndrome [34], but in general, the involvement of chemosensory systems in these diseases is currently receiving little attention in clinical practice. A possible explanation is that the levels of this hormone are not considered vital for humans. Therefore, the hormone study is insignificant for the routine medical examination [23].

Perhaps two strikingly opposite conditions need to be considered: cachexia (which involves elevated resting energy expenditure, anorexia) and obesity.

These multifactorial syndromes share common biochemical characteristics. In addition to altered food intake, similar metabolic changes are present, such as insulin resistance, loss of muscle tissue, altered energy expenditure (in both increased or decreased diseases depending on the patient), increased lipolysis, unregulated excess protein catabolism, chronic inflammation [35], and dysregulation of the immune system [36, 37]. Most patients present similar multiple endocrine dysfunctions and, in particular, elevated peripheral serotonin levels [38]. Both central and peripheral levels of this hormone from enterochromaffin cells are a major component of metabolic regulation [39]. All of these parameters are largely under the control of chemosensory systems. The cause-and-effect relationships have not yet been established, but they are worth studying.

INFLUENCE OF GUSTATORY RECEPTIVITY ON NEUROENDOCRINE MECHANISMS OF APPETITE REGULATION

Gustatory receptors of the tongue respond to different taste stimuli by releasing different combinations of neuropeptides. These peptides are recognized by receptors located on the taste bud cells themselves for intercellular communication (autocrine/paracrine) or on adjacent afferent sensory nerve fibers to transmit taste information to the brain [40]. Examples of neuropeptides produced by gustatory receptor cells are glucagon, glucagon-like peptide 1 (GLP-1), cholecystokinin (CCK), NPY, peptide tyrosine tyrosine (PYY), vasoactive intestinal peptide, ghrelin and galanin [40]. Although adenosine triphosphate (ATP) is the main neurotransmitter that transmits signals to afferent nerve fibers. These peptides can function as cotransmitters that form the physiological response to various stimuli [41].

Some of these peptides are involved in food intake and energy utilization, suggesting that oral nutrient perception may influence whole body metabolism through neural and endocrine pathways. When these hormones enter the extracellular space of the lamina propria, they can affect neighboring cells (paracrine) or penetrate intestinal capillaries and lymphatic vessels to affect other peripheral organs (endocrine) [42].

However, it is still unclear whether these gustatory receptor peptides actually cause endocrine effects by entering the bloodstream and affecting other organs [41]. Interestingly, peptide receptors on gustatory cells can also be a target for peptides produced in the intestine, adipose tissue or other tissues [41]. Leptin receptors on gustatory cells have been shown to respond to systemic leptin content, causing decreased sensitivity to sweet stimuli without affecting the response to sour, salty and bitter substances [43]. The above suggests that postingestin hormone release is capable of regulating the peripheral gustatory apparatus, for example, by modulating the response to sweet stimuli [41].

Animal and human studies demonstrate an inverse correlation between fatty acid sensitivity and fat intake [42].

Recognition of fatty acids by intestinal lipid-sensitive receptors triggers signaling cascades that lead to the release of hormones such as GLP-1, CCK and PYY [42]. However, obese patients have impaired sensitivity to dietary fat in the oral cavity and gastrointestinal tract [42]. Desensitization of these receptors in response to chronic dietary fat intake may be a potential mechanism that contributes to decreased receptor sensitivity to consumed fat. Therefore, fatty acid recognition dysfunction in the gastrointestinal tract may contribute to satiety response dysfunction, leading to overeating and obesity.

Therefore, future studies should investigate how these gustatory receptors and their signaling pathways are altered by impaired metabolism and what signaling

molecules may be targeted to restore gustatory receptor function.

OBESITY AND AFFERENT CONDUCTION DYSFUNCTION

In addition to impaired nutrient transport, obesity also shows changes in vagus nerve responses to appetite-regulating hormones [42, 44]. Obese mice on high-fat diet have a reduced vagus nerve response to leptin [42, 45]. M. Covasa and R. Ritter in 2000 demonstrated a decrease in sensitivity to CCK in obesity models on rats [42, 46]. In a study by D. Daly et al. (2011) long-term feeding of mice with a high-fat diet led to a decrease in mechanosensitivity of intestinal afferents and a decrease in excitability of membranes of nodose ganglion neurons [42, 47]. Therefore, dysfunction of afferent excitability of the vagus nerve may be a mechanism for loss of sensitivity to hormones.

Like vagus nerve afferents, the primary afferents of the enteric nervous system (ENS) are also able to perceive intestinal hormones such as GLP-1, GLP-2, CCK and PYY due to their close proximity to enteroendocrine cells (EECs) and expression of the corresponding receptors [48]. The ENS is a network of nerves and glial cells organized into two main plexuses: the submucosal plexus, located between the submucosa and the circular muscle, and the myenteric plexus, located between the circular and longitudinal muscles. It functions to regulate gastric motor activity.

Changes in ENS during a high-fat diet also resulted in decreased sensitivity to gastrointestinal hormones. E. Grasset et al. (2017) found that the lack of response to GLP-1 in mice fed on a high-fat diet is due to the abundance of a particular set of intestinal bacteria that disrupt GLP-1-induced nitric oxide production in enteric neurons, which prevents activation of the intestine-brain-peripheral release axis to control insulin secretion [49].

In addition, these peptides can activate sensory afferent nerve fibers. Vagal nerve fibers do not project into the intestinal lumen but have been shown to respond to nutrients including glucose, amino acids and lipids in a postabsorptive manner [50]. The additional regulation of their neural signaling by nutrient-stimulated hormones from the intestine is supported by the presence of intestinal peptide receptors on afferent fibers (such as CCK and GLP-1R receptors) and the dependence of the effects of gastric emptying and CCK saturation on vagal signaling [51].

In conclusion, receptor perception of nutrients in the intestine and vagus nerve responses to them serve as important mediators of energy homeostasis and represent distinct steps in which high-fat diets and obesity can disrupt proper functioning.

ASSESSMENT OF GUSTATORY AND OLFACTORY FUNCTIONS IN CLINICAL PRACTICE

Unfortunately, quantitative testing of gustation and olfaction is rarely performed in clinical practice. The accuracy of a patient's chemosensory complaint cannot be definitively established without diagnosis. Indeed, most people do not accurately assess the nature and extent of their sense of different tastes and smells, and significant recovery of function can occur, often without patients being aware of it [52]. For example, the study by H. Tomita et al. (2002) showed that only 18 % of patients with bilateral taste loss after transection of both *chorda tympani* nerves were aware of their deficit [52, 53]. It is almost impossible to detect gustatory and olfactory dysfunctions without testing [52, 54], nor is it possible to determine whether the perceived decline in function is normal for the patient's age and gender [52, 55]. Without testing, the efficacy of pharmacological, surgical, or other therapeutic interventions cannot be accurately established.

Quantitative evaluation has shown that olfactory dysfunction is more prevalent than gustatory dysfunction [52]. In fact, most patients who clinically complain of gustatory dysfunction have pathology related to olfactory function [52]. The taste of foods, which is often interpreted as "flavor", is largely dependent on volatile substances that reach olfactory receptors through the nasopharynx during swallowing [52].

Along with sweet, sour, bitter, salty, savory ("umami"), it is likely that chalky or metallic sensations in the oral cavity are olfactory sensations [52].

CORRELATION BETWEEN OBESITY AND OLFACTORY AND GUSTATORY DYSFUNCTIONS

Reduced gustatory and olfactory perception causes high consumption of palatable foods, which will either lead to obesity or aggravate existing obesity [19, 56, 57], although the consequences of the food addiction component should not be ignored, especially for sweet and fatty food [19, 58].

The study by A.S. Khan et al. (2020) demonstrated that obese people have a lower sensitivity to sweet-sour taste compared to healthy people [19]. Mice with obesity induced by a 10-week high-fat diet had reduced gustatory sensitivity to the tastes [19, 59]. Similar results have been obtained for bitter and salty tastes in obese patients [19].

Decreased sensitivity to various gustatory stimuli may be related to deficient functionality of gustatory receptors/sensors caused by obesity [59], genetic polymorphisms [19, 59] or epigenetic patterns [19, 60].

A similar situation is characteristic of olfactory function. For example, olfactory threshold increases with body weight of obese individuals [19, 61]. Z. Patel et al. reported that high body mass index (BMI) was associated with subjective olfactory dysfunction of obese patients [62].

Decreased olfactory perception during obesity is a multicomponent phenomenon that involves abnormalities not only of the nasal epithelial receptors but also of various brain regions such as the limbic system, thalamus and piriform cortex, and amygdala, which project to the orbitofrontal cortex [19, 63].

In addition to the above-mentioned factors leading to gustatory and olfactory perception dysfunction, the role of cytokine-induced (generalized or specific) inflammation during obesity cannot be overlooked.

The study by A. Kaufman et al. showed that the increase in the level of tumor necrosis factor α (TNF-α) in the area of tongue papillae of obese mice was associated with a significant decrease in the number of taste bud cells and their precursors [64]. Moreover, TNF-α-null mice were protected from obesity-induced reduction in taste bud cell number, and administration of exogenous TNF-α caused taste buds to degenerate [64]. The Sel1L (Suppressor/Enhancer of Lin-12-like) deletion specific for adipose tissue in mice maintained on a high-fat diet resulted in reduced adipose tissue levels and showed neither an increase in TNF-α concentration nor any evidence of taste bud cell atrophy. These observations clearly indicate that TNF-α released from hyperplastic/inflamed adipose tissue in obesity may cause loss of gustatory perception. Moreover, inflammation has also been found to reduce the lifespan of mature taste bud cells.

It has also been demonstrated that chemosensory perception in mammals is regulated by bacterial metabolites, and the microbiota of the tongue mucosa may also be involved in taste formation by influencing food intake and metabolism [65, 66]. A recent study of the human microbiome showed that commensal bacteria have evolved strategies to stimulate chemosensory receptors and trigger host cell functions [65]. Therefore, the tongue microbiota may influence metabolic systems through interaction with chemosensory receptors, similar to how this process occurs in the intestine [67, 68].

It has been reported that obese children have significantly reduced gustatory discrimination (level of taste recognition) and fewer mushroom papillae have been identified, which is accompanied by a decrease in the α-diversity of the microbiota of the tongue membrane, which may affect gustatory perception [66]. Studies involving healthy subjects showed that the microbiota of the tongue mucosa was associated with gustatory function, thereby influencing dietary habits such as preference for salty baked goods and foods rich in saturated fats [66].

The microbiota of the tongue mucosa, one of the important components of the oral microbiome, is characterized by high sampling stability and is more accessible for research. Therefore, it is a promising subject for studies.

In addition, it has been shown that tongue mucosal microbiota disorders can lead to increased levels of various markers of chronic inflammation, and thus are closely associated with the development of a number of chronic noncommunicable diseases (NCDs) such as obesity, type 2 diabetes mellitus, and cardiovascular pathology [66, 69, 70].

However, the number of studies on the association between the mechanisms of metabolic disorders and microbiota of the tongue mucosa is limited.

Considering the above, it can be hypothesized that the microbiota of the tongue mucosa will be a new, simple and non-invasive biological marker that can contribute to diagnostic and prognostic studies of obesity and other NCDs.

CONCLUSION

Thus, further study of olfactory and gustatory disorders associated with obesity is relevant and may make it possible to predict the risks of developing metabolic disorders and carry out their correction before the realization of the obesity phenotype in the future.

Conflict of interest

The authors declare no apparent and potential conflicts of interest related to the publication of this article.

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POSSIBILITIES OF USING BRANCHED-CHAIN AMINO ACIDS FOR THE TREATMENT AND PREVENTION OF SARCOPENIA IN ELDERLY AND OLD PATIENTS (LITERATURE REVIEW)

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ABSTRACT

Due to the high prevalence of sarcopenia among elderly and old patients, early prevention and treatment of sarcopenia and its complications are relevant. Protein supplements can be used to maintain muscle strength and mass during aging. The possibility of using branched-chain amino acids (BCAAs) in the treatment and prevention of sarcopenia in geriatric patients is of scientific interest. BCAAs promote the synthesis and inhibit the degradation of muscle tissue proteins, are involved in the regulation of tissue sensitivity to insulin, ammonia utilization, the tricarboxylic acid cycle, etc.

Search strategy. *The search for scientific articles for literature review was carried out in the PubMed and PubMed Central databases. The selection criterion was scientific articles published up to December 2022. We used the following search keywords: "branched-chain amino acids", "BCAA", "body composition", "sarcopenia", "aging". The 2019 European Working Group on Sarcopenia in Older People 2 (EWGSOP2) Consensus was included in the list of articles.*

Conclusions. *The possibility of using BCAAs in elderly and old patients for the prevention and treatment of sarcopenia is a relevant topic that continues to be actively studied. The effectiveness of BCAA supplementation in the diet is debatable as long as sufficient protein is consumed daily. On the other hand, BCAA supplementation may be justified in cases where it is not possible to consume enough high-quality protein in the diet. More research is needed on this topic.*

Key words: *branched-chain amino acids, BCAA, gerontology, body composition, sarcopenia, aging, metabolism*

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ВОЗМОЖНОСТИ ПРИМЕНЕНИЯ АМИНОКИСЛОТ С РАЗВЕТВЛЁННЫМИ БОКОВЫМИ ЦЕПЯМИ (BCAA) ДЛЯ ЛЕЧЕНИЯ И ПРОФИЛАКТИКИ САРКОПЕНИИ У ПАЦИЕНТОВ ПОЖИЛОГО И СТАРЧЕСКОГО ВОЗРАСТА (ОБЗОР ЛИТЕРАТУРЫ)

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РЕЗЮМЕ

В связи с высокой распространённостью саркопении среди пациентов пожилого и старческого возраста актуальными являются ранняя своевременная профилактика и лечение саркопении и её осложнений. Для сохранения мышечной силы и массы при старении могут применяться пищевые добавки белков. Представляет научный интерес возможность применения аминокислот с разветвлённой цепью (BCAA, branched-chain amino acids) в лечении и профилактике саркопении у гериатрических пациентов. BCAA способствуют синтезу и замедляют деградацию белков мышечной ткани, участвуют в процессах регуляции чувствительности тканей к инсулину, утилизации аммиака, цикле трикарбоновых кислот и т. д.

Стратегия поиска. *Поиск научных статей для обзора литературы производился в базах «PubMed» и «PubMed Central». Критерием выбора являлись научные статьи, опубликованные в период до декабря 2022 г. включительно. Ключевые слова для поиска: «branched-chain amino acids», «BCAA», «body composition», «sarcopenia», «aging». В список статей был включён Консенсус Европейской рабочей группы по саркопении второго пересмотра (European Working Group on Sarcopenia in Older People 2, EWGSOP2) 2019 г.*

Выводы. *Возможность применения BCAA у пожилых и старых пациентов для профилактики и лечения саркопении является актуальной темой, которая продолжает активно изучаться. Эффективность добавок BCAA в рацион питания является спорной при условии ежедневного потребления достаточного количества белка. С другой стороны, добавление BCAA может быть оправдано в тех случаях, когда невозможно потреблять достаточное количество высококачественного белка с пищей. Необходимы дополнительные исследования по этой теме.*

Ключевые слова: *аминокислоты с разветвлённой цепью, BCAA, геронтология, композиционный состав тела, саркопения, старение, метаболизм*

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INTRODUCTION

Due to the high prevalence of sarcopenia among elderly and old patients, early prevention and treatment of sarcopenia and its complications are relevant [1]. Nutrition, along with physical exercise and treatment of chronic non-communicable diseases, plays a significant role in the comprehensive prevention and treatment of sarcopenia. To increase the strength and mass of skeletal muscles, nutrition should fully compensate not only for the energy requirements of Basal Metabolic Rate (BMR), but also for the additional calorie expenditure and metabolites during exercise [2]. Protein supplements can be used to maintain muscle strength and mass during aging. In addition, it is of interest to add branched-chain amino acids (BCAAs) to the diet, which promote the synthesis of muscle tissue proteins, slow down the degradation of muscle tissue proteins, participate in the processes of regulation of tissue sensitivity to insulin, ammonia utilization, tricarboxylic acid cycle, etc. [3].

BCAAs (valine, leucine and isoleucine) are common amino acids in humans and animals, making up approximately 35 % of the essential amino acids in most mammals. The functional R-groups of all three BCAAs are branched (hence their name), small and hydrophobic, making them essential components of most proteins. Collectively, BCAAs account for about 18 % of all amino acids and 63 % of the hydrophobic amino acids in protein. The molar relative abundance of BCAAs to each other is nearly always approximately 1.6:2.2:1.0 (Val:Leu:Ile), reflecting the linked nature of their synthesis and oxidation [4]. BCAAs are not only used for protein synthesis by the body, but also play an important role in signaling pathways [5]. BCAAs induce an anabolic response of muscle protein synthesis and stimulate muscle mass growth [6]. Along with skeletal muscles, white adipose tissue is also involved in BCAA metabolism [7].

Overall, BCAAs play several important metabolic and physiological roles besides substrates for protein synthesis.

For example, S. Gancheva et al. (2018) suggest that BCAAs play a key role in interorgan metabolic interactions, and dysregulation of BCAA catabolism may play a significant role in a number of metabolic diseases [8].

F. Vanweert et al. (2022) showed in a literature review that impaired BCAA catabolism plays a major role in the development of insulin resistance in obese people with type 2 diabetes mellitus (T2DM). Among these patients, BCAA levels are significantly elevated in plasma and tissues [9].

Much of the evidence for the effects of BCAAs has come from rodent studies. Some foreign studies have shown that BCAAs in rats can limit the skeletal muscle protein degradation rate, and thus, adding BCAAs to the diet can promote hypertrophy of rat musculature [10, 11]. O.V. Turtikova et al. (2014) performed experimental work on laboratory rats. The effect of BCAA administration on *m. gastrocnemius medialis* of rats was investigated during a 30-day recovery period after 16-week alcohol intoxication. BCAA usage promoted more efficient recovery of fast muscle fiber size, resulted in complete restoration of phosphorylated p90RSK content and did not significantly affect overall proliferative processes and myonuclear number in the rat medial musculus gastrocnemius [12]. However, according to R.R. Wolfe (2017), the results of rodent studies have questionable generalizability to humans. In particular, rats have a much lower percentage of skeletal muscle mass compared to humans. In addition, the processes involved in regulating muscle protein synthesis and myoprotein degradation differ from those in humans at both the initiation and translation stages [13].

When evaluating the effectiveness of BCAA supplementation for increasing muscle mass and strength in humans, it is important to consider the various factors against which BCAA intake occurs. There is a number of aspects that can affect the results, including nutrition (total macronutrient and energy intake), presence or absence of strength training, timing of BCAA intake, consumption of other amino acids, demographics, measurement protocols, etc. [3].

Of interest is the possibility of using BCAAs in the treatment and prevention of sarcopenia in geriatric patients.

BIOCHEMICAL AND PHYSIOLOGICAL ASPECTS OF BCAAS

Branched-chain amino acids (BCAAs) are leucine, valine, and isoleucine. BCAAs are the most hydrophobic of the amino acids and play a key role in the structure of spheroproteins as well as in the interaction of the transmembrane domains of integral membrane proteins with phospholipid bilayers. The evolutionary occurrence of BCAAs in nature is due to their primary role in protein structure rather than their secondary metabolic role. BCAAs typically make up approximately 20–25 % of most dietary proteins [14]. C. Nie et al. (2018) showed that BCAAs act as signaling molecules that regulate glucose, lipid and protein metabolism [15]. BCAAs are unique in that they largely bypass first-pass metabolism in the liver, and utilization occurs primarily in the mitochondria of skeletal myocytes. The first steps in their catabolism are common to all three amino acids, including BCAA aminotransferase (BCAT) and branched-chain alpha-keto acid dehydrogenase (BCKD). Their further metabolism uses different pathways to produce different end products (glucose and/or ketone bodies) [14]. Unlike other amino acids, BCAAs are primarily metabolized in muscle tissue, where they serve as precursors to other amino acids and as an energy substrate. In the presence of hyperammoniemia, BCAA metabolism in muscle tissue is altered and promotes ammonia detoxification through glutamine formation [16]. Also in brain tissue,

BCAAs are used as an alternative pathway for ammonia detoxification [17].

Muscular hypertrophy results from an increase in myofibrillar proteins (actin, myosin, troponin, etc.). The rate of synthesis increases with strength exercise and increased protein intake with food [18].

E.L. Glynn et al. (2010) showed that the beneficial effects of protein supplementation include increased synthesis and/or suppression of muscle protein degradation [19].

Skeletal muscle mass growth occurs as a result of a positive balance of muscle protein, i. e., the predominance of synthesis over degradation [2].

G. Biolo et al. (1997) observed that BCAA supplementation increased anabolism, leading to a positive muscle protein balance with little change in degradation, both at rest and after exercise [20]. According to I. Rieu et al. (2006), of the three BCAAs, leucine is the most prominent key regulator of muscle protein synthesis [21].

Protein synthesis is regulated by a network of intracellular signaling cascades that modulate mRNA translation during initiation and elongation. The target of rapamycin (mTOR) or, more specifically, the mTOR-1 complex, a key regulator of protein synthesis, plays an important role in this regulatory network [4, 22, 23]. Energy and protein intake from food is a major nutritional effector of the mTOR-1 complex, which acts as a "nutrient sensor" and thus increases muscle tissue protein synthesis. BCAAs activate signaling pathways that converge in the mTOR-1 complex [24]. S.R. Kimball and L.S. Jefferson (2006) confirmed that BCAA and, in particular, leucine stimulate mTOR and increase muscle protein synthesis [25].

The important role of strength exercises as a powerful stimulator of mTOR and, as a consequence, an activator of muscle protein synthesis has been noted [26, 27]. Human studies with C^{13}-labeled leucine have shown that BCAA oxidation increases 2–4-fold during exercise [28]. BCAA catabolism in muscles increases and plasma BCAA concentration decreases during physical exercise [26].

In addition to stimulating muscle anabolism, BCAAs are involved in the processes of regulating tissue sensitivity to insulin. For example, M.S. Yoon (2016) showed that BCAAs improve the ability of muscle fibers to absorb glucose from blood plasma and modulate insulin signaling [29]. F. Vanweert et al. (2022) confirmed that rodent studies and to a lesser extent human ones strongly suggest that increased BCAA catabolism improves glucose homeostasis in metabolic disorders such as obesity and DM2 [9]. L. Breen, S.M. Phillips (2012) believe that leucine plays the role of the so-called "leucine trigger". This "leucine trigger" hypothesis is that some minimum, threshold level of dietary leucine intake is required, below which stimulation of muscle protein synthesis does not occur [30]. According to O.C. Witard et al. (2016), the minimum "threshold level" for leucine in the diet is 2–3 g per day [31].

However, excessive dietary leucine intake does not further increase muscle protein synthesis [30]. There is probably an upper threshold of BCAA concentration in plasma, beyond which the action of BCAA can lead to adverse effects [3]. So, D.E. Lackey et al. (2013) believe that elevated blood BCAA levels are associated with insulin resistance and DM2, which may be the result of decreased cellular utilization and/or incomplete oxidation of BCAA [7]. On the other hand, the thresholds for BCAA and leucine among the elderly have not yet been clearly defined.

The question of the need for BCAA supplementation in the diet is an interesting one. According to C. Giezenaar et al. (2016), leucine supplementation can promote skeletal muscle hypertrophy when it is impossible to consume sufficient protein [32]. However, some researchers believe that muscle mass growth depends not only on BCAAs, but also on sufficient amounts of other essential amino acids in the diet. For example, R.W. Morton et al. (2017) showed in a systematic review and meta-analysis that healthy adults should consume at least 1.6 g per kg of body mass per day of complete protein containing all nine essential amino acids in order to improve strength performance and body composition during physical training [33]. T.A. Churchward-Venne et al. (2014) showed that the need for essential amino acids increases in the period after intense physical exercise [34].

D.L. Plotkin et al. (2021) suggest that there is no benefit from additional leucine supplementation in the presence of adequate daily protein intake or with respect to measures of muscular hypertrophy. However, when there is no source of whole protein or sufficient essential amino acids available, a higher dose of leucine or BCAAs may slow the degradation and increase muscle protein synthesis [3]. The anabolic effects of whole food protein depend on a number of factors, such as physical training, nutritional status, body mass and skeletal muscle condition [35]. L.S. Macnaughton et al. (2016) believe that the optimal dose of high-quality protein to stimulate muscle anabolism is between 20 and 40 g [36].

Of interest is the question of whether plasma levels of essential amino acids during protein supplementation differ from those that can be obtained after protein intake with normal food.

In 2014, O. Bouillanne et al. published a randomized controlled trial involving 66 elderly people with malnutrition admitted to a rehabilitation unit. The authors found that a single supplement of concentrated powdered plasma protein enriched with leucine (20 g whey protein and 2.8 g leucine) increased the postprandial plasma concentration of essential amino acids by 50 % more compared to a normal diet [37].

The results of several studies suggest that young and middle-aged people who consume sufficient protein with food do not derive additional benefit from BCAA supplementation [35, 38–40].

A.F. Aguiar et al. (2017) showed that during 8 weeks of weight training, leucine supplementation did not increase muscle mass and strength with adequate protein intake (more than 1.6 g of protein per kg of body mass per day) [38]. C.B. Mobley et al. (2017) reported no differences in muscular hypertrophy between groups of participants supplemented with placebo, or 3 g of leucine alone, or 25 g of whey protein (standardized for leucine content) for 12 weeks of physical training. All study participants reported an intake of ~1.8 g protein per kg body mass per day, so the authors believe that supplementation did not confer any additional benefits for building muscle mass. Interestingly, the group that consumed only whey protein had a higher number of satellite cells, suggesting an increased potential for long-term/sequential muscle mass growth [40]. I.T. de Andrade et al. (2020) showed that supplementation with 10 g of leucine per day did not increase muscle mass or strength gains compared to a control group during 12 weeks of weight training and residual dietary protein intake [39]. Thus, it can be assumed that BCAA supplemention is ineffective in increasing muscle mass during weight training with adequate dietary protein intake.

On the other hand, loss of muscle mass can be minimized by using BCAAs during physical training against a calorie-restricted diet. S. Mettler et al. (2010) believe that increased consumption of protein supplements leads to the maintenance of muscle mass during weight training and calorie-restricted diet [41]. However, this study was conducted with athletes and the results should be interpreted on older adults with caution.

In addition, according to D.L. Plotkin et al. (2021), it is questionable whether BCAA supplementation will enhance muscle growth against energy restriction. Whole high-quality protein is superior to BCAAs in performance, so there is no logical justification for taking BCAAs in place of a more complete protein source, regardless of energy status [3].

BCAAS AND SARCOPENIA IN GERIATRIC PATIENTS

Sarcopenia is often associated with malnutrition whether it is related to low food intake (starvation, inability to eat), reduced nutrient bioavailability (diarrhea, vomiting) or high nutrient requirements (chronic inflammatory disease, oncopathology or organ failure with cachexia) [1]. Therefore, optimal nutrition is essential for geriatric patients with sarcopenia because the availability of the building components necessary for muscle recovery is crucial [42]. To halt and possibly reverse the loss of muscle mass and function with age, several authors recommend weight training, optimizing protein intake, and correcting vitamin D deficiency [1, 43, 44]. Nutritional recommendations for older adults (> 65 years) suggest increasing daily protein intake (1–1.2 g/kg/day; 1.2–1.5 g/kg/day for inflammatory diseases), preferably high-quality protein (e. g. whey protein) high in essential amino acids such as leucine [43, 44].

A number of authors have reported positive effects of BCAA supplementation on preservation of muscle strength, mass, and function in geriatric patients. There is evidence for the potential benefit of BCAAs, particularly leucine, for older adults in the treatment of sarcopenia [45–48].

I.F. Kramer et al. (2017) conducted a study to assess whether muscle tissue protein synthesis differs between elderly men with and without sarcopenia. 15 healthy men (mean age – 69 years) and 15 men with sarcopenia (mean age – 81 years) received a single leucine-enriched whey protein supplement (21 g protein). Basal and postprandial muscle protein concentrations were measured using stable isotopes and collection of blood and muscle biopsy samples. After leucine-enriched whey protein supplementation, muscle protein synthesis increased significantly in both the sarcopenia group and the control group without statistically significant intergroup differences [45].

A meta-analysis published by B. Komar et al. (2015) studied the effect of leucine supplementation on anthropometric and body composition parameters in elderly and sarcopenia-prone individuals. Leucine supplementation significantly increased body weight gain (1.02 kg; 95% confidence interval (95% CI): 0.19–1.85; $p = 0.02$), lean body mass (0.99 kg; 95% CI: 0.43–1.55; $p = 0.0005$) and body mass index (0.33 kg/m^2; 95% CI: 0.13–0.53; $p = 0.001$) compared to the respective control groups. In terms of total body weight and lean mass, leucine supplementation was more effective in the subgroup of study participants with severe sarcopenia. However, the addition of leucine to the diet did not affect all other body composition parameters investigated. The authors concluded that intake of various protein products containing at least 2 g of leucine per day, regardless of exercise, had a beneficial effect on body composition indices among individuals prone to sarcopenia [46].

M.K. Park et al. (2022) showed a positive effect of BCAA supplementation on the treatment of sarcopenia after stroke. It is known that after stroke, brain damage activates the systemic catabolic pathway and structural changes in muscle lead to a rapid decrease in muscle mass. In addition, hypodynamia due to weakness and poor nutrition caused by dysphagia accelerates the loss of muscle mass. The authors assessed stroke-related functional status using the Korean version of Modified Barthel Index (K-MBI), Berg Balance Scale (BBS), Functional Ambulation Categories (FAC) assessment test, and Manual Function Test (MFT), which are well established in stroke patients.

Swallowing function was assessed using the Functional Dysphagia Scale (FDS) and the Penetration-Aspiration Scale (PAS) based on the results of the Videofluoroscopic Swallow Study (VFSS). As a result, the group of patients with BCAA supplementation in their diet showed an increase in hand grip strength and skeletal muscle mass index. Mobility, activities of daily living and swallowing function improved. The authors suggest that there is a potential benefit of BCAA supplementation in providing functional improvement by increasing skeletal muscle mass index. The results of this study suggest that a comprehensive rehabilitation intervention combined with BCAA supplementation may be a useful option during the critical period of post-stroke neurological recovery [47].

Proper dosage of BCAA supplementation in the dietary intake of geriatric patients is also important. For example, C.S. Katsanos et al. (2006) noted that supplementation of 1.7 g of leucine to the dietary intake of older adults did not result in activation of muscle protein synthesis. However, daily supplementation of 2.8 g leucine has been shown to be sufficient for this purpose [48]. Similar conclusions were reached by L. Breen, S.M. Phillips (2011). The authors believe that due to the effects of age-related anabolic resistance, older adults require higher doses of leucine than younger adults to maximize the muscle protein synthesis response. Moreover, even with aging, anabolic resistance in the elderly may be reduced by exercise and consumption of higher doses of protein and/or leucine [30].

Although the exact etiology of age-related anabolic resistance during aging has not yet been fully elucidated, it is probably the result of the interaction of many factors at the level of muscular, nervous, cardiovascular, and other body systems [1, 49]. Decreases in motoneurons, anabolic hormones, and increased chronic inflammation likely contribute to the decreased sensitivity to anabolic stimuli with aging [3].

Several authors believe that older adults can increase muscle protein synthesis by consuming an additional ~3 g of leucine per day or ~30–35 g of high-quality protein in the diet [44, 50–52].

J.E. Morley et al. (2010) advise that to overcome anabolic resistance of aging muscles and increase skeletal muscle protein synthesis, 25–30 g of high-quality protein and up to 2.8–3 g of leucine (the minimum recommended dose of leucine is 78.5 mg/kg of the body mass per day) should be consumed at each meal and at least twice a day [44]. The recommended daily amount of protein can be effectively achieved by combined supplementation of high-quality protein, BCAA (including leucine) and vitamin D [43]. According to R.W. Morton et al. (2017), if total protein intake requirements are met, there is no apparent benefit of supplemental BCAA intake because muscle mass growth requires the full complement of essential amino acids [33].

CONCLUSIONS

The possibility of using BCAAs in elderly and old patients to prevent and treat sarcopenia is a hot topic that continues to be actively studied. The effectiveness of BCAA supplementation in the diet is debatable as long as sufficient protein is consumed daily. On the other hand, BCAA supplementation may be justified in cases where it is not possible to consume enough high-quality protein in the diet. However, more research is needed on this topic in older and aged adults.

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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

SOME PROBLEMATIC ISSUES OF REHABILITATION OF PATIENTS WITH THE STROKE CONSEQUENCES AT THE PRESENT STAGE

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ABSTRACT

Background. *Acute cerebrovascular disorders are one of the main causes of severe disability in modern society.*
Russian and foreign researchers register the emerging trend towards rejuvenation of the patients with stroke, which, in turn, significantly increases the relevance of the treatment and rehabilitation of these patients at various stages.
The aim. *To study the social and hygienic characteristics of the stroke patients and to determine their need for medical rehabilitation in the early recovery period.*
Materials and methods. *We studied the social and hygienic characteristics of 109 patients who had stroke in 2020 and 2021 and underwent rehabilitation in the specialized rehabilitation department of the Southern District Medical Center of the Federal Medical and Biological Agency, and determined their need for medical rehabilitation using the analytical method, the method of expert assessments and questionnaires.*
Results. *The average portrait of a patient with a stroke in the early recovery period is as follows: this is a man aged 61 to 70 years with a higher or secondary specialized education, unemployed, living in the city. Up to 30 % of stroke survivors were recognized as disabled; among which, the persons with more severe disability (groups I and II) predominate – up to 90 %. Implementation of rehabilitation measures in the acute period in the hospital and in the early recovery period in the outpatient clinic is carried out at a fairly low level. The percentage of patients being examined by a multidisciplinary rehabilitation team ranges from 17.4 to 10.1 %.*
Conclusion. *The availability of medical rehabilitation measures in a specialized rehabilitation center is quite limited; more than half of the surveyed contingent (52.3 %) experienced problems in obtaining a referral to treatment, about half of the respondents (46.8 %) waited up to 6 months for a referral, which indicates the need to expand the network of such institutions at the regional level.*

Key words: *stroke, disability, rehabilitation, medical rehabilitation, rehabilitation department, hospital, out-patient clinic*

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НЕКОТОРЫЕ ПРОБЛЕМНЫЕ ВОПРОСЫ РЕАБИЛИТАЦИИ ПАЦИЕНТОВ С ПОСЛЕДСТВИЯМИ ИНСУЛЬТА НА СОВРЕМЕННОМ ЭТАПЕ

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РЕЗЮМЕ

Обоснование. *Острые нарушения мозгового кровообращения являются одной из основных причин тяжёлой инвалидности в современном обществе. Отечественные и зарубежные исследователи отмечают наметившуюся тенденцию к омоложению контингента больных инсультом, что в свою очередь значительно повышает актуальность лечения и реабилитации этой категории лиц на различных этапах.*

Цель исследования. *Изучить социально-гигиеническую характеристику контингента лиц, перенёсших инсульт, и определить их потребность в мероприятиях по медицинской реабилитации в раннем восстановительном периоде.*

Материалы и методы. *Изучена социально-гигиеническая характеристика 109 пациентов, перенёсших инсульт в 2020 и 2021 гг. и проходивших реабилитацию в условиях специализированного отделения реабилитации ФГБУЗ «Южный окружной медицинский центр Федерального медико-биологического агентства», и определена их потребность в мероприятиях по медицинской реабилитации с использованием аналитического метода, метода экспертных оценок и анкетирования.*

Результаты. *Среднестатистический портрет пациента с перенесённым инсультом в раннем восстановительном периоде выглядит следующим образом: это мужчина в возрасте от 61 до 70 лет с высшим или средним специальным образованием, неработающий, проживающий в городе. До 30 % лиц, перенёсших инсульт, признаются инвалидами, среди которых превалируют лица с более тяжёлой инвалидностью (I и II группы) – до 90 %. Проведение реабилитационных мероприятий в остром периоде в стационаре и раннем восстановительном периоде в амбулаторно-поликлиническом учреждении осуществляется на достаточно низком уровне. Процент осмотра пациентов мультидисциплинарной реабилитационной командой колеблется от 17,4 до 10,1 %.*

Заключение. *Доступность мероприятий по медицинской реабилитации в условиях специализированного реабилитационного центра достаточно ограничена; более половины исследуемого контингента (52,3 %) испытывали проблемы при получении направления, около половины респондентов (46,8 %) ожидали направление до 6 месяцев, что свидетельствует о необходимости расширения сети подобных учреждений на региональном уровне.*

Ключевые слова: *инсульт, трудоспособность, инвалидность, реабилитация, медицинская реабилитация, реабилитационное отделение, стационар, поликлиника*

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INTRODUCTION

Objectives

The priority national tasks in the field of health care at the present stage are: promotion of public health; development of disease prevention; reduction and prevention of disability; introduction of highly effective medical technologies; development of rehabilitation [1–3].

In order to improve the situation with the preservation of citizens' health, it is necessary to ensure a qualitative breakthrough in the health care system through the development of innovative technologies in the field of prevention, diagnosis and treatment of diseases, including rehabilitation [4–6].

Stroke remains the most urgent medical and social problem at the present stage. The incidence of stroke in Russia is 3–4 cases per 1,000 population per year. The mortality rate from this disease in the acute period reaches 35 %. Within the first 5 years after a stroke, 44 % of patients die. The number of people who have suffered a stroke is more than 1 million annually in Russia, 80 % of whom are disabled. Only up to 25 % of patients return to work after a stroke, and the same number of patients remain disabled for the rest of their lives [7, 8].

Acute cerebrovascular diseases are one of the main causes of severe disability in modern society [9].

At the present stage, a number of Russian and foreign researchers register the emerging trend toward rejuvenation of the patients with stroke, which, in turn, significantly increases the relevance of the treatment and rehabilitation of these patients at various stages [10, 11].

THE AIM OF THE STUDY

To study the social and hygienic characteristics of the stroke patients and to determine their need for medical rehabilitation in the early recovery period.

MATERIALS AND METHODS

We studied the social and hygienic characteristics of 109 patients who had stroke in 2020 and 2021 and underwent rehabilitation in the specialized rehabilitation department of the Southern District Medical Center of the Federal Medical and Biological Agency, and determined their need for medical rehabilitation using the analytical method, the method of expert assessments and questionnaires. By means of analytical method the social and hygienic characteristics of the studied patients were described. The availability of rehabilitation measures for these patients was studied and assessed by the method of expert assessments and questionnaires.

RESULTS AND DISCUSSION

Patients are admitted to the medical rehabilitation department of the Southern District Medical Center of the Federal Medical and Biological Agency (Russia) in the early recovery period.

The majority of patients in the acute phase of the disease – 77 (70.6 %) people – were residents of the city, treated in vascular departments of Rostov-on-Don (N.A. Semashko City Hospital No. 1 in Rostov-on-Don, City Emergency Hospital in Rostov-on-Don, City Hospital No. 20 in Rostov-on-Don) 32 (29.4 %) people, who were villagers, were hospitalized in central district hospitals at their place of residence, and in some cases, depending on the severity of the state of health and the presence of appropriate indications, were transported by air ambulance to the Regional Vascular Center, Rostov Regional Clinical Hospital.

Upon completion of inpatient treatment, patients with the consequences of acute cerebrovascular accidents (ACVA) were discharged for further outpatient treatment by specialists of the appropriate profile with recommendations for rehabilitation measures in specialized institutions. Local practitioners, in accordance with their available quotas, referred patients to specialized rehabilitation centers, where a set of rehabilitation measures developed by a multidisciplinary rehabilitation team (MDRT) was carried out, followed by assessment of the measures taken at the second stage of rehabilitation and development of a rehabilitation plan for the third outpatient stage.

The patients studied were distributed by sex as follows: men – 75 (68.8 %), women – 34 (31.2 %). There were 14 (12.9 %) between 31 and 40 years of age, 8 (7.3 %) between 41 and 50 years, 28 (25.7 %) between 51 and 60 years, 41 (37.6 %) between 61 and 70 years, and 18 (16.5 %) above 70 years. In terms of education level, the following gradation is observed: patients with secondary education – 25 (22.9 %) people, with specialized secondary education – 39 (35.8 %) people, with higher education – 45 (41.3 %) people. At the same time, more than half of the respondents – 66 (60.5 %) people – did not work anywhere; 26 (23.9 %) people were engaged in mental work and 17 (15.6 %) in physical work.

The following concomitant diseases were registered in the patients: carotid artery atherosclerosis (stenosis from 15 to 40–50 %); dyslipidemia, arterial hypertension (stage II or III, more often stage II) – 95 (87,2 %) patients; type 2 diabetes mellitus – 23 (21,12 %) patients; atrial fibrillation – 5 (4,6 %) patients. Hemophilia in 9 (8.3 %) and aneurysm rupture in 4 (3.7 %) were recorded in young age (31–40 years) individuals who underwent ACVA.

According to the NIHSS (National Institutes of Health Stroke Scale), the respondents were distributed according to the severity of their stroke as follows: more than one-third of patients – 37 (33.9 %) people – scored from 3 to 8 points, which corresponds to a mild degree of disease severity; more than half – 56 (51.4 %) people – scored from 9 to 12 points, which corresponds to a moderate degree of severity; 16 (14.7 %) people scored from 13 to 15 points, which indicates a severe degree of the disease.

The terms of temporary incapacity to work at all stages of treatment and rehabilitation, including the outpatient stage, in the studied population ranged from 1 to 10 months depending on the severity of the disease and the severity of functional disorders of organs and systems. Up to 1 month incapacity to work was experienced by 14 (12.8 %) individuals, 2 to 4 months – by 56 (51.4 %), 5 to 10 months – by 39 (35.8 %). Due to the lack of adequate effect from the conducted treatment and rehabilitation measures and the presence of persistent marked impairment of body functions, 30 (27.5 %) patients were referred for examination to the Bureau of Medical and Social Expertise to determine the disability group and the need for social protection measures, including rehabilitation.

Of the total number of patients recognized as disabled (30 individuals), there were 3 (10.0 %) patients aged 31–40 years, 3 (10.0 %) patients aged 41–50 years, 16 (53.3 %) patients aged 51–60 years, 6 (20 %) patients in the age group 61–70 years, and 2 (6.7 %) patients over 70 years. Most of them – 14 (46.7 %) patients – were diagnosed with disability group I, 13 (43.3 %) – with disability group II, 3 (10 %) – with disability group III, i. e. the patients under study are characterized by more severe disability groups (I and II), which emphasizes the social significance of the problem. It should be noted that 6 (20.0 %) of those recognized as disabled continued to work, 24 (80.0 %) individuals did not work.

Among the patients admitted for rehabilitation in the specialized rehabilitation department in the early recovery period, the following disorders of body functions were determined according to the results of rehabilitation diagnostics performed by the MDRT: paralysis – in 26 (23.9 %) patients, paresis – in 108 (99.1 %), walking disorders – in 91 (83.5 %), cognitive disorders – in 79 (72.5 %), epilepsy – in 8 (7.3 %), sensory disorders – in 6 (5.5 %), disorders of higher cerebral function (speech, gnosis, praxis) – in 51 (46.8 %). It should be noted that, as a rule, one patient had a combination of several disorders of the above-mentioned functions of varying severity, requiring appropriate correction.

In the course of MDRT rehabilitation diagnostics, a questionnaire survey of the study population was conducted in order to investigate the possible implementation of rehabilitation measures in the acute period at the first stage and in the early recovery period at the second stage. It should be noted that close relatives and direct caregivers took part in the patient questionnaire. In particular, only 33 (30.3 %) people answered positively to question No. 1 "Did you undergo rehabilitation measures during your stay in hospital?", 39 (35.8 %) people answered negatively, and 37 (33.9 %) people found it difficult to answer this question. Only 19 (17.4 %) people answered positively to question No. 2 "Have you been examined by the MDRT in hospital?"; the majority of respondents – 51 (46.8 %) people – answered it negatively; 39 (35.8 %) people found it difficult to answer. 35 (32.1 %) people answered positively, 38 (34.9 %) people answered negatively, and 36 (33.0 %) people found it difficult to answer question No. 3 "Were you subjected to rehabilitation measures in outpatient conditions?". Regarding question No. 4 "Have you been examined by the MDRT in the polyclinic?" only 11 (10.1 %) people answered positively, 68 (63.4 %) answered negatively and 30 (27.5 %) found it difficult to answer this question.

The fifth question of the questionnaire concerned the problem of getting a referral to the specialized rehabilitation department. Of note, more than half of the respondents, 57 (52.3 %) people, experienced various problems in obtaining this referral; 31 (28.4 %) people responded negatively and 21 (19.3 %) people found it difficult to answer this question. At the same time, the majority of respondents – 51 (46.8 %) people – noted that they had to wait for a referral for rehabilitation to the specialized department for up to 6 months, 32 (29.4 %) – up to 3 months, 26 (23.8 %) people received a referral within 1 month.

Based on the results of MDRT examination, a set of rehabilitation measures is determined for each patient, taking into account their individual characteristics and rehabilitation diagnosis, including drug therapy (nootropics, botulinum toxin for severe spasticity, hypotensive drugs, statins), physiotherapy (magnetotherapy, correction of motor disorder, robotic mechanotherapy, low-frequency pulsed electromagnetic field exposure, including massage of the affected limbs), rehabilitation exercises for diseases of the central nervous system and brain, the method of functional programmed electrical muscle stimulation, mechanotherapy of the lower limbs, active-passive mechanotherapy of the upper and lower limbs, correction of walking and balance. Along with the use of the above-mentioned technologies, a clinical psychologist worked with each patient individually. The duration of patients' stay in the specialized rehabilitation department varies from 9 to 14 bed-days depending on the severity of the patient's health condition. No doubt, it is difficult to achieve significant improvement in health status in post-stroke patients in the early recovery period in such a short period of time. At the same time, the complex of rehabilitation measures allows stabilizing the patient's state of health, preventing further progression of movement disorders and, most importantly, developing a plan of further rehabilitation measures on the basis of objective assessment and determining their scope. There is a high need for medical rehabilitation activities among the study population. In particular, 82 (75.2 %) subjects need rehabilitation therapy in outpatient conditions, 93 (85.3 %) subjects needed regular medical check-ups at the moment of discharge from the rehabilitation department; for the remaining 16 (14.7 %) subjects the issue of regular medical check-ups will be decided after the course of rehabilitation treatment in a 24-hour hospital. A total of 108 (99.1 %) patients needed drug treatment, 1 person categorically refused drug therapy, having made an informed voluntary refusal. 100 (91.7 %) people need treatment in the conditions of a 24-hour hos-

pital: it should be noted that the majority of respondents needed planned hospitalization in a specialized department. As a rule, the terms of planned hospitalization ranged from 1 to 2 months. 16 (14.7 %) subjects needed emergency medical care, and the issue of their hospitalization was resolved within 1–2 days, in connection with which the issue of regular medical check-ups was also postponed. 105 (96.3 %) people need physiotherapy, 103 (94.5 %) people need exercise therapy, 61 (56.0 %) people need psychotherapeutic help, 51 (46.8 %) people need restoration of speech functions (classes with a speech therapist), 21 (19.3 %) people need health resort treatment. The high demand for rehabilitation treatment in specialized rehabilitation centers – 105 (96.3 %) people – also draws attention. At the same time, it should be noted that the availability of this type of rehabilitation services, taking into account the data we obtained from the results of the questionnaire, is very limited, which, of course, has a negative impact on achieving the maximum effect of measures for comprehensive rehabilitation of patients with stroke consequences and requires its resolution at the regional level.

Based on the above, the following conclusions can be drawn:

1. The average portrait of a patient with a stroke in the early recovery period is as follows: this is a man aged 61 to 70 years with a higher or secondary specialized education, unemployed, living in the city.

2. Up to 30 % of stroke survivors were recognized as disabled; among which, the persons with more severe disability (groups I and II) predominate – up to 90 %.

3. Implementation of rehabilitation measures in the acute period in the hospital and in the early recovery period in the outpatient clinic is carried out at a fairly low level. The percentage of patients being examined by a multidisciplinary rehabilitation team ranges from 17.4 to 10.1 % in inpatient and outpatient conditions, respectively.

4. The availability of medical rehabilitation measures in a specialized rehabilitation center is quite limited; more than half of the surveyed contingent (52.3 %) experienced problems in obtaining a referral to treatment, about half of the respondents (46.8 %) waited up to 6 months for a referral, which indicates the need to expand the network of such institutions at the regional level.

Conflict of interest

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

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RISK FACTORS FOR VASCULAR DEMENTIA

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ABSTRACT

Background. *An increase in the number of older people with cognitive disorders, including dementia, is expected.*
The aim. *To study the risk factors for vascular dementia.*
Material and methods. *39 patients with vascular dementia (ICD-10 diagnosis code F01) were examined. The comparison group consisted of 167 people. According to the MMSE (Mini Mental State Examination) scale, moderate dementia was detected in 56.4 % of cases, severe – in 43.6 %; in the comparison group, 94 % had mild and 6 % – moderate cognitive impairment.*
Results. *In the vascular dementia group, the following risk factors were registered significantly more often: ischemic heart disease – at the age of 70–79 years (57.1 %; p = 0.000) and in the group as a whole (56.4 %; p = 0.000); diabetes mellitus – at the age of 60–69 years (100 %; p = 0.005); arterial hypertension – at the age of 70–79 years (100 %; p = 0.000) and in the group as a whole (87.2 %; p = 0.000). In the comparison group, the frequency of body mass index over 25 was significantly higher at the age of 70–79 years (60 %; p = 0.000), 80 years and older (64.3 %; p = 0.037) and in the group as a whole (68.9 %; p = 0.000). Among people with moderate cognitive impairment, the following risk factors were significantly more common: coronary heart disease – at the age of 60–69 years (10 %; p = 0.001); diabetes mellitus – at the age of 70–79 years (40 %; p = 0.025) and in the group as a whole (50 %; p = 0.033), the frequency of body mass index over 25 – at the age of 80 years and older (70 %; p = 0.000) and in the group as a whole (100 %; p = 0.000).*
Conclusion. *Risk factors for the development of cognitive disorders (coronary heart disease, hypertension, diabetes mellitus, overweight) are potentially reversible; their timely detection can reduce the development of cognitive disorders and dementia.*

Key words: *cognitive impairment, vascular dementia, risk factors, prevention*

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ФАКТОРЫ РИСКА РАЗВИТИЯ СОСУДИСТОЙ ДЕМЕНЦИИ

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РЕЗЮМЕ

Обоснование. *Прогнозируется увеличение количества людей пожилого возраста с когнитивными расстройствами, в том числе с деменцией.*

Цель исследования. *Изучение факторов риска развития сосудистой деменции.*

Методы. *Обследованы 39 пациентов с сосудистой деменцией (F01 по МКБ-10); средний возраст – 75,2 ± 9,9 года. Группу сравнения составили 167 человек, посещающие школы памяти, у которых выявлено снижение когнитивных функций (средний возраст – 70,01 ± 3,03 года. По шкале MMSE (Mini Mental State Examination) умеренная степень деменции выявлена в 56,4 % случаев, тяжёлая – в 43,6 %; в группе сравнения в 94 % случаев отмечены лёгкие и в 6 % – умеренные когнитивные нарушения.*

Результаты. *В группе сосудистой деменции статистически значимо чаще выявлялись: ишемическая болезнь сердца (ИБС) в возрасте 70–79 лет (57,1 %; p = 0,000) и в группе в целом (56,4 %; p = 0,000); сахарный диабет в возрасте 60–69 лет (100 %; p = 0,005); артериальная гипертензия (АГ) в возрасте 70–79 лет (100 %; p = 0,000) и в группе в целом (87,2 %; p = 0,000). В группе сравнения частота индекса массы тела (ИМТ) более 25 была статистически значимо выше в возрасте 70–79 лет (60 %; p = 0,000), 80 лет и старше (64,3 %; p = 0,037) и в группе в целом (68,9 %; p = 0,000). Среди лиц с умеренными когнитивными расстройствами статистически значимо чаще встречались: ИБС в возрасте 60–69 лет (10 %; p = 0,001), сахарный диабет в возрасте 70–79 лет (40 %; p = 0,025) и в группе в целом (50 %; p = 0,033), ИМТ более 25 в возрасте 80 лет и старше (70 %; p = 0,000) и в группе в целом (100 %; p = 0,000). Общим фактором риска как для пациентов с сосудистой деменцией, так и для группы с умеренными когнитивными расстройствами оказался возраст 80 лет и старше (20,5 % (p = 0,027) и 70 % (p = 0,005) соответственно).*

Заключение. *Факторы риска развития когнитивных нарушений – ИБС, АГ, сахарный диабет, избыточная масса тела – являются потенциально обратимыми; их своевременное выявление позволит уменьшить развитие когнитивных расстройств и деменции.*

Ключевые слова: *когнитивные нарушения, сосудистая деменция, факторы риска, профилактика*

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Memory impairment is very common in elderly people; preservation of intellectual functioning is a current problem. The number of elderly people is predicted to increase as well as cognitive impairment, including dementia [1–4]. Changes in higher functions due to cerebropathy affect all spheres of life (social, professional, domestic) and can cause maladaptation [5–8]. However, even with pronounced cognitive impairment, the possibility of helping patients is limited due to the irreversibility of pathological processes [9, 10].

Cerebrovascular and neurodegenerative diseases hold the leading position among the causes of cognitive impairment, with vascular dementia being the second most frequent cause of mental deficiency, behind Alzheimer's disease [11, 12]. Due to the scale of prevalence, the difficulty of dementia therapy, correction of risk factors will reduce the prevalence of dementia by 8.5 % by 2050 [13].

Modifiable risk factors for dementia formation are arterial hypertension, alcohol abuse, smoking, depression, diabetes mellitus, heart disease, and excessive body weight [13]. Elevation in blood pressure in middle age increases the risk of developing dementia during the 18-year follow-up period by 60 % [14]. If arterial hypertension persists past the age of 60, the risk of developing dementia increases by up to 100 %. Blood pressure control medications are recommended for the prevention of dementia [15].

Patients having diabetes mellitus are 2 times more likely to develop dementia; the risk of dementia is related to the duration and severity of diabetes [16–18]. It is believed that patients with type 2 diabetes mellitus may have elevated amyloid levels [19, 20].

Obesity is associated with a 30 % increased risk of developing dementia in advanced age [21]. Cerebral atrophy is more likely to form in the case of obesity [22]. Metabolic syndrome also contributes to cognitive impairment [23].

Education, professional activities, and active leisure time contribute to the preservation of cognitive abilities [24]. A low level of education achieved in youth increases the risk of dementia formation by 1.72 times; the protective role of a high level of education will reduce the possibility of dementia development by 4 % [25]. The risk of dementia is 40 % higher in the elderly and 20 % higher in the widowed compared to the married; in contrast, social contact, especially in old age, increases cognitive reserve [26, 27].

Increased awareness of modifiable risk factor prevention has a positive impact on the prevalence of cognitive decline. For example, information about risk factors for dementia formation obtained through the Internet motivated 55.6 % of users to change their lifestyle and 27 % to visit a doctor for treatment of existing diseases [28].

Thus, the provision of timely care to patients with vascular dementia is closely related to early diagnosis, prevention and treatment of cognitive and related disorders in old age, awareness of risk factors for their formation [29, 30].

The aim of the study was to investigate risk factors for the development of vascular dementia.

MATERIALS AND METHODS

39 patients with vascular dementia (ICD-10 diagnosis code F01) were studied: men – 18, women – 21; mean age at the time of examination – 75.2 ± 9.9 years. Patients were examined and treated in general departments of the Irkutsk Regional Clinical Psychiatric Hospital No. 1. Vascular post-stroke dementia was diagnosed in 7 (17.9 %) patients and vascular subcortical dementia in 32 (82.1 %) patients. The vascular dementia diagnosis was confirmed by the diagnostic criteria for vascular cognitive impairment [31]. Neuropsychological study was performed using the scales of the Mini Mental State Examination (MMSE), Montreal Cognitive Assessment (MoCA). Patients with moderate (11–19 points as per the MMSE scale) and severe dementia (10 or less) were included in the study. The frequency of risk factors for the development of vascular dementia – arterial hypertension, coronary heart disease, type 2 diabetes mellitus – was studied on the basis of patient history and clinical examination results, and body mass index was calculated.

Patients with organic amnestic syndrome, delirium, paranoid hallucinatory syndrome, Alzheimer's disease, epilepsy, toxic, drug, metabolic encephalopathy, somatic pathology in decompensation stage, brain injuries were excluded from the study.

The comparison group consisted of seminar and school attendees on the prevention of memory disorders as part of the exhibition "Sibzdravookhranenie" and on the basis of the Center for Preventive Medicine "Zdorovye" (Irkutsk), in whom cognitive decline was detected (n = 167; men – 11, women – 156; mean age – 70.01 ± 3.03 years). The study to identify cognitive impairment and risk factors was conducted by interview method using a written questionnaire. The questionnaire included complaints about memory decline and other cognitive dysfunctions, socio-demographic characteristics, data on the presence of somatic pathology (arterial hypertension, coronary heart disease, diabetes mellitus); body mass index was calculated. In the neuropsychological study, the MMSE, MoCA scales were used. Patients with mild (24–27) and moderate (20–23) cognitive impairment were included in the study.

This study was conducted in accordance with the provisions of the World Medical Association Declaration of Helsinki on Medical Ethics, respecting the rights, interests and personal dignity of the participants. A local ethics committee approved the conduct of the study (Research Ethics Committee of Irkutsk State Medical Academy of Postgraduate Education – branch of the Russian Medical Academy of Continuous Professional Education of the Ministry of Healthcare of the Russian Federation; extract from the protocol No. 1 of 24.01.2019). All subjects who underwent cognitive diagnosis at the School of Memory signed voluntary informed consent for the reporting of their data. An informed consent for data reporting was obtained from relatives of patients with vascular dementia.

Statistical analysis of the study results was performed using Statistica 10.0 for Windows software package (StatSoft Inc., USA). Pearson's χ^2 test, Fisher's test (in case $n < 4$) were used for statistical calculations.

RESULTS

In the group of patients with vascular dementia as per the MMSE scale, the severity of dementia corresponded to a moderate degree of severity (11–19; mean – 14.5 ± 1.3) in 22 (56.4 %) people, severe (0–10; mean – 6.7 ± 2.5) – in 17 (43.6 %). All patients showed a decrease in scale score according to the MoCA scale (mean – 13.3 ± 1.1).

Among patients having vascular dementia, arterial hypertension stage II–III was detected in 87.2 % of cases; coronary heart disease (CHD) – in 56.4 %, including stable angina II–III functional class (FC) – in 33.3 %, coronary artery atherosclerosis – in 15.4 %; atrial fibrillation – in 7.7 %. CHD was complicated by congestive heart failure (CHF) II FC in 41 % of cases, III FC – in 15.4 %. Type 2 diabetes mellitus was detected in 15.4 % of cases. The mean duration of follow-up for arterial hypertension was 16.3 ± 2.4 years, for coronary heart disease – 13.8 ± 1.9 years, and for diabetes mellitus – 14.6 ± 3.8 years. Body mass index (BMI) > 25 was determined in 6 (15.4 %) patients having vascular dementia.

Magnetic resonance imaging (MRI) studies of patients with post-stroke vascular dementia revealed focal changes in the frontoparietal localization of both hemispheres, temporo-occipital region in the basal ganglia of the left hemisphere, thalamus; subcortical foci of leukoareosis in the anterior parts of the brain, frontal lobe white matter, basal ganglia, thalamus (84.6 %). Patients with vascular subcortical dementia had leukoaraiosis in the anterior, frontal and posterior parts of the brain in 35.9 % of cases, with partially or total foci in the basal ganglia, frontal lobes, thalamus region of the left hemisphere – in 25.6 %, in the thalamus region with bilateral localization – in 20.5 %.

In the comparison group (n = 167), a neuropsychological study, based on the MMSE scale, revealed mild cognitive impairment (24–27 points) in 157 (94 %) people, moderate cognitive impairment (23 points) – in 10 (6 %); as per the MoCA scale, a decrease was noted in all attendees, the mean value was 22.1 ± 1.1 points. Stage II–III arterial hypertension was detected in 44.3 % of cases, CHD in 7.8 %, type 2 diabetes mellitus in 10.2 %, and body mass index over 25 in 68.9 %.

As a result of the comparative study, the mean age of patients with vascular dementia was higher than in the comparison group, but the differences were not statistically significant (Table 1). Males were statistically significantly more common in the vascular dementia group (46.2 %; χ^2 = 40.9207; p = 0.000) and females in the comparison group (93.4 %; χ^2 = 40.9207; p = 0.000). Comparative analysis of age periods revealed a statistically significant higher number of subjects in schools of memory aged 60–69 years (46.7 %; χ^2 = 20.1697; p = 0.000) compared with patients having vascular dementia (7.7 %). In contrast, ages 70–79 years (71.7 %; χ^2 = 9.1407; p = 0.003) and 80 years and older (20.5 %; χ^2 = 4.8764; p = 0.027) were statistically significantly more prevalent in the vascular dementia group. Patients with vascular dementia were characterized by higher values of secondary education than the comparison group (51.3 % and 17.9 %, respectively; χ^2 = 78.0598; p = 0.000). In contrast, there were statistically significantly more people with vocational secondary education (67.1 % and 28.2 %, respectively; χ^2 = 19.8466; p = 0.000) and higher education (29.9 % and 20.5 %, respectively; no statistical difference) among those attending schools of memory than among patients with vascular dementia. Widows and widowers were statistically significantly more common in the group with vascular dementia (76.9 %; χ^2 = 60.1253; p = 0.000). In contrast, people in the comparison group were statistically significantly more likely to be married (78.4 %; χ^2 = 52.3227; p = 0.000). Absence of disability was statistically significantly more common in the memory school attendee group compared with the vascular dementia group (89.8 % and 17.9 %, respectively; χ^2 = 90.088; p = 0.000). Among patients, 38.5 % had the first disability group due to a diagnosis of vascular dementia, while 1 patient (0.6 %) in the comparison group; and it was formalized due to a general disease (χ^2 = 63.2701; p = 0.000). The second disability group was observed in 43.7 % of patients with vascular dementia (χ^2 = 47.6778; p = 0.000) and 4.2 % in the comparison group; in both cases due to a general disease. The third disability group was formalized in 8 (4.8 %) people attending the school of memory.

A comparative study of risk factors in patients with vascular dementia and in persons of the comparison group was carried out (Table 2). Among patients with vascular dementia, the incidence of CHD was statistically significantly higher than in the comparison group at ages 70–79 years (57.1 % and 10.7 %, respectively; χ^2 = 24.642; p = 0.000) and in the overall group (56.4 % and 7.8 %, respectively; χ^2 = 53.005; p = 0.000). The incidence of diabetes mellitus among patients having vascular dementia was statistically significantly higher at ages 60–69 years (100 % and 15.4 %, respectively; p = 0.005) and with no statistical difference at ages 70–79 years (10.7 % and 6.7 %, respectively). The incidence of BMI greater than 25 was statistically significantly more frequent in the comparison group at ages 70-79 years (60 %; p = 0.000), 80 years and older (64.3 %; p = 0.037), and in the overall group (68.9 %; χ^2 = 37.3068; p = 0.000). The highest incidence of BMI greater than 25 was between the ages of 60–69 years in both groups – patients with vascular dementia (100 %) and the comparison group (78.2 %). Arterial hypertension in patients diagnosed with vascular dementia was statistically significantly more frequent in the overall group (87.2 %; χ^2 = 23.2547; p = 0.000) and in those aged 70–79 years (100 %; χ^2 = 42.377; p = 0.000) than in the comparison group (44.3 % and 28 %, respectively).

The results of risk factors in patients with vascular dementia and in the comparison group with moderate cognitive impairment are given in Table 3.

A statistically significant higher proportion of patients with vascular dementia were aged 70-79 years (71.7 %; p = 0.004); in contrast, the group with moderate cognitive impairment was aged 80 years or older (70 %; χ^2 = 9.1764; p = 0.002). Individuals having moderate cognitive impairment were statistically significantly more likely to have vocational secondary education (70 %; χ^2 = 5.9823; p = 0.014).

TABLE 1

SOCIO-DEMOGRAPHIC CHARACTERISTICS OF PATIENTS WITH VASCULAR DEMENTIA AND COMPARISON GROUP

Indicators	Vascular dementia group (n = 39)		Comparison group (n = 167)		p
	abs.	%	abs.	%	
Mean age	75.2 ± 9.9		70.01 ± 3.03		0.617
Men	18	46.2	11	6.6	0.000 (χ^2 = 40.9207)
Women	21	53.8	156	93.4	0.000 (χ^2 = 40.9207)
60–69 years	3	7.7	78	46.7	0.000 (χ^2 = 20.1697)
70–79 years	28	71.7	75	44.9	0.003 (χ^2 = 9.1407)
80 years and older	8	20.5	14	8.4	0.027 (χ^2 = 4.8764)
Education					
Secondary	20	51.3	3	17.9	0.000 (χ^2 = 78.0598)
Vocational secondary	11	28.2	112	67.1	0.000 (χ^2 = 19.8466)
Higher	8	20.5	50	29.9	0.239 (χ^2 = 1.3890)
Incomplete higher	–	–	2	1.2	–
Family status					
Married	7	17.9	131	78.4	0.000 (χ^2 = 52.3227)
Divorced	2	5.1	10	5.9	0.836 (χ^2 = 0.0426)
Widowed	30	76.9	26	15.6	0.000 (χ^2 = 60.1253)
Disability					
No	7	17.9	150	89.8	0.000 (χ^2 = 90.088)
First disability group	15	38.5	1	0.6	0.000 (χ^2 = 63.2701)
Second disability group	17	43.7	7	4.2	0.000 (χ^2 = 47.6778)
Third disability group	–	–	8	4.8	–

A body mass index greater than 25 was more frequently detected at all ages studied in the group with moderate cognitive impairment, including a statistically significant difference between those aged 80 years and older (70 %; p = 0.000) and the overall group (100 %; χ^2 = 25.9135; p = 0.000). Arterial hypertension was statistically significantly more frequently reported in patients having vascular dementia aged 70–79 years (71.8 %; p = 0.004), in the group with moderate cognitive impairment – aged 80 years and older (70 %; p = 0.000). In the comparison group with moderate cognitive impairment, CHD with a statistically significant difference was more frequently detected at ages 60–69 years (10 %; p = 0.001), while in patients having vascular dementia it was detected at ages 70–79 years (41 %). Diabetes mellitus among subjects with moderate cognitive impairment was statistically significantly more common in those aged 70–79 years (40 %; p = 0.025) and in the overall group (50 %; χ^2 = 5.4780; p = 0.033).

All patients with vascular dementia had psychopathological disorders including psychomotor retardation, difficulty in refocusing, and impaired working memory. Dysregulatory disorders in the form of difficulties in decision-making and planning were found in 84.6 % of cases, confabulations – in 43.6 %, elements of amnestic aphasia – in 28.2 %. Depressive symptoms were detected in 43.6 % of patients in the form of sad mood with a dreary tinge, indifference, apathy. The clinic of vascular dementia was characterized by flickering symptoms with intensification of confused mental state in the evening and at night, disturbance of temporal-spatial gnosis, psychopathy-like behavioral disorders in the form of irritability and aggression. In 15.4 % of cases, psychotic symptomatology included paranoid delusion of small range and episodes of visual hallucinosis. All patients were non-critical of their condition.

The therapy of patients diagnosed with vascular dementia in a psychiatric hospital for symptomatic improvement of cognitive functions and maintenance of their daily activity included the use of memantine. Among neuroprotective drugs were used ethylmethylhydroxypyridine succinate (79.5 %), deproteinized hemoderivative of calf blood (69.2 %), vinpocetine (69.2 %), aminophenyl-butyric acid hydrochloride (48, 7 %), cerebrolysin concentrate (41 %). The antipsychotic agents, such as chlorprotixen

TABLE 2

RISK FACTORS IN PATIENTS WITH VASCULAR DEMENTIA AND IN THE COMPARISON GROUP

Age	Comparison group (n = 167)		Vascular dementia group (n = 39)		p
	abs.	%	abs.	%	
			CHD		
60–69 years	5/78	6.4	1/3	33.3	0.209
70–79 years	8/75	10.7	16/28	57.1	0.000 (χ^2 = 24.642)
80 years and older	–	–	5/8	62.5	–
Total	13/167	7.8	22/39	56.4	0.000 (χ^2 = 53.005)
			Diabetes mellitus		
60–69 years	12/78	15.4	3/3	100	0.005
70–79 years	5/75	6.7	3/28	10.7	0.375
80 years and older	–	–	–	–	–
Total	17/167	10.2	6/39	15.4	0.353
			BMI > 25		
60–69 years	61/78	78.2	3/3	100	0.488
70–79 years	45/75	60	2/28	7.1	0.000
80 years and older	9/14	64.3	1/8	12.5	0.037
Total	115/167	68.9	6/39	15.4	0.000 (χ^2 = 37.3068)
			Arterial hypertension		
60–69 years	43/78	55.1	3/3	100	0.254
70–79 years	21/75	28	28/28	100	0.000 (χ^2 = 42.377)
80 years and older	10/14	71.4	3/8	37.5	0.1836
Total	74/167	44.3	34/39	87.2	0.000 (χ^2 = 23.2547)

(28.2 %), quetiapine (15.4 %), risperidone (7.7 %), were used for the treatment of behavioral and psychotic disorders. Such antidepressants as pipofezine (28.2 %), agomelatine and escitalopram (7.7 % each) were used for the treatment of affective disorders. Antiepileptic Drugs – valproic acid (15.4 %), carbamazepine (12.8 %) – were used in agitated patients.

CONCLUSION

Thus, risk factors for the formation of vascular dementia have been investigated as a result of this study. Moderate dementia was found in 56.4 % of patients with vascular dementia and severe dementia in 43.6 %; mild (94 %) and moderate (6 %) cognitive impairment was diagnosed in the comparison group.

Patients with vascular dementia were statistically significantly more likely to be male (46.2 %; p = 0.000), age ranges 70–79 years (71.7 %; p = 0.003) and 80 years and older (20.5 %; p = 0.027), persons having secondary education (51.3 %; p = 0.000), widows and widowers (76.9 %; p = 0.000). Patients had first and second disability groups (38.5 % (p = 0.000) and 43.7 % (p = 0.000), respectively).

The comparison group had statistically significantly more females (93.4 %; p = 0.000), individuals with vocational secondary education (67.1 %; p = 0.000) and higher education (29.9 %; no statistical difference). Memory school attendees were married 78.4 % of the time (p = 0.000) and 89.8 % had no disability group (p = 0.0000).

Among risk factors, CHD was statistically significantly more frequent in patients with vascular dementia compared with memory school attendees at age 70–79 years and in the overall group (p = 0.000, p = 0.000) and diabetes mellitus at age 60–69 years (p = 0.005); the incidence of arterial hypertension was statistically significantly higher at age 70–79 years (p = 0.000) and in the overall group (p = 0.000). In the comparison group, the incidence of BMI greater than 25 was statistically significantly higher at ages 70–79 years (p = 0.000), 80 years and older (p = 0.037), and in the overall group (p = 0.000) than in patients having vascular dementia.

TABLE 3

RISK FACTORS IN PATIENTS WITH VASCULAR DEMENTIA AND IN THE COMPARISON GROUP WITH MODERATE COGNITIVE IMPAIRMENT

Risk factors	Vascular dementia group (n = 39)		Comparison group – moderate cognitive impairment (n = 10)		p
	abs.	%	abs.	%	
Mean age	75.2 ± 9.9		78.3 ± 6.3		0.793
Men	18	46.2	3	30	0.126
Women	21	53.8	7	70	0.290
60–69 years	3	7.7	1	10	0.612
70–79 years	28	71.7	2	20	0.004
80 years and older	8	20.5	7	70	0.002 (χ^2 = 9.1764)
Education					
Secondary	20	51.3	2	20	0.076
Vocational secondary	11	28.2	7	70	0.014 (χ^2 = 5.9823)
Higher	8	20.5	1	10	0.403
Family status					
Married	7	17.9	–	–	–
Divorced	2	5.1	1	10	0.504
Widowed	30	76.9	9	90	0.335
Disability					
No	7	17.9	–	–	–
First disability group	15	38.5	1	10	0.086
Second disability group	17	43.7	4	40	0.565
Third disability group	–	–	4	40	–
BMI > 25					
60–69 years	3	7.7	1	10	0.612
70–79 years	2	5.1	2	20	0.180
80 years and older	1	2.6	7	70	0.000
Total	6	15.4	10	100	0.000 (χ^2 = 25.9135)
Arterial hypertension					
60–69 years	3	7.7	1	10	0.612
70–79 years	28	71.8	2	20	0.004
80 years and older	3	7.7	7	70	0.000
Total	34	87.2	10	100	0.302 (χ^2 = 1.4277)
Coronary heart disease					
60–69 years	1	2.6	1	10	0.001
70–79 years	16	41.0	2	20	0.066
80 years and older	5	12.8	–	–	–
Total	22	56.4	3	30	0.127
Diabetes mellitus					
60–69 years	3	7.7	1	10	0.612
70–79 years	3	7.7	4	40	0.025
80 years and older	–	–	–	–	–
Total	6	15.4	5	50	0.033 (χ^2 = 5.4780)

The group of persons with moderate cognitive impairment in comparison with patients with vascular dementia revealed a statistically significantly higher number of subjects aged 80 years and older ($p = 0.002$), with vocational secondary education (70 %; $p = 0.014$); there were also statistically significantly higher incidence of CHD at the age of 60–69 years ($p = 0.001$), diabetes mellitus at the age of 70–79 years ($p = 0.025$) and in the overall group ($p = 0.033$), body mass index greater than 25 – at the age of 80 years and older ($p = 0.000$) and in the overall group ($p = 0.000$). A common risk factor for both patients with vascular dementia and the group with moderate cognitive impairment was age 80 years or older ($p = 0.027$ and $p = 0.005$, respectively).

Clinical features of patients diagnosed with vascular dementia in a psychiatric hospital were characterized by severe cognitive impairment, affective, psychotic and behavioral disorders, which required prescription of combined therapy (memantine, antipsychotic agent, antidepressant, normothymic agent).

The organization of health schools for both patients with memory impairment and those with risk factors will increase the level of knowledge on preventing the development of cognitive impairment. Risk factors for cognitive decline (education level, marital status (social contacts), cardiovascular diseases (coronary heart disease, arterial hypertension), diabetes mellitus, overweight) are potentially reversible, and their timely detection will reduce the development of cognitive impairment and dementia.

Conflict of interest

The author of this article declares the absence of a conflict of interest.

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ONCOLOGY

CASTLEMAN DISEASE. A RARE CLINICAL CASE OF RETROPERITONEAL TUMOR LOCALIZATION IN AN ELDERLY PATIENT

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ABSTRACT

Castleman disease is an extremely rare benign disease of the lymphatic system with an estimated incidence of 1–9 cases per 1,000,000. Its etiology remains unknown; interleukin 6 (IL-6) plays an important role in pathogenesis. Castleman disease has two clinical forms: localized (up to 90 % of cases) with a favorable prognosis, treated predominantly by surgical method; generalized (up to 10 % of cases) with less favorable prognosis, treated by pharmacological therapy. The diagnosis is rarely established at the preoperative stage.

The aim. *To present a clinical case of diagnosis and treatment of Castleman tumor of a rare topical localization.*

Results. *A 66-year-old patient was admitted at the Abdominal Oncology Department of the G.E. Ostroverkhov Kursk Oncology Scientific and Clinical Center. Diagnosis: Retroperitoneal mass on the right found at the preventive examination; no peripheral lymphadenopathy was detected on ultrasound and computed tomography (CT). After the examination, a preliminary diagnosis was made: Gastrointestinal stromal tumor (GIST) of the small intestine mesentery.*

Based on the results of the case conference, the decision was taken to perform a surgery – laparoscopic removal of the tumor under endotracheal anesthesia. For surgical approach, a fan-shaped arrangement of ports was chosen. The surgery had no complications. Intraoperative blood loss was 50.0 ml. The total operating time was 98 minutes.

According to the results of the histological study, the following diagnosis was made: Castleman disease, unicentric form, hyaline-vascular variant.

En bloc surgery is the standard method for the treatment of localized forms of the Castleman disease. In all cases, long-term follow-up shows a long relapse-free period in almost all patients.

During follow-up examinations (ultrasound of the abdominal cavity and retroperitoneal space, CT of the abdominal cavity with contrast enhancement, CT of the chest), no disease recurrence was detected during the year of observation.

Castleman disease is a rare non-clonal lymphoproliferative disease of unknown etiology. A rare case of its retroperitoneal localization indicates that in cases with an uncertain nature of the peritoneal mass, Castleman disease should be included in the differential diagnostic search.

Key words: *Castleman disease, retroperitoneal lymphadenopathy, immunodeficiency, hyaline-vascular variant, interleukin 6, computed tomography, laparoscopy*

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БОЛЕЗНЬ КАСТЛЕМАНА. РЕДКИЙ КЛИНИЧЕСКИЙ СЛУЧАЙ ЗАБРЮШИННОЙ ЛОКАЛИЗАЦИИ ОПУХОЛИ У ПАЦИЕНТКИ ПОЖИЛОГО ВОЗРАСТА

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РЕЗЮМЕ

Болезнь Кастлемана относится к крайне редким доброкачественным заболеваниям лимфатической системы с приблизительной заболеваемостью 1–9 случаев на 1 000 000. Этиология заболевания остаётся неизвестной; в патогенезе важную роль играет интерлейкин 6 (IL-6). Заболевание имеет две клинические формы: локализованная (до 90 % случаев) – имеет благоприятный прогноз, лечение преимущественно хирургическое; генерализованная (до 10 % случаев) – прогноз менее благоприятный, применяются медикаментозные методы лечения. Диагноз редко устанавливается на дооперационном этапе.

***Цель**. Представить клинический случай диагностики и лечения опухоли Кастлемана редкой топической локализации.*

***Результаты.** В отделение абдоминальной онкологии ОБУЗ «Курский онкологический научно-клинический центр им. Г.Е. Островерхова» поступила пациентка 66 лет с диагнозом: Объёмное образование забрюшинного пространства справа, выявленное в ходе профилактического осмотра; по данным ультразвукового исследования (УЗИ) и компьютерной томографии (КТ) перефирической лимфаденопатии выявлено не было. После обследования пациентке был выставлен предварительный диагноз: Гастроинтестинальная стромальная опухоль (GIST) брыжейки тонкой кишки.*

По результатам консилиума было принято решение выполнить оперативное вмешательство – лапароскопическое удаление опухоли под эндотрахеальным наркозом. Для оперативного доступа была выбрана веерообразная расстановка портов. Оперативное вмешательство прошло без осложнений. Интраоперационная кровопотеря составила 50,0 мл. Общее время операции составило 98 минут.

По результатам гистологического заключения был выставлен диагноз: Болезнь Кастлемана, уницентричная форма, гиалиново-сосудистый вариант. Хирургическое вмешательство «en bloc» является стандартом лечения локализованных форм болезни, во всех случаях долгосрочного наблюдения показывает длительный безрецидивный период практически у всех пациентов. При контрольных обследованиях (УЗИ брюшной полости и забрюшинного пространства, КТ брюшной полости с контрастным усилением, КТ грудной клетки) в течение года наблюдения рецидива заболевания не выявлено.

Таким образом, болезнь Кастлемана представляет собой редкое неклональное лимфопролиферативное заболевание неизвестной этиологии. Редкий случай забрюшинной локализации указывает на то, что в случаях с неопределённым характером объёмного поражения брюшиной полости в дифференциально-диагностический поиск должная входить болезнь Кастлемана.

***Ключевые слова:** болезнь Кастлемана, забрюшинная лимфаденопатия, иммунодефицит, гиалиново-сосудистый вариант, интерлейкин 6, компьютерная томография, лапароскопия*

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INTRODUCTION

Castleman disease (angiofollicular nodular hyperplasia) is a rare benign lymphoproliferative disease. This pathology was first described in 1954 by Benjamin Castleman as a localized enlargement of mediastinal lymph nodes with a characteristic increase in the number of follicles, involution of their central part, and a significant vascular network with endothelial hyperplasia [1].

According to the literature, the incidence is 1–9 cases per 1,000,000 population. There are no precise incidence data; the average incidence is 1 case per 150,000 population. The incidence among male and female population is approximately the same, but precise data are not available. The age group of 40 years or less is more common among patients diagnosed with Castleman disease [2]. Precise data on the incidence of the disease in the Russian Federation are not available.

Three main morphological variants of the tumor were described: hyaline-vascular, plasma cell and mixed [3]. Currently, a 4th variant, HHV-8-associated, is distinguished. Hyaline-vascular variant is characterized by disorder of lymph node architectonics due to hyperplasia of lymphatic follicles, reduction of their size, atrophy and hyalinization of the central zones, wide mantle part, interfollicular hypervascularization; externally, this variant resembles an «onion skinning». Mitoses, as a rule, are absent. A vessel runs to the center of the hyalinized follicle, increased vascularization is noted in the interfollicular zones (the most frequent type of vessel is a postcapillary venule with hyperplastic endotheliocytes), single or grouped lymphocytes, plasmacytes, plasmacytic monocytes are found between the vessels [4]. In the plasma cell variant, the structure of lymph nodes is disturbed due to hyperplastic follicles with a narrow mantle zone of lymphocytes. In the interfollicular zones and cerebral layer there are wide areas of mature and immature plasma cells, sinuses are dilated [5].

Thus, the occurrence of hyaline-vascular variant is 80–90 %, of plasma cell variant – 8–10 % [6]. The literature also identifies a mixed variant (about 5 % of cases). Recently, the HHV-8-associated variant has been identified as new data on the influence of human herpesvirus type 8 on the development of Castleman disease have been obtained [7].

The clinical classification is based on the number of affected lymph nodes or groups of lymph nodes. Thus, two forms of the disease are distinguished: localized (unicentric) (one region or lymph node is affected) and generalized (multicentric) (several regions are affected) [8]. The unicentric form is the most common one (80–90 %). Thoracic lymph nodes are most commonly affected (about 70 %), particularly in the mediastinum. Abdominal and retroperitoneal lymph nodes are the next most frequently affected (approximately 10 %). The rarest localizations are the tonsils, orbit, lymphatic tissue of the nasopharynx and tongue (no more than 8 %). The generalized form has a more significant clinical picture and a less favorable prognosis. In the multicentric form, most cases manifest in patients 50–60 years of age, and some cases have secondary renal and pulmonary involvement, paraneoplastic vesicular disease, and peripheral neuropathy. Laboratory findings include anemia, thrombocytosis, hypergammaglobulinemia, and elevated C-reactive protein [9–12].

There are no established risk factors for the localized variant, and the disease is idiopathic. At the same time, the main risk factor for multicentric variant of the lesion is an immunodeficiency state of varying severity, and in some cases, the presence of human herpes virus type 8 (HHV-8) [13].

Therefore, the presence of immunodeficiency caused by HIV infection would be a predisposing factor for the occurrence of Castleman disease. In the vast majority of cases, HIV-positive patients will be HHV-8-positive, whereas the rate of HHV-8 detection among HIV-negative patients ranges from 2 % to 50 %. Although HHV-8 status is directly related to viral load in the population, patients in this group have an increased risk of transformation into HHV-8-positive plasmablastic lymphoma and the occurrence of Kaposi's sarcoma [14–16].

Although the etiopathogenesis of Castleman disease is still unclear, indolent chronic inflammation, immunodeficiency and/or autoimmune conditions are considered as likely factors. In 1993, S. Akira et al. first pointed out the role of interleukin 6 (IL-6). The effect of IL-6 overproduction and dysregulation on stimulation of acute phase protein production by hepatocytes, stimulation of B-cells, macrophages, fibroblasts, direct and indirect stimulation of neoangiogenesis and endothelial cell proliferation has been established. Therefore, anti-IL6-immunotherapy is used as one of the treatment options [17].

In the vast majority of cases, the localized form of the disease is treated surgically. This report presents a clinical case of a rare retroperitoneal paraduodenal localization of Castleman disease, its diagnosis and treatment.

CASE HISTORY

A 66-year-old female patient was admitted at the Abdominal Oncology Department of the G.E. Ostroverkhov Kursk Oncology Scientific and Clinical Center. Diagnosis: Retroperitoneal mass on the right. She had no complaints. She was referred to the department by the medical and preventive institution at her place of residence due to a retroperitoneal mass lesion detected during a preventive examination, which coincides with reports of a prolonged asymptomatic course of the process [18, 19]. General condition on admission was satisfactory, no bad habits, body mass index – 31.24 kg/m^2. She reported that a preventive ultrasound of the abdominal cavity revealed a right-sided mass lesion up to 6.0 cm. In 2012, she underwent a surgical intervention – "open" appendectomy, without complications. No plasma or hemotransfusions were performed. Laboratory tests are within reference values. The tests for Wassermann reaction (WR), hepatitis B surface antigen (HBS Ag), antibodies to hepatitis C virus (HCV), HIV were negative. Associated disease: hypertension.

The patient was examined at our center. Chronic atrophic gastritis was detected during fibrogastrodu-

odenoscopy. Fibrocolonoscopy revealed no organic pathology. No thrombosis or reflux on ultrasound examination of the lower limb veins. Mammography: fibrotic breast changes (BIRADS-2). No gynecological pathology was detected during the examination. No coronavirus COVID-19 RNA was detected.

Ultrasound of peripheral, abdominal, retroperitoneal lymph nodes showed no lymphadenopathy. Changes on pelvic ultrasound are within the age-related normal range.

Abdominal ultrasound: in the area of the head of the pancreas there is a round-shaped hyperechogenic mass lesion with a clear even contour, 5.2 × 3.7 × 4.4 cm in size, well vascularized. Computed tomography (CT) of the chest organs revealed fibrotic changes. Subsequent computed tomography of the abdominal cavity organs with intravenous contrast (Ultravist 100 ml): in the root of the mesentery on the right side, in front of the lower horizontal part of the duodenum, a space-occupying mass with a rather clear contour (5.4 × 3.7 × 4.6 cm), soft tissue density, contrast-accumulating, with a vascular network along the contour, without clear organ affiliation was detected (Fig. 1). The findings may be consistent with gastrointestinal stromal tumor (GIST) of the small intestine mesentery. The differential diagnosis of abdominal and retroperitoneal localizations of Castleman disease is most often made with GIST or neuroendocrine tumors [18].

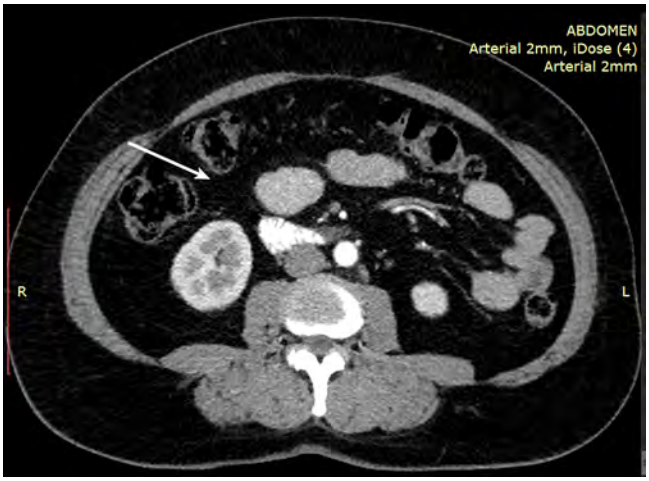

FIG. 1.
CT scan of the abdominal cavity with intravenous contrast, axial projection, arterial phase: round-shaped mass lesion, adjacent to the duodenum

The CT scan revealed precise topical localization of the tumor, no signs of invasion of surrounding structures, and vascular anatomy – both anatomical landmarks and vessels of the tumor (Fig. 2). There were no signs of dissemination during the study, in particular, there were no focal liver mass lesions, which was confirmed by CT scans in different projections (Fig. 3). However, CT findings for Castleman disease are nonspecific, and due to the rare occurrence of retroperitoneal localization, GIST is most commonly identified. The mesenteric unicentric form of the disease, usually appears on CT as a mass lesion of soft tissue density, without satellite nodes [20, 21].

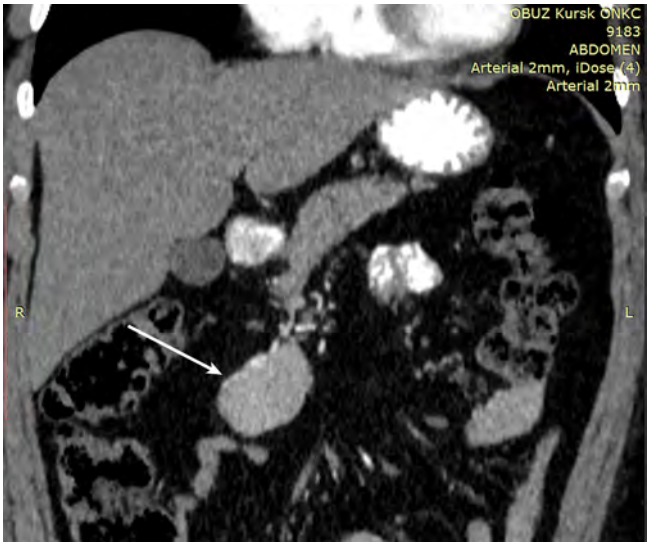

FIG. 2.
CT scan of the abdominal cavity with intravenous contrast, coronary projection, arterial phase: no proved invasion of the duodenum, pancreas, vessels of the small intestine mesentery detected

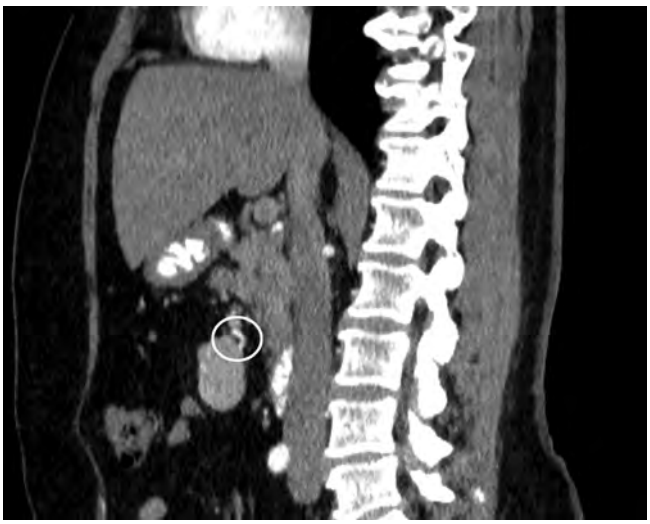

FIG. 3.
CT scan of the abdominal cavity with intravenous contrast, sagittal projection, arterial phase: an arterial vessel along the lateral contour enters the mass lesion in the upper contour

Pancreaticoduodenal endosonography data were concordant with abdominal CT data. After examination, the patient was given a provisional diagnosis of GIST of the small intestine mesentery.

After a consilium, it was decided to perform surgical intervention – laparoscopic tumor removal under endotracheal anesthesia.

A fan-shaped port arrangement was chosen for surgical approach: 10 mm optical port was located parumbilically below the umbilicus; 10 mm port in the right lateral region along the midclavicular line; 5 mm port – in the right subcostal region; 5 mm port – in the left lateral region along the midclavicular line.

Revision of the abdominal cavity revealed no signs of dissemination, including visual metastatic foci in the liver, pelvic organs without pathology (Fig. 4).

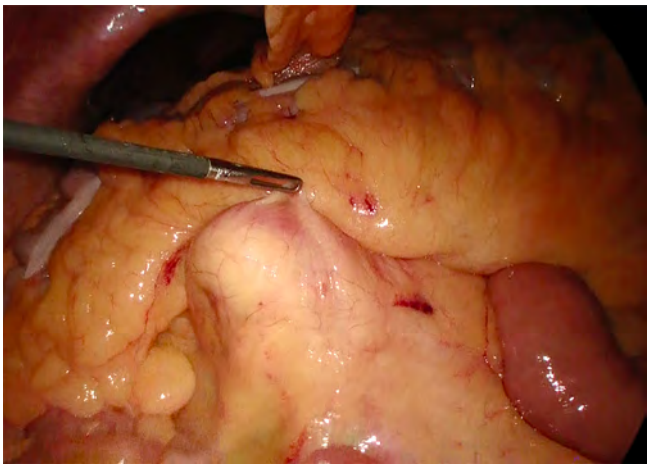

FIG. 4.
Intraoperative photo. Laparoscopic picture: a tumor was found; no carcinomatosis, no ascites; no signs of invasive growth. The lesion is located retroperitoneally, without involving small intestine loops

The classic fan-shaped port arrangement allows dissection in various planes. After mobilization of the mass lesion, clipping and vessel crossing, tumor removal was performed (Fig. 5, 6). Revision of the abdominal cavity and bed was performed, and hemostasis was controlled (Fig. 7).

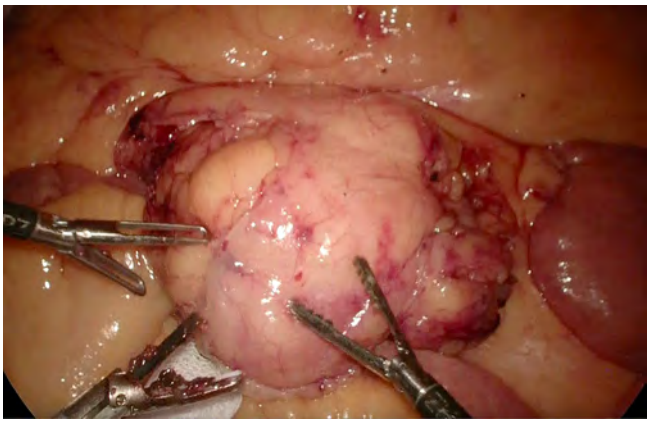

FIG. 5.
Intraoperative photo. The peritoneum was opened along the periphery of the tumor; the tumor is floating; mobilization was performed along the lower contour of the tumor

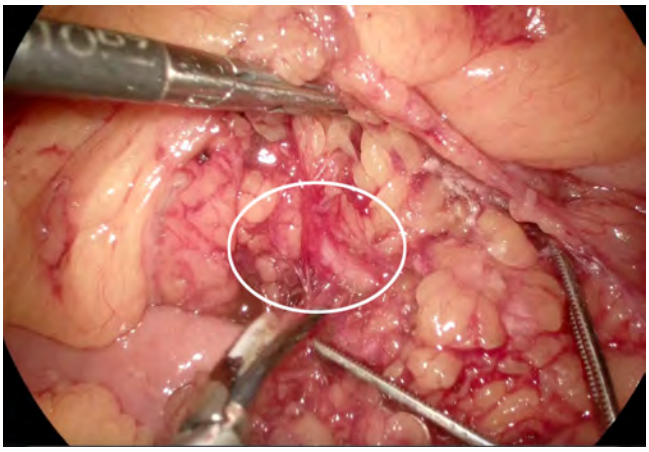

FIG. 6.
Intraoperative photo. A vessel from the superior mesenteric artery system is isolated in the area of upper pole of the tumor, being visualized by CT of the abdominal cavity

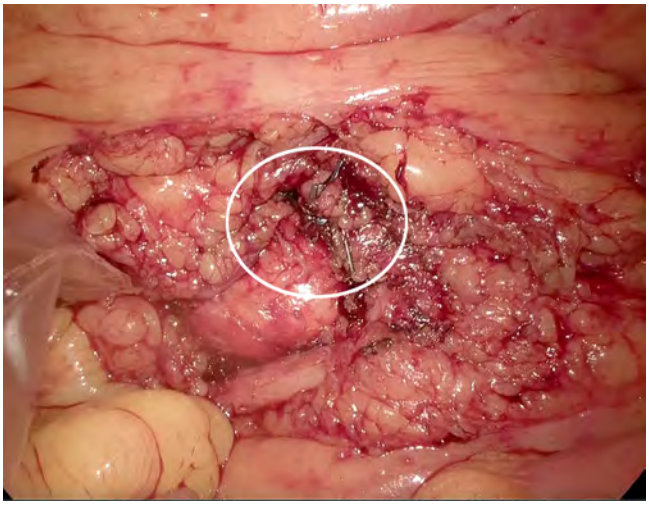

FIG. 7.
Intraoperative photo. Final view: the bed of the removed tumor; clips are put on the vessels (artery, vein)

DISCUSSION

The surgery had no complications. Intraoperative blood loss was 50.0 ml. The total operating time was 98 minutes. Due to the absence of bleeding and signs of connection with the pancreas, abdominal drainage was not performed. The container with the gross specimen was extracted through a minilaparotomy incision in the right iliac region with excision of the scar after appendectomy.

Despite the previously established diagnosis (GIST of the small intestine mesentery), our preoperative diagnosis was consistent with M. Ohta et al. who indicated that the preoperative diagnosis never included Castleman disease [22]. The en bloc surgery performed is the standard of treatment for localized forms of the disease. In all cases, long-term follow-up shows a long relapse-free period in almost all patients [23, 24].

Based on the results of the histological study, the following diagnosis was made: Castleman disease, unicentric form, hyaline-vascular variant.

There is increased vascularization of interfollicular zones, mainly due to postcapillary venules, with altered endothelial cells (Fig. 8). Scattered or grouped plasmacytes, small lymphocytes and monocytes are located among the vessels, sometimes eosinophils are included in the interfollicular infiltrate. The histological data obtained were full compliance with those of many investigators [25–29].

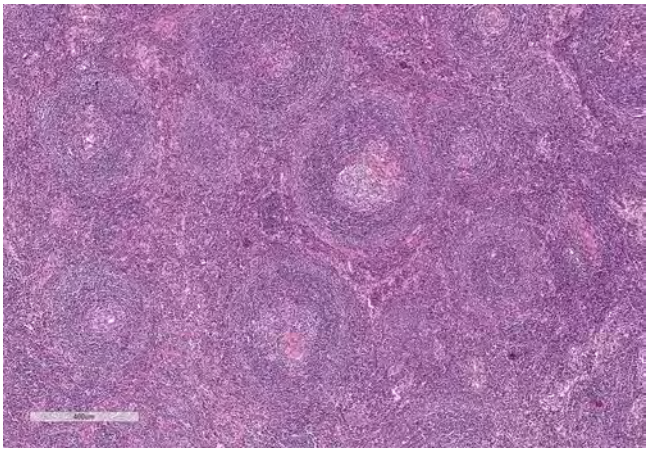

FIG. 8.

Micrograph. Alteration of the histologic structure of the lymph nodes due to an increase in the number of lymphatic follicles and interfollicular zones hypervascularization. The follicles are reduced and have a transformed, atrophic, hyalinized germinal center. Hematoxylin and eosin staining; magnification ×20

The germinal centers are significantly cell-depleted, consisting of single lymphocytes, follicular dendritic cells, and hyaline deposits (Fig. 9, 10). Immunohistochemical study: cells of interfollicular space consist of $CD68^+$ plasma cells, dendritic cells, endotheliocytes of blood vessels; cells of germinal centre consist of $CD3^+$, $CD21^+$, $CD23^+$ [30].

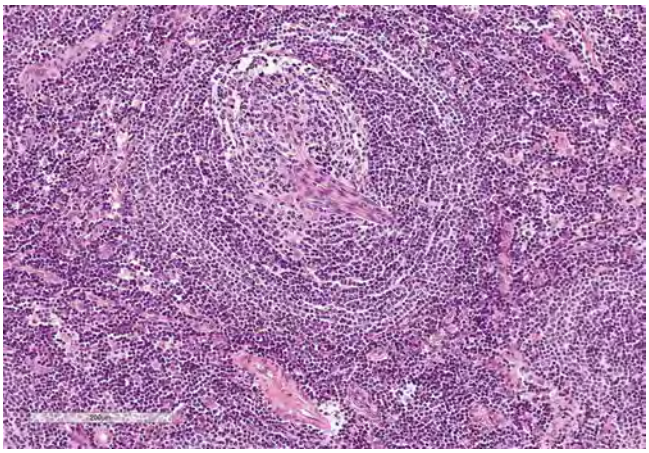

FIG. 9.

Micrograph. Lymphatic follicle. A wide, concentric mantle zone formed by small lymphocytes – "onion skinning". No mitoses detected. Hematoxylin and eosin staining; magnification ×100

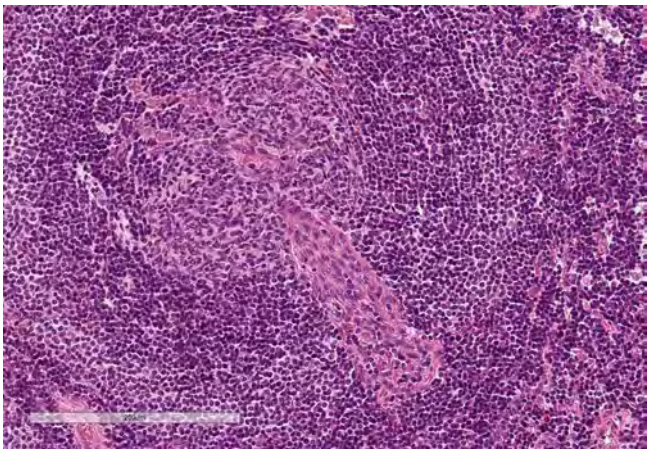

FIG. 10.

Micrograph. Lymphatic follicle. A hyalinized capillary-type vessel passes through the center of the atrophic follicle, the structure resembles a lollipop. Hematoxylin and eosin staining; magnification ×100

RESULTS

After the histological report, the patient underwent PCR test to detect HHV-8. The result was negative, which was in compliance with literature data and laboratory and instrumental findings. The patient was discharged from the ward on the 8th day after surgery, referred for consultation to a hematologist: the diagnosis was confirmed, no additional therapy was indicated.

During follow-up examinations (ultrasound of the abdominal cavity and retroperitoneal space, CT of the abdominal cavity with contrast enhancement, CT of the chest), no disease recurrence was detected during the year of observation.

CONCLUSION

Therefore, Castleman disease is a rare lymphoproliferative disease of unknown etiology pathogenetically associated with IL-6 hyperexpression. It has characteristic histological variants. The diagnosis is often established after morphological examination of the removed surgical specimen. It has a favorable prognosis, especially in the localized form.

The presented case is unusual due to the location of the tumor in the paraduodenal, retroperitoneal, and parapancreatic areas. Laparoscopic removal of the tumor was performed due to the obvious advantages of this surgical approach [31]. Despite the short follow-up time (18 months), there were no signs of disease recurrence, but further dynamic follow-up is necessary due to the rare localization in this case [32].

In cases with indeterminate abdominal and retroperitoneal mass lesions, laparoscopic approach is considered as the last stage of diagnosis and the first stage of treatment.

There is a need to develop CT criteria to suspect Castleman disease in order to include it in the differential di-

agnostic series for abdominal and retroperitoneal mass lesions. Further study of the etiopathogenesis of the disease will make it possible to develop an optimal therapeutic and diagnostic algorithm.

Conflict of interest

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

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RESECTION INTERVENTIONS IN THE TREATMENT OF HEPATOCELLULAR CANCER IN A SPECIALIZED SURGERY CENTER

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ABSTRSACT

Background. *The relevance of the treatment of hepatocellular cancer (HCC) is determined by a significant increase in the incidence rate and its high prevalence among primary malignant hepatic tumors.*
The aim of the study. *To summarize the experience of providing specialized medical care to patients with hepatocellular cancer.*
Methods. *We studied the direct results of treatment of patients with primary hepatic cancer treated at the Kuzbass Regional Hepatological Center for the period from January 2015 to August 2022. The materials for the study were medical records of patients with an established diagnosis of primary hepatic cancer, surgical records, results of pathohistologic examination. The exclusion criterion was diagnosed cholangiocellular carcinoma.*
Results. *During the period from 2015 to 2022, 59 patients with primary hepatic cancer were treated at the Kuzbass Regional Hepatological Center. Among them, hepatocellular cancer was diagnosed in 48 cases, cholangiocellular cancer – in 11 cases; radical surgery was performed in 12 patients with hepatocellular cancer; fatal outcome was noted in 1 (2.1 %) patient, complications in the postoperative period developed in 5 (41.7 %) cases and were ranked as I-3, IIIb-1 and IVb-1 according to Clavien – Dindo classification.*
Conclusion. *The degree of risk of severe post-resection hepatic failure should be considered one of the main criteria in choosing a strategy for radical surgical treatment. New surgical approaches (laparoscopic vascular isolation of the portal blood flow, using temporary hemostasis in laparoscopic hepatic resections and the device for its implementation) in resection surgery of primary hepatic cancer can improve the immediate results of treatment.*

Key words: *hepatocellular carcinoma, two-staged hepatic resection, post-resection hepatic failure, methods of hemostasis*

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РЕЗЕКЦИОННЫЕ ВМЕШАТЕЛЬСТВА В ЛЕЧЕНИИ ГЕПАТОЦЕЛЛЮЛЯРНОГО РАКА В СПЕЦИАЛИЗИРОВАННОМ ЦЕНТРЕ ХИРУРГИИ

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РЕЗЮМЕ

Обоснование. *Актуальность вопроса лечения гепатоцеллюлярного рака (ГЦК) определяется значимым приростом показателя заболеваемости им и его высокой частотой встречаемости среди первичных злокачественных опухолей печени.*

Цель исследования. *Обобщение опыта оказания специализированной помощи больным гепатоцеллюлярным раком.*

Методы. *Изучены непосредственные результаты лечения больных первичным раком печени, пролеченных в Кузбасском областном гепатологическом центре (КОГЦ) за период с января 2015 по август 2022 г. Материалами для исследования были медицинские карты больных с установленным диагнозом первичного рака печени, протоколы хирургических вмешательств, результаты патолого-гистологического исследования. Критерий исключения – диагностированный холангиоцеллюлярный рак.*

Результаты. *За период с 2015 по 2022 г. в КОГЦ пролечено 59 пациентов с первичным раком печени, из них гепатоцеллюлярный рак диагностирован в 48 случаях, холангиоцеллюлярный рак – в 11; радикальное хирургическое лечение проведено 12 больным ГЦК; летальный исход отмечен у 1 (2,1 %) пациента, осложнения в послеоперационном периоде развились в 5 (41,7 %) случаях и имели градацию тяжести по Clavien – Dindo I-3, IIIb-1, IVb-1.*

Заключение. *Степень риска развития тяжёлой пострезекционной печёночной недостаточности следует считать одним из основных критериев в выборе стратегии радикального хирургического лечения. Новые хирургические подходы (лапароскопическая сосудистая изоляция воротного кровотока, использование способа временного гемостаза при лапароскопических резекциях печени и устройства для его осуществления) в резекционной хирургии первичного рака печени позволяют улучшить непосредственные результаты лечения.*

Ключевые слова: *гепатоцеллюлярный рак, двухэтапная резекция печени, пострезекционная печёночная недостаточность, способы гемостаза*

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INTRODUCTION

Hepatocellular carcinoma (HCC) is a liver disease of tumorigenic nature, the prognosis of which is unfavorable, the five-year survival rate does not exceed 15 % [1, 2]. There is a correlation between the number of viral hepatitis B cases in a population and the incidence of primary hepatic cancer, with rates in Asian and central African countries higher than the global average of 150–500 per 100,000 population [3, 4]. Our country has primary hepatic cancer incidence rates of 4–5 cases per 100,000 population, which is 13th in the structure of cancer morbidity and 11th among causes of death. Every year in Russia more than 6 thousand new cases of primary hepatic cancer are detected, usually at a stage that is not subject to special types of medical care. The number of early (first and second) stages is detected in less than 10 % of cases; approximately 58 % of diagnoses correspond to stage 4 of the disease [5–7]. Morphologic verification with the diagnosis of primary hepatic cancer was performed in only 48.9–56.4 % of cases [6, 7]. Thus, the relevance of the treatment of hepatocellular cancer (HCC) is determined by a significant increase in the incidence rate, its high prevalence among primary malignant hepatic tumors, aggressive course of the disease and low figures of 5-year survival. Late disease diagnosis determines insufficient coverage of this group of patients with specialized treatment [8].

MATERIAL AND METHODS

To analyze the immediate results of treatment, medical records of patients diagnosed with primary hepatic cancer, protocols of surgical interventions, and the results of pathohistological examination were studied. From 2015 to 2022, 48 patients with HCC were treated at the Kuzbass Regional Hepatological Centre (KRHC). There were 32 males (66.7 %) and 16 females (33.3 %); the mean age was 59.9 ± 2.6 years. HCC was diagnosed by pathohistological examination. Thus, males were predominant among those who suffered from HCC.

All the patients admitted to the KRHC were examined according to the clinical guidelines of the Russian Ministry of Health and the standard of care, which included both "routine" methods of blood examination (Complete Blood Count and Biochemical Blood Test) and special methods, which were considered to be the main ones in diagnostics – an alpha-fetoprotein (AFP) test, radiological methods (abdominal ultrasound, multislice-CT of the abdomen, MRI of the abdomen). Liver fibroelastometry was also performed in all cases. In all patients diagnosed with cirrhosis, the functional class of hepatic function according to Child – Pugh was determined, the MELD model (Model for End-Stage Liver Disease) was investigated, and liver fibroelastometry was performed. Patients with diagnosed liver neoplasms less than 1 cm, unavailable for biopsy under ultrasound control, were subjected to dynamic follow-up with subsequent monthly follow-up examinations and ultrasound (U/S); in case of growth, they were subjected to histological verification by a fine needle aspiration (FNA) of the liver under U/S. In all other cases, histological verification was performed by needle biopsy under ultrasound control or by diagnostic laparoscopy. 39 patients underwent FNA of the liver under U/S; 9 patients underwent diagnostic laparoscopy and biopsy.

In the course of diagnostics, we used clarifying methods and rating scales of the patient's functional state, the state of liver function, studied the correlation of the actual liver volume with the criteria for the application of resection method and transplantation technologies, which allowed us to choose the most rational method of treatment.

HCC patients were staged according to the Barcelona classification of HCC (BCLC, Barcelona – Clinic Liver Cancer), which is the most frequently used classification, taking into account the prevalence of the tumor process, functional state of the liver, objective condition of the patient and the expected effectiveness of treatment. However, BCLC has certain disadvantages associated with difficulties in the allocation of patients to stage B: only transarterial chemoembolization (TACE) is recommended in case of ambiguous criteria and recommended disease management [1].

ECOG status (Eastern Cooperative Oncology Group), assessment of liver function in patients with liver cirrhosis combined with HCC according to the following criteria: Child-Pugh classification, MELD, Milan criteria; TNM (Tumor, Nodus, Metastasis) staging was also performed when planning surgical treatment.

Such approach allowed to differentiate the strategy of surgical treatment according to the degree of possible risk of post-resection hepatic failure development, and where it was estimated as statistically significant, we applied stage surgical treatment, the essence of which consists in the formation of vicarious hypertrophy of the planned liver remnant after resection by reduction of portal blood flow of the resected liver lobe.

As a result of staging according to the Barcelona classification of HCC, the patients were distributed as follows: BCLC 0/A – 8 patients, BCLC B – 7, BCLC C – 32, BCLC D – 1.

As a result of TNM staging, the patients were distributed as follows: T1bN0M0 – 2 patients, T2N0M0 – 5, T2N1M1 – 2, T2NxM1 – 4, T3N0M0 – 5, T3N1M1 – 16, T4N1M1 – 14.

Liver resections: Atypical (3 liver resections – by laparoscopic method) – 5; anatomical (right-sided hemihepatectomy) – 3; ALLPS (Associated Liver Partition and Portal vein ligation for staged hepatectomy) – 1; laparoscopic vascular isolation of portal vein – 2; staged extended right-sided hemihepatectomy – 2; liver transplantation – 1.

In 3 cases the operation was performed in the volume of atypical liver resection by laparoscopic method, the method of instrumental liver clamping was used for parenchyma separation. Blood loss during surgery

ranged from 50 to 250 ml, with an average of 150 ± 58 ml (Fig. 1–3).

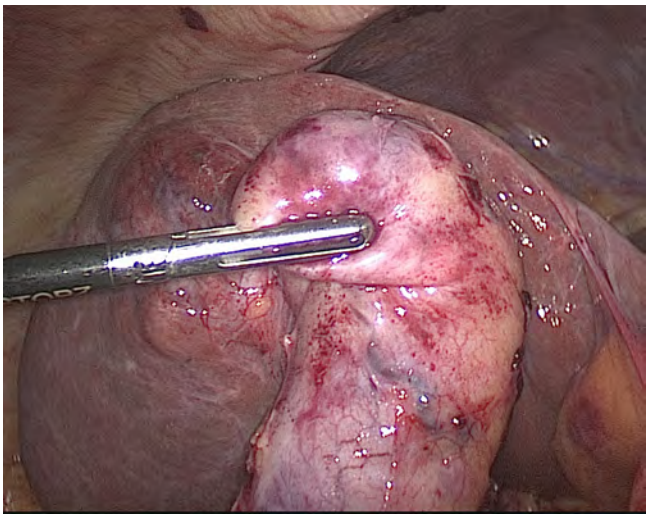

FIG. 1.
Tumor of the right lobe of the liver with gallbladder invasion

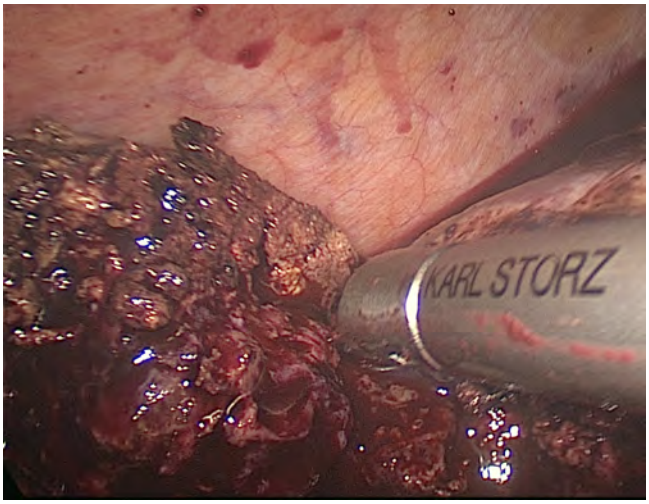

FIG. 2.
Stage of laparoscopic atypical resection of a liver area with a tumor

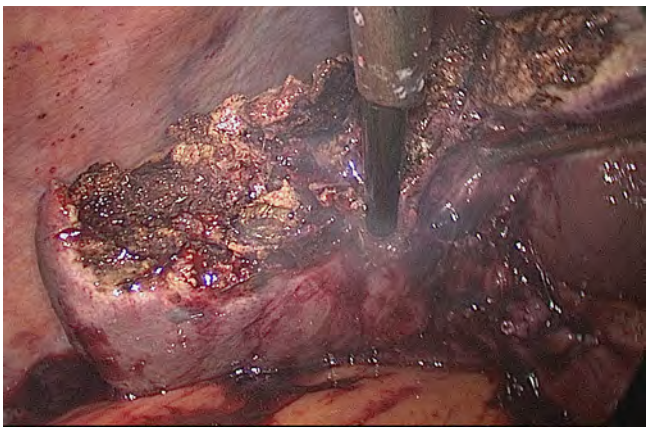

FIG. 3.
The stage of final hemostasis after laparoscopic atypical liver resection

During laparoscopic liver resection the ports were inserted in standard points for the resection method; in order to reduce surgical blood loss, we used the method of laparoscopic hemostasis and a device for its implementation (patent for an invention No. 2772189) (Fig. 4), which allowed to minimize blood loss and thus to achieve optimal immediate results of surgical treatment – absence of general and local complications in this group of patients.

In all cases of the resection method, surgical ultrasound was performed during the intervention in order to accurately localize the focal liver lesion, which allowed for precision liver resection.

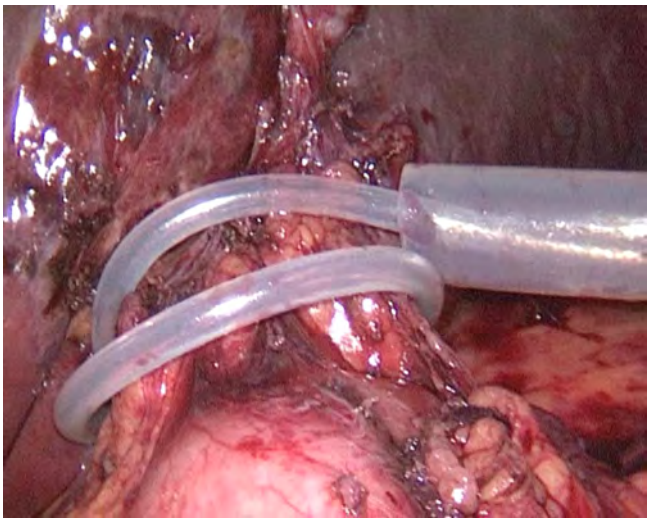

FIG. 4.
Device for temporary hemostasis in laparoscopic liver resections

In 2 cases due to technical impossibility to perform laparoscopic liver resection the intervention was performed through laparatomy access. The liver digitoclasia method was used to separate the parenchyma; blood loss during surgery ranged from 50 to 300 ml, averaging 175 ± 125 ml.

In 3 cases, right-sided hemihepatectomy was performed, when the risk of post-resection hepatic failure in case of extensive liver resections was estimated as minimal; blood loss during surgery ranged from 300 to 1,500 ml, with an average of 766 ± 371 ml (Fig. 5).

The necessary conditions for this were functional class A of concomitant cirrhosis (Child – Pugh) and sufficient volume of liver parenchyma remaining after resection.

In 2 cases radical surgical treatment of HCC was performed by a stage method, the essence of which was the formation of vicarious hypertrophy of the remaining left lobe of the liver by laparoscopic vascular isolation of the portal vein of the liver. Subsequently, after a compensatory pause and formation of vicarious hypertrophy confirmed by CT with liver

volumetry, we performed a radical stage of surgical treatment – extended right-sided hemihepatectomy. Blood loss during surgery ranged from 500 to 1,000 ml, with an average of 750 ± 250 ml (Fig. 6).

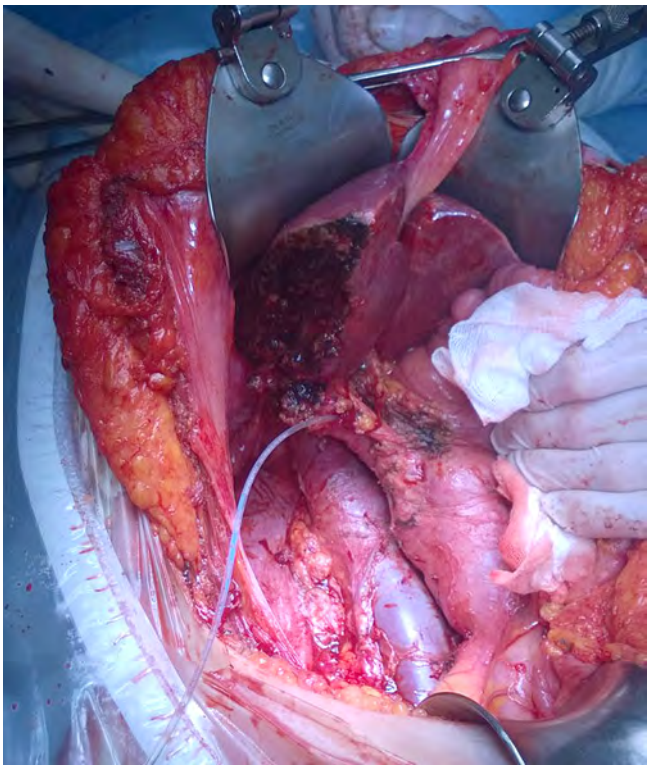

FIG. 5.
The final view of the surgical field after right-sided hemihepatectomy

The 1st stage of liver resection was performed in one of the patients in order to clarify the clinical site for ALLPS technique and early formation of vicarious hypertrophy of the remaining liver stump; subsequently, the development of the Multiple Organ Dysfunction Syndrome (MODS) in the patient and his death from the developed complication were noted. Blood loss during surgery amounted to 500 ml.

In case of impossibility to use the resection method in HCC (invasion of the hepatic vein confluence area), orthotopic liver transplantation was performed in one case, uncomplicated course of the postoperative period was stated, and the patient was discharged on the 12th day of the postoperative period.

IMMEDIATE RESULTS

Twelve patients underwent radical surgery. Postoperative mortality was 1 (8.3 %) case and total mortality was 1 (2.1 %) case.

Complications developed in 5 (41.7 %) cases and had Clavien – Dindo I-3, IIIb-1 and IVb-1 grading of severity.

DISCUSSION

As a result of generalization of the 7-year experience of rendering specialized medical care in the profile section to the patients with hepatocellular cancer it is necessary to state the shortcomings of early disease diagnostics, which is evidenced by a relatively small number of performed radical surgical interventions due to the diagnosis of predominantly advanced forms of the disease in 36 (75 %) cases.

The selection of patients for surgery should be considered problematic due to significant risks of hepatic failure after liver resection, taking into account the fact that HCC

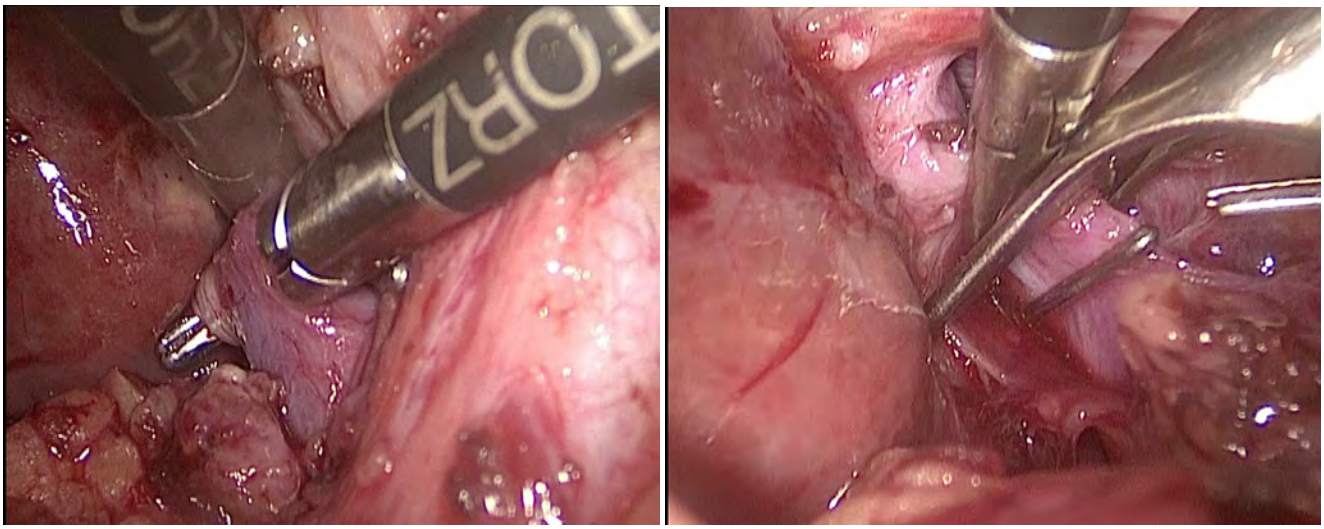

FIG. 6.
Stages of laparoscopic vascular isolation of portal blood flow in the right hepatic vein

relatively rarely develops in an intact organ, and more often – on the background of pre-existing cirrhosis. To solve the problem of post-resection hepatic failure, a group of patients with indications for a staged method of surgical treatment should be singled out.

Laparoscopic liver resections with the use of blood-saving techniques, as well as methods of staged surgical treatment, allowing to effectively prevent the development of severe forms of post-resection hepatic failure, should be considered promising in the treatment of HCC.

CONCLUSION

The degree of risk of severe post-resection hepatic failure should be considered one of the main criteria in choosing a strategy for radical surgical treatment.

A new surgical approach (laparoscopic vascular isolation of portal blood flow) in operations to create vicarious hypertrophy of the group of two-stage surgical treatment in resection surgery of primary hepatic cancer, as well as the use of new methods of precision hemostasis during laparoscopic liver resections (method of temporary hemostasis during laparoscopic liver resections and a device for its implementation) allow to improve the immediate results of treatment of patients by reducing the number of severe forms of post-resection hepatic cancer, as well as the use of new methods of hemostasis during laparoscopic liver resections.

Resection techniques for HCC patients should be performed in a timely manner; laparoscopic liver resections should be considered a priority where possible [9].

Staged surgical treatment of HCC allows to perform extensive liver resections, bypassing the development of severe post-resection hepatic failure [10].

The use of ALPPS in the treatment of HCC is limited by the risks of unfavorable outcomes due to the development of severe postoperative complications [11].

Unsatisfactory results of HCC treatment require improvement of early diagnosis approaches.

Financing

The study was conducted without sponsorship.

Ethical review

The study complies with the principles of the Declaration of Helsinki (1964) and has been reviewed by the local ethical committee (meeting minutes No. 132B dated 01.11.2022).

Conflict of interest

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

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OPHTALMOLOGY

CASE REPORT OF SCLEROCHOROIDAL CALCIFICATION

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ABSTRACT

Background. *Sclerochoroidal calcification is an idiopathic rare benign lesion of the sclera or choroid characterized by histological deposition of calcium pyrophosphate. Taking into consideration its similar clinical manifestations with other diseases of the sclera, the most dangerous of which are malignant, timely verification of the diagnosis with the appointment of a further observation period is important.*
The aim. *The description of a clinical case of sclerochoroidal calcification to improve the efficiency of disease detection through the use of multimodal diagnostics.*
Material and methods. *A 62-year-old patient with complaints of "bright flashes" in her left eye for the past few months, who underwent a standard complex of ophthalmological examinations, supplemented according to indications by optical coherence tomography of peripapillary nerve fibers, macular zone, B-scan, Dopplerography in color Doppler mapping mode. Auxiliary diagnostic methods were magnetic resonance imaging of the orbits and extraocular muscles, computed tomography of the orbits and a biochemical blood test.*
Results. *Considering the anamnesis, the absence of progression of complaints, the data of instrumental diagnostic methods, the absence of pathological blood flow in the area of both eyes formations, the correct diagnosis is most likely to be sclerochoroidal calcification of both eyes, despite the difficulties of the diagnostic process, which consisted in the absence of visualization of foci during ophthalmoscopy.*
Conclusion. *Sclerochoroidal calcification is of interest to practicing ophthalmologists due to the difficulties of diagnostic search and differential diagnosis with malignant neoplasms. Modern medicine has a sufficient set of instrumental and laboratory research methods for making an accurate diagnosis.*

Key words: *sclerochoroidal calcification, mineral metabolism, computed tomography*

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КЛИНИЧЕСКИЙ СЛУЧАЙ СКЛЕРОХОРИОИДАЛЬНОЙ КАЛЬЦИФИКАЦИИ

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РЕЗЮМЕ

Обоснование. *Склерохориоидальная кальцификация – это идиопатическое редкое доброкачественное образование склеры или сосудистой оболочки, характеризующееся гистологическим отложением пирофосфата кальция. Учитывая схожие клинические проявления с другими заболеваниями склеры, большую опасность из которых представляют злокачественные, имеет значение своевременная верификация диагноза с назначением дальнейшего периода наблюдения.*

Цель. *Описание клинического случая склерохориоидальной кальцификации для повышения эффективности выявления заболевания путём применения мультимодальной диагностики.*

Материал и методы. *Пациентка 62 лет с жалобами на «яркие вспышки» перед левым глазом последние несколько месяцев, которой был проведён стандартный комплекс офтальмологического обследования, дополненный по показаниям оптической когерентной томографией перипапиллярных нервных волокон, макулярной зоны, В-сканированием, допплерографией в режиме цветного доплеровского картирования. Вспомогательными методами диагностики являлись магнитно-резонансная томография орбит и экстраокулярных мышц, компьютерная томография орбит и биохимический анализ крови.*

Результаты. *Учитывая анамнез, отсутствие прогрессии жалоб, данные инструментальных методов диагностики, отсутствие патологического кровотока в области образований обоих глаз правомерным диагнозом, вероятнее всего, будет склерохориоидальная кальцификация глаз, несмотря на трудности диагностического процесса, которые заключались в отсутствии визуализации очагов при офтальмоскопии.*

Выводы. *Склерохориоидальная кальцификация представляет интерес для практикующих офтальмологов ввиду трудностей диагностического поиска и дифференциальной диагностики со злокачественными новообразованиями. Современная медицина располагает достаточным набором инструментальных и лабораторных методов исследования для постановки точного диагноза.*

Ключевые слова: *склерохориоидальная кальцификация, минеральный обмен, компьютерная томография*

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OBJECTIVES

Sclerochoroidal calcification (SCC) is an idiopathic rare benign lesion of the sclera or choroid characterized by histological deposition of calcium pyrophosphate. Morphologically, SCC is visualized as whitish-yellow foci in the thickness of the sclera, usually located at the medial periphery in the superior temporal and superior nasal quadrants, with a symmetrical location in both eyes. Often, diagnostic difficulties lie in the similar clinical manifestations of diseases such as choroidal osteoma, choroidal nevus, metastatic choroidal lesion, choroidal hemangioma, and retinal astrocytoma [1–4]. According to a study by C.L. Shields, the average age at the time of diagnosis was 69 years. It was most common in Caucasian women, with unilateral and bilateral eye lesions occurring in almost equal percentages of cases (48 and 52 % respectively). Patients usually had no complaints, and the detected calcium deposition did not lead to a decrease or loss of vision acuity, changes in the size of the focus, decalcification and associated subretinal fluid, and neovascularization for 4 years of follow-up [1, 3–6]. According to the results of foreign studies, optical coherence tomography (OCT) with an extended depth imaging module showed that the lesion foci are located in the sclera rather than being of scleral and choroidal origin, as previously believed [7–9]. Among Russian scientists, A.S. Stoyukhina confirms the fact of scleral origin of SCC in the course of studies on high-resolution OCT data with the study of deep tissues using the scan averaging function [2, 10]. In rare unique cases described by foreign scientists, SCC is accompanied by neovascular membrane formation with subsequent need for treatment with angiogenesis inhibitors [11–13]. A case of SCC with detected metabolic disorders in a 70-year-old patient with chronic renal failure and a follow-up period of 7 months is described. The results of the follow-up showed no growth of visualized foci [14].

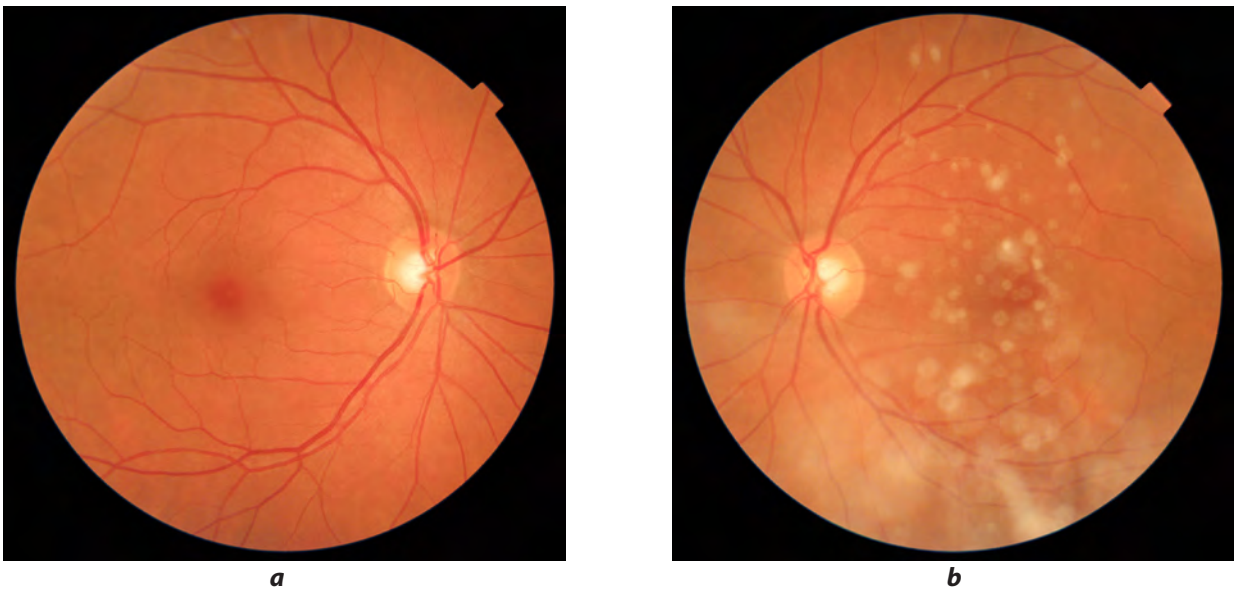
THE AIM

Description of a case report of SCC to improve the detection of sclerochoroidal calcification by using multimodal diagnosis.

MATERIALS AND METHODS

A 62-year-old female patient came to the Orenburg Branch of the Eye Microsurgery Institution in October 2021 with complaints of "bright flashes" in her left eye that had been bothering her for several months.

Medical history: she was registered with iris nevus of the left eye at the place of residence. At her follow-up examination in April 2021, the patient underwent ultrasound of the orbit and duplex ultrasound of the ophthalmic artery territory using the GE Logiq e machine. Along the posterior contour of the left eyeball at the edge of the optic nerve a focal choroideal lesion was detected with a length of 4.2 mm and a prominence of 2.1 mm of hyperechogenic homogeneous structure, with clear and irregular contours. Within the focal area there were single arterial and venous vessels with linear blood flow velocity (LBFV) in arteries 3.1 cm/s, RI – 0.50, in veins – up to 8.3 cm/s. The patient underwent the control examination in October 2021. The results with regard to the foci in the left eye showed no negative dynamics. When performing duplex ultrasound of the right eye in the right orbit, a focus of similar characteristics was found, 4.6 × 1.5 mm in size, with LBFV in arteries up to 16.1 cm/s, in veins – up to 7.9 cm/s. The ophthalmic artery (OA) hemodynamics was asymmetric, with some hemodynamic predominance in the central retinal artery (CRA) in the right orbit, in OA – in the left orbit – sufficient with markedly increased tone.

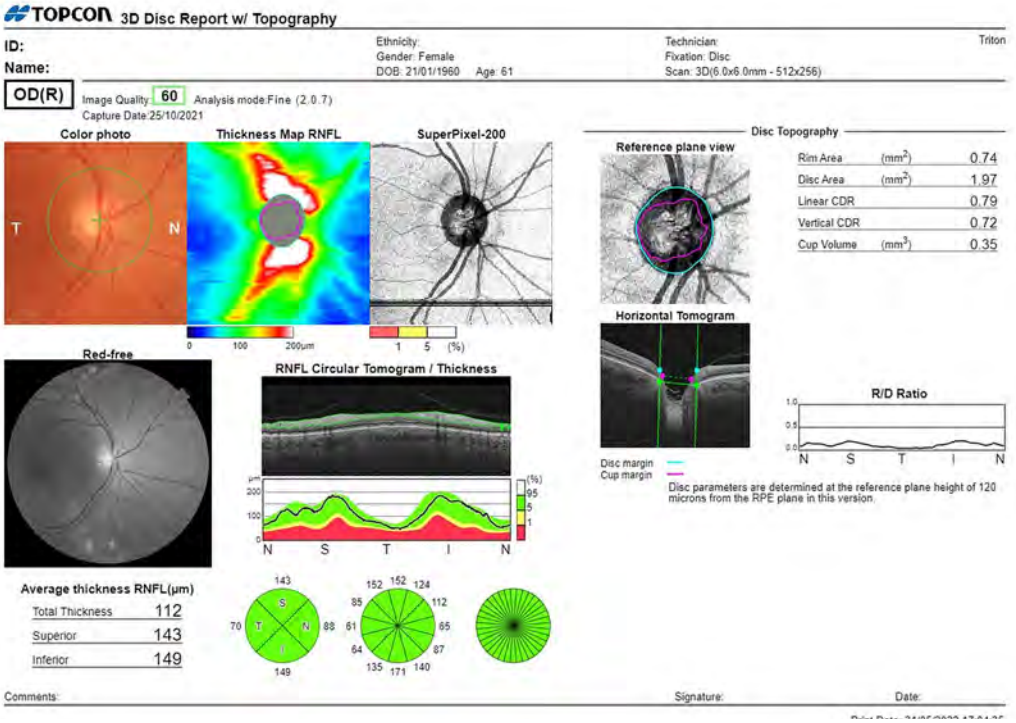


a ***b***

FIG. 1.
Ophthalmoscopic picture of the fundus: ***a*** *– right eye;* ***b*** *– left eye*

When the patient came to the Orenburg Branch of the Eye Microsurgery Institution, the visual acuity of the right eye was 0.8 with hyperopic complex correction of best corrected visual acuity (BCVA) to 1.0, and the left eye was 0.7 with hyperopic complex correction to 1.0.

Ophthalmoscopy: right eye without features, left eye – flat iris nevus at 8 o'clock position, pseudoexfoliative syndrome, vitreous degeneration by the type of asteroid hyalopathy (Fig. 1). Examination with the Goldmann three-mirror lens in the right eye from 7 to 9 o'clock postitions, retinoschisis with the necessity for retinal laser photocoagulation was detected in the extreme periphery; the extreme periphery of the left eye was unremarkable.



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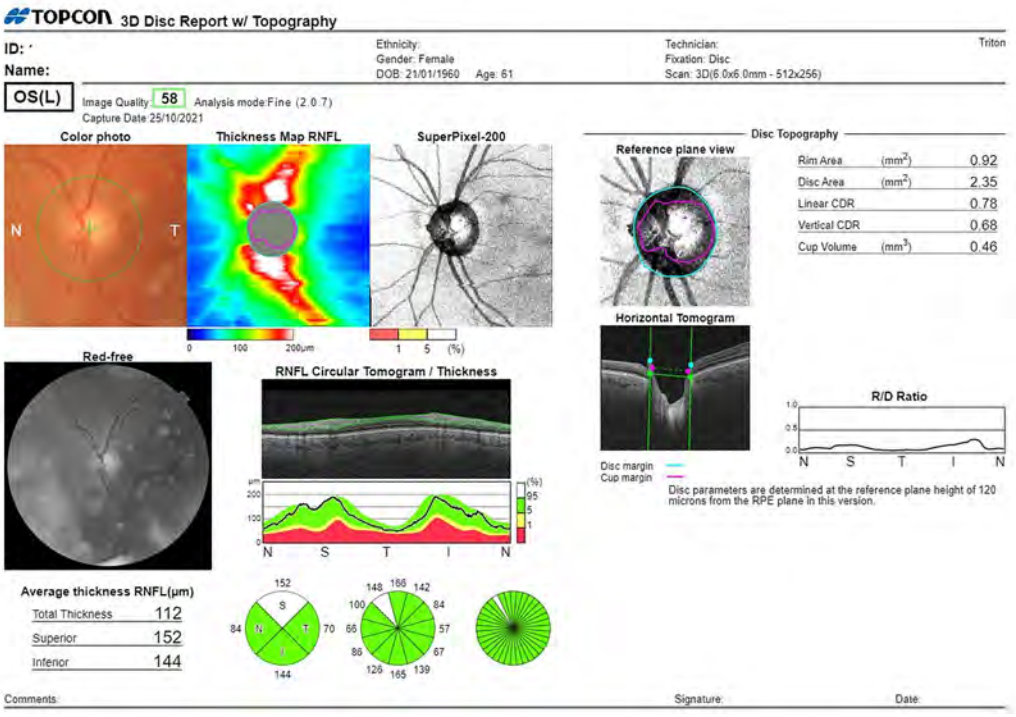


b

FIG. 2.
OCT of peripapillary nerve fibers: ***a*** *– right eye;* ***b*** *– left eye*

The patient underwent OCT of peripapillary nerve fibres (Topcon DRI OCT Triton), macular zone (Spectralis HRA + OCT (Heidelberg), B-scan (Accutome B-scan Plus) and Doppler ultrasound (GE Logiq e) in colour Doppler imaging (CDI) mode.

According to OCT data of peripapillary nerve fibres, the thickness of peripapillary nerve fibres of the right eye is within normal limits (Fig. 2a), thickened in the upper segment of the left eye (Fig. 2b).

According to OCT data of the macular zone of both eyes, the retinal thickness is within normal limits, the pigment epithelium is preserved (Fig. 3).

The B-scan of the right eye (Fig. 4a) revealed single threads of low echogenicity in the vitreous body. A high echogenicity inclusion with Hmax = 0.81 mm, 2.17 mm in length is scanned in the middle periphery in the membrane thickness at 12 o'clock meridian. According to the B-scan of the left eye (Fig. 4b), there are many clumps of high echoicity in the vitreous body (the "golden rain" type of degeneration). At 12–1:30 meridian in the membrane thickness at the middle periphery a high echoicity inclusion giving a shadow with Hmax = 0.92 mm, extent 2.33 mm, is scanned.

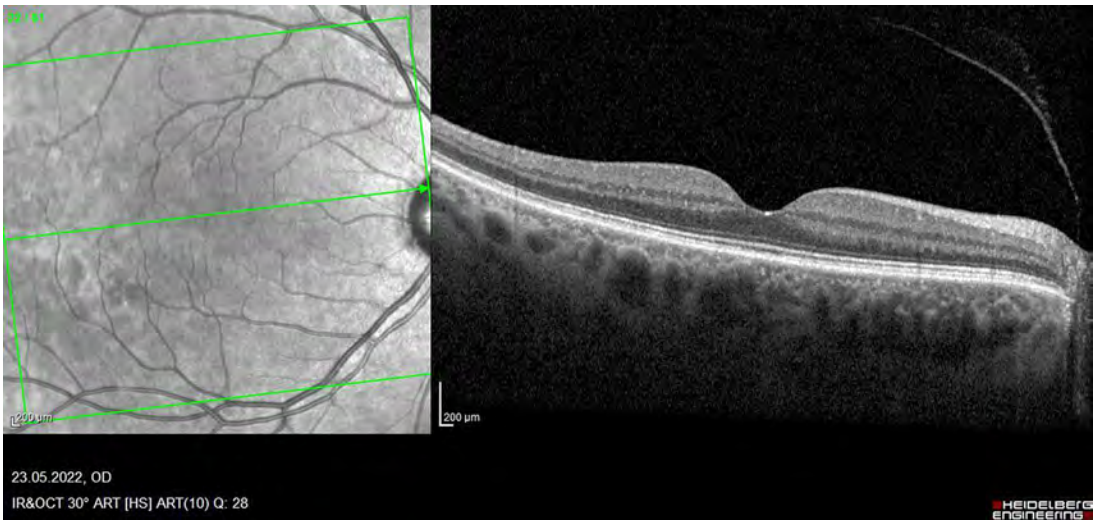
According to the Doppler ultrasound of both eyes (Fig. 5), a high echogenicity lesion with acoustic shadow (3.9 × 1.9 mm – right eye, 3.9 × 1.1 mm –left eye) with no signs of blood flow in the CDI mode was scanned in the upper anterior segment in the membrane thickness (Fig. 6).

At the follow-up examination in January 2022 (4 months later), the size of the lesion in the right eye had increased to 4.3 × 2.1 mm, in the left eye – no negative dynamics, still without signs of blood flow.

The patient was recommended to consult an endocrinologist and a biochemical blood test was scheduled to search for mineral metabolism disorders (Table 1).

According to the examination results, no mineral metabolism disorders were found, and no endocrinological pathology was detected.

In January 2022, the patient underwent focal retinal laser photocoagulation of the retinoschisis zone at 7–9 o'clock of the right eye at the Orenburg Branch of the Eye Micro-



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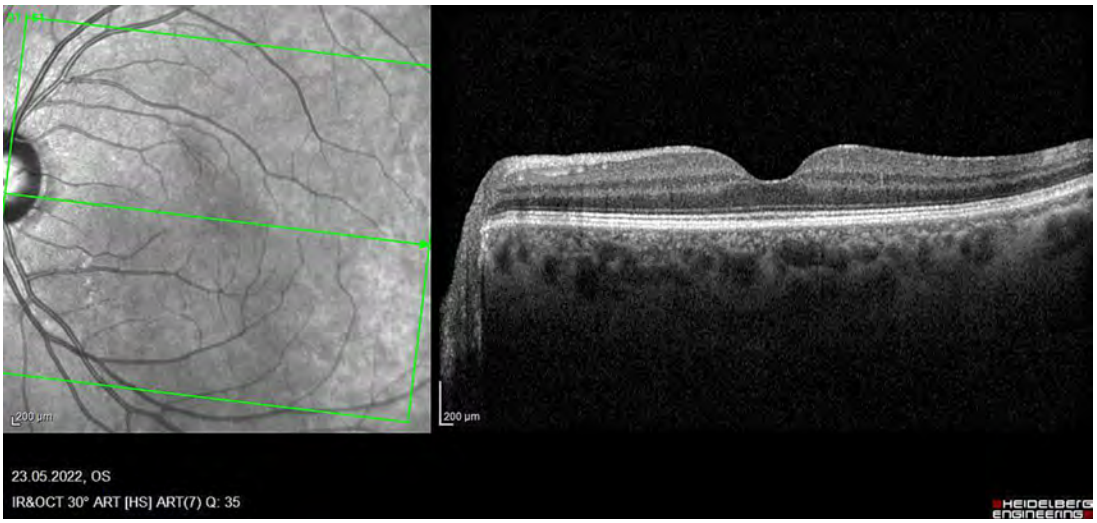
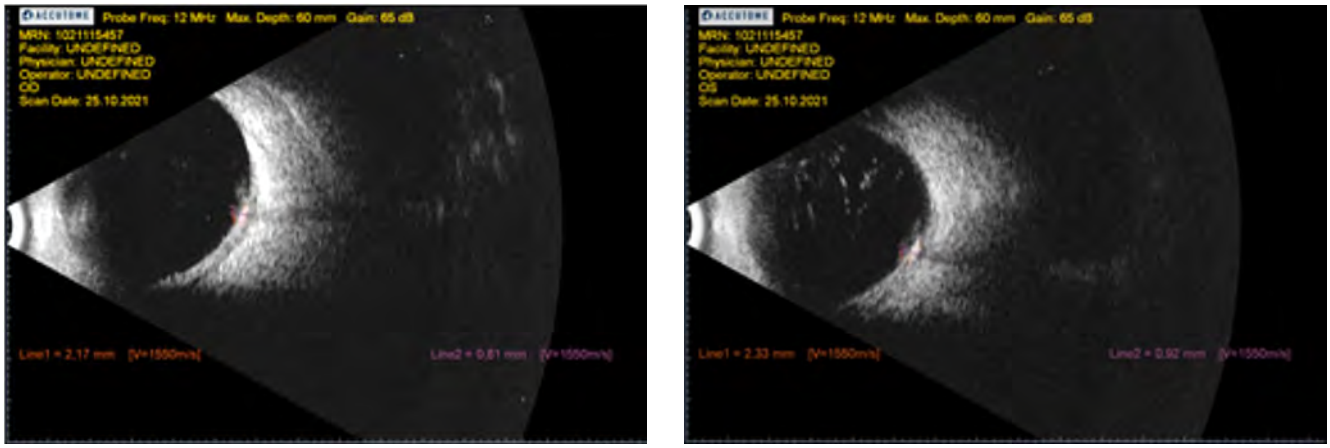


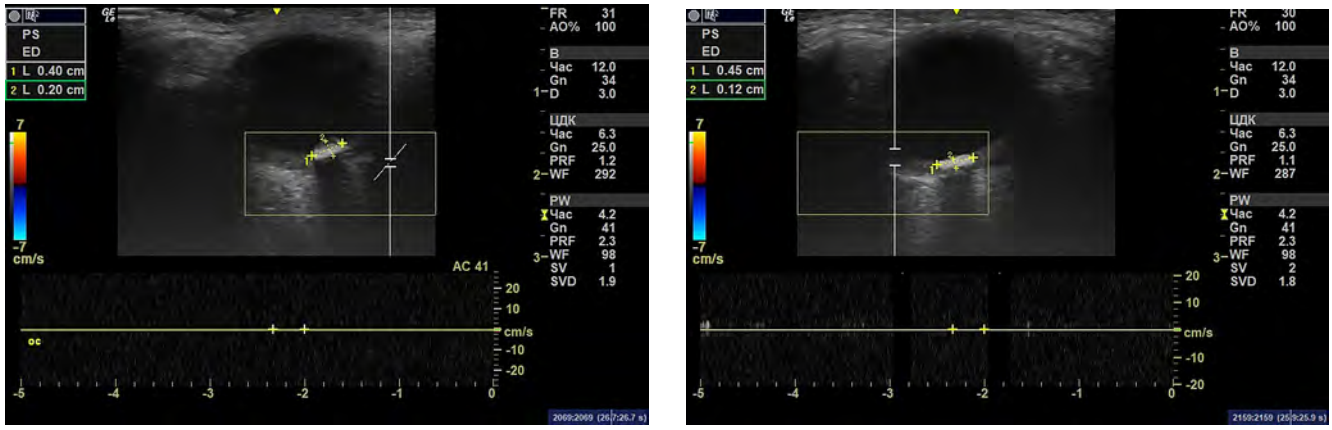
b

FIG. 3.
*OCT of the macula: **а** – right eye; **б** – left eye*



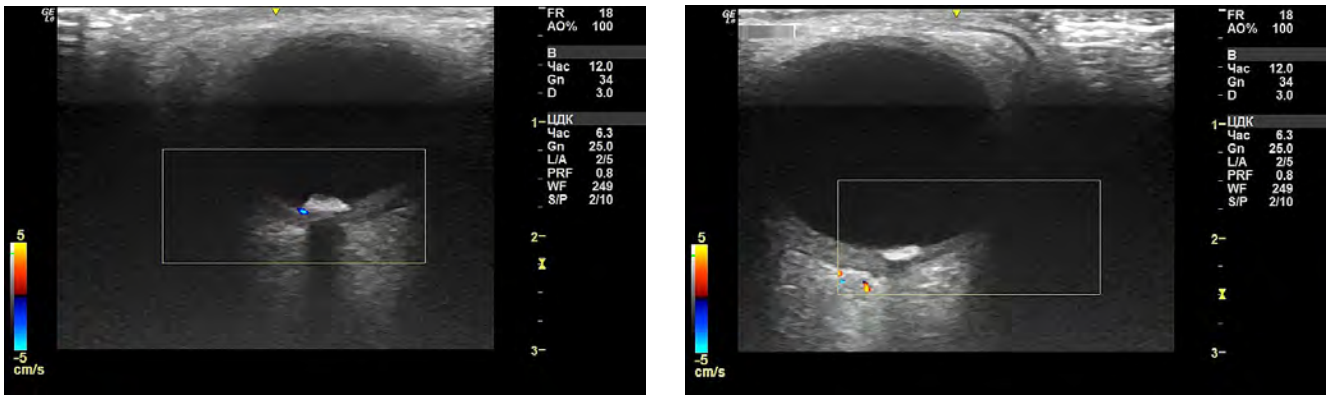
a ***b***

FIG. 4.
B-scan: ***a*** *– right eye;* ***б*** *– left eye*



a ***b***

FIG. 5.
Dopplerography: ***a*** *– right eye;* ***б*** *– left eye*



a ***b***

FIG. 6.
Dopplerography in the color duplex mapping mode: ***a*** *– right eye;* ***б*** *– left eye*

surgery Institution. She was also recommended to undergo computed tomography (CT) of the orbits at her place of residence.

The patient returned for a follow-up examination in May 2022. She presented no complaints. At the time of examination, the BCVA of the right eye was 1.0, the BCVA of the left eye was 0.7 with previous correction to 1.0. Due to the impossibility to perform the prescribed CT examination at her place of residence, the patient provided magnetic resonance imaging (MRI) data of the orbits and extraocular muscles on a Siemens Magnetom Essenza 1.5 T in T1 Vibe Fs Ttra mode with a slice thickness of 1 mm.

TABLE 1

THE RESULTS OF A BIOCHEMICAL ANALYSIS OF THE BLOOD OF THE EXAMINED PATIENT, INDICATING THE REFERENCE VALUES

Study	Result	Unit	Reference values
Calcium	2.37	mmol/L	2.20–2.55
Potassium	4.1	mmol/L	3.5–5.1
Sodium	141	mmol/L	136–145
Chlorine	106	mmol/L	101–110
Magnesium	0.88	mmol/L	0.66–1.07
Organic phosphorus	1.16	mmol/L	0.74–1.52
Parathyroid hormone	6.8	pmol/L	1.45–10.41
Glomerular filtration CKD-EPI Creatinine	95	ml/min/1.7 m^2	> 60
25-OH vitamin D	32	ng/ml	< 10 ng/ml – severe deficiency; < 20 ng/ml – deficiency; 20–30 ng/ml – insufficiency; 30–100 ng/ml – adequate level; > 150 ng/ml – possible toxic effect

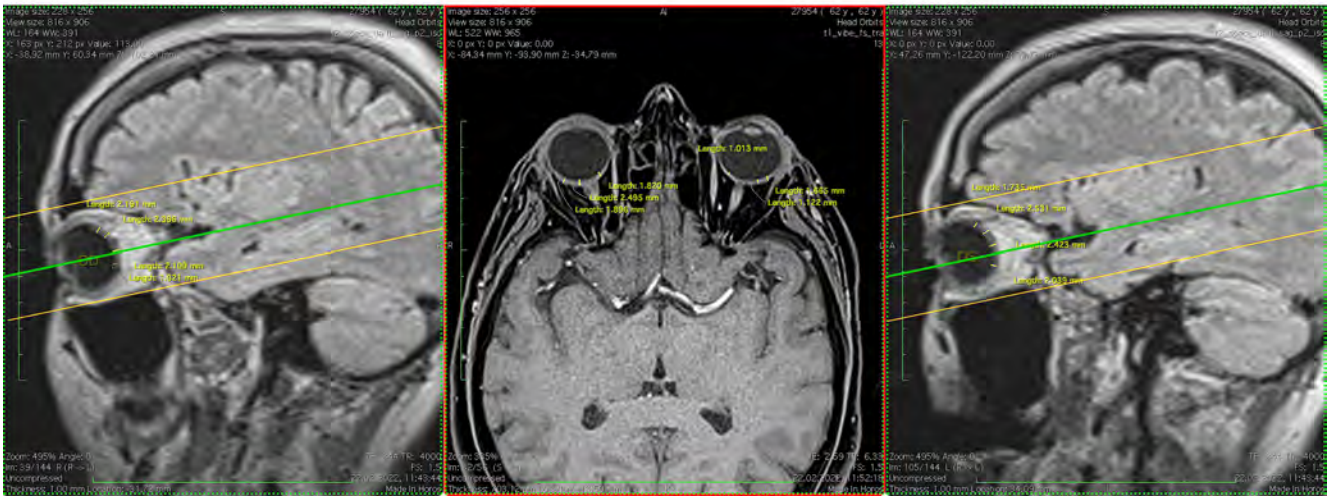


FIG. 7.

MRI of thickening of the sclera

MRI revealed MR signs of uneven thickness of the sclera of both eyeballs, inhomogeneous structure (Fig. 7) with point-like inclusions (Fig. 8).

As a final method of examination for the most accurate visualization of calcification foci in the sclera, CT of the orbits on a Philips Mx-16 device in bone and brain modes with a slice thickness of 1 mm in sagittal, frontal and vertical planes was chosen.

According to the CT findings, almost symmetrical areas of calcification located at 11 o'clock, at a distance of about 6 mm from the optic disc (OD) of the right eye are determined in the posterior hemispheres of the orbits and the sclera structure (Fig. 9) the size of the calcificates is 2.3 × 4 × 1.6 mm.

A focus measuring 3.8 × 3.7 × 1.5 mm was detected at a distance of 6.5 mm from the OD of the left eye during 12 o'clock CT in the posterior hemispheres of the orbits of the left eye (Fig. 10).

These calcificates have a maximum density of about +200 HU, repeat the course of the sclera, without bulging beyond its outer contour, with minimal bulging towards the chorioidea on both sides, their contours are clear, slightly irregular on the left side. A questionable, but not excluded, forming small clumpy calcificate about 1 mm in diameter was found at the border of the anterior and posterior OS hemispheres at 3 o'clock. There were no signs of other areas of pathological density in the sclerae.

On the next Doppler ultrasound in May 2022, the size of the lesion in the right eye was negative, and in the left eye it was 4.3 × 5 mm with no signs of blood flow.

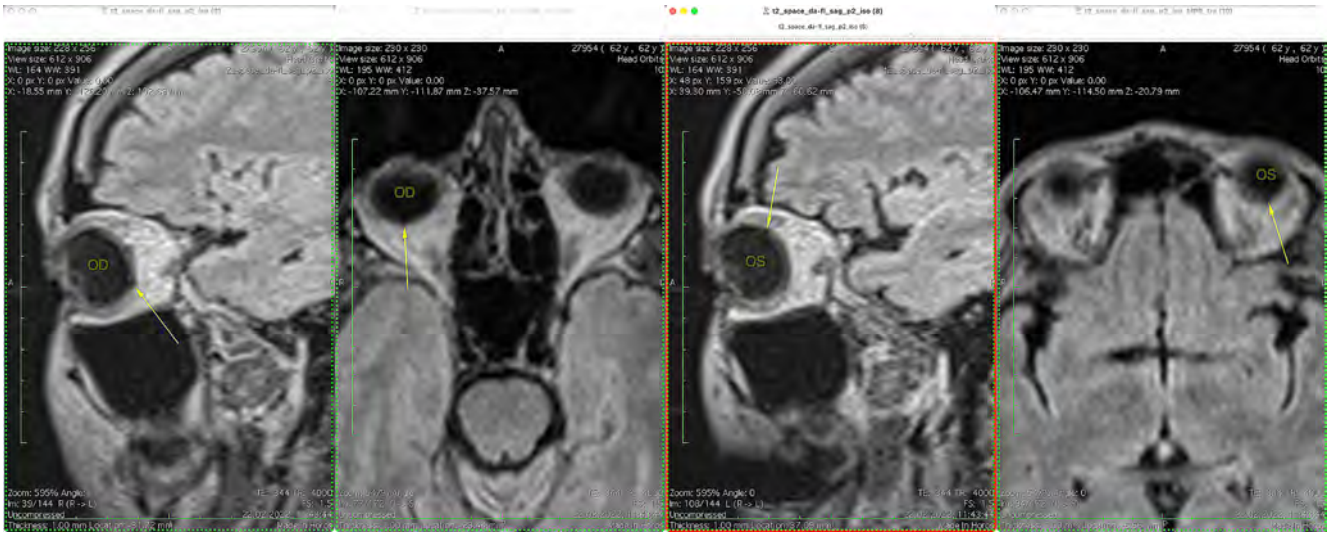


FIG. 8.
MRI of inclusions suspicious of calcifications

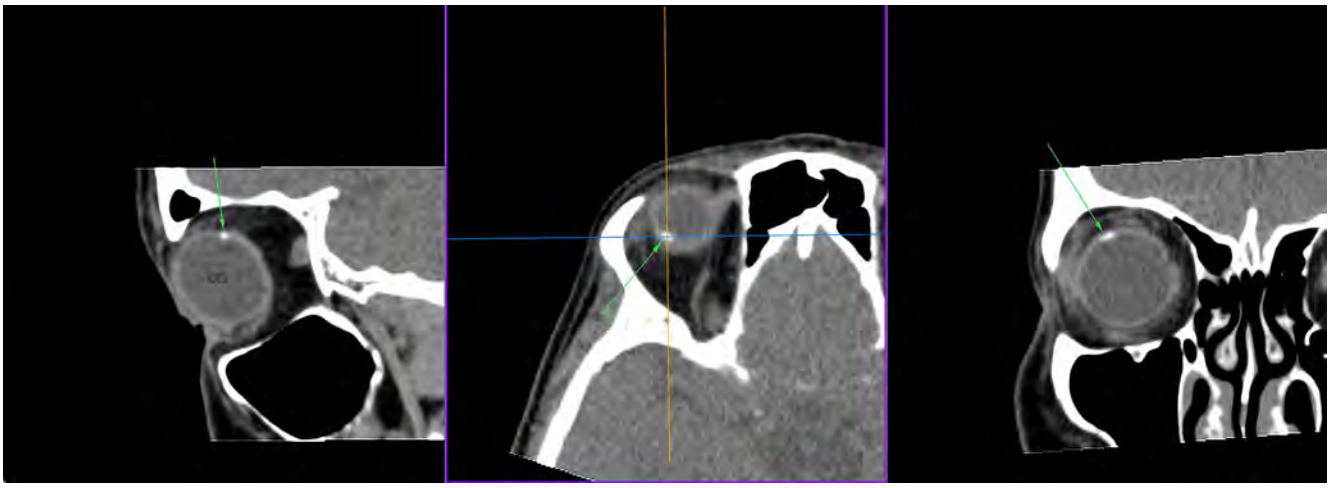


FIG. 9.
CT of calcifications in the structure of the sclera of the right eye

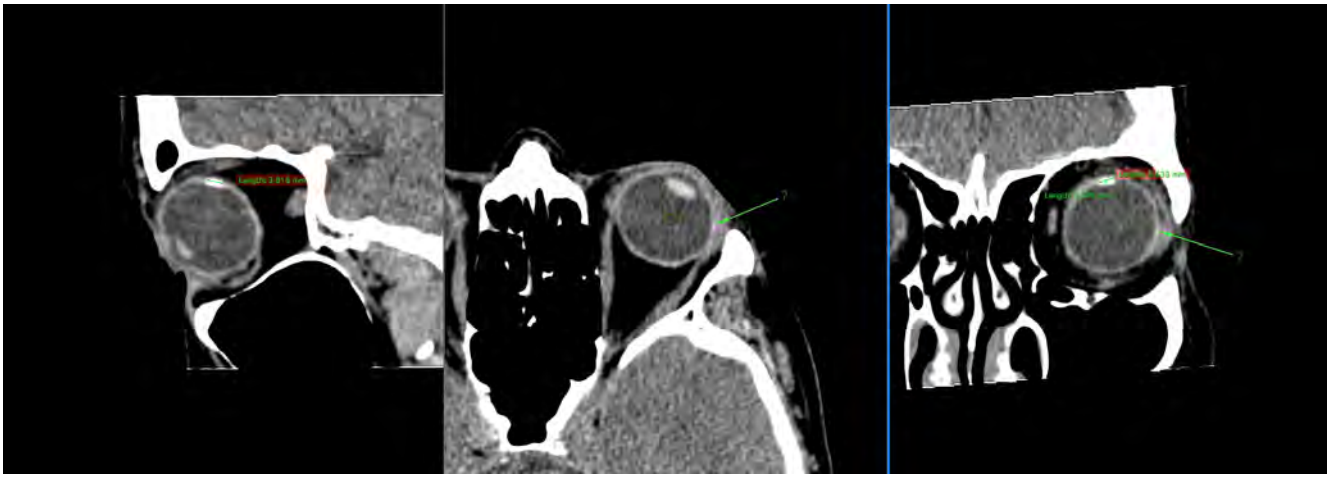


FIG. 10.
CT of calcifications in the structure of the sclera of the left eye

RESULTS AND DISCUSSION

Considering the anamnesis, the absence of progression of complaints, the data of instrumental diagnostic methods, the absence of pathological blood flow in lesion areas of both eyes according to Doppler ultrasound in CDI mode, the correct diagnosis is most likely to be sclerochoroidal calcification of both eyes, despite the difficulties of the diagnostic process, which consisted in the absence of visualization of foci during ophthalmoscopy. Taking into account the follow-up period of 9 months and the growth of the lesions by 0.4 × 0.2 mm and 0.4 × 3.9 mm according to Doppler ultrasound, the patient was recommended dynamic follow-up in 3 and 6 months with control biochemical blood test. Thanks to the above-described algorithms of diagnostic search of Russian and foreign colleagues, today the diagnosis of SCC for ophthalmologists together with CT-diagnosticians is a feasible task, which is confirmed by the result of the work done.

CONCLUSION

SCC is an idiopathic benign disease of the sclera that is difficult to diagnose. Diagnosis is made on the basis of patient's medical history, instrumental and laboratory tests. Given the uniqueness of the disease, as well as the difficulties in diagnosis due to its similarity to other malignant diseases of the sclera and vasculature, the correct diagnosis, determination of management methods, prescription of appropriate treatment and establishment of a follow-up period are of great importance. Modern medicine has a sufficient set of instrumental and laboratory methods of examination to make an accurate diagnosis of patients with SCC.

Conflict of interest

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

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DENTISTRY

FUNCTIONAL ACTIVITY OF THE ORAL ENDOTHELIUM IN PERSONS WITH CHRONIC PERIODONTITIS DURING TREATMENT WITH PLASMOLIFTING

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ABSTRACT

Chronic periodontitis as an osteoimmune disease of the oral cavity is accompanied by a change in the functional activity of endotheliocytes. Moreover, abnormal vascularization exacerbates periodontal inflammation, as it promotes the transmigration of a larger number of immunocompetent cells, the influx of inflammatory mediators and cytokines.

The aim of our work *was to study the functional activity of the endothelium of the vessels of the oral cavity in persons suffering from chronic periodontitis in the treatment of plasmolifting.*

Materials and methods. *Under observation were 30 patients diagnosed with chronic generalized periodontitis of moderate severity at the age of 35 (32.50; 40.00) years, with no severe somatic pathology (main group). The comparison group included 20 people aged 38 (34.00; 45.00) years with no inflammatory diseases in the oral cavity. All patients underwent local anti-inflammatory therapy and sanitation of periodontal pockets, correction of occlusal contacts, curettage, plasma lifting. Oral fluid concentration of soluble adhesion molecules ICAM-1 and VCAM-1, endothelin-1, qualitative and quantitative composition of microflora were determined.*

Results. *After the treatment with plasmolifting, a noticeable relief of the activity of the inflammatory process was observed. In patients with chronic periodontitis, Porphyromonas gingivalis was found in 100 % of cases in a titer of 5.73 (4.9; 6.7) lg (gEq/sample), in 62.5 % – Prevotella intermedia in a titer of 4.5 (3.0; 5.5) lg (gEq/sample). Against the background of therapy, decrease of the occurrence of the microorganism and of the number of microorganisms was observed. The concentration of the soluble form of VCAM-1 in the oral fluid of patients with chronic periodontitis exceeded the values of the control group by 38.3 times (p = 0.000001), and ICAM-1 – by 18.1 times (p = 0.00001). Against the background of plasmolifting therapy, the level of the studied substances decreased, but exceeded the control values by 25.2 and 6.4 times, respectively. The content of endothelin in the oral fluid in patients with periodontitis exceeded the values of healthy individuals by 40.7 % (p = 0.003), during therapy its values decreased, but did not reach the level of healthy volunteers (p = 0.04).*

Key words: *endothelial dysfunction, chronic periodontitis, plasmolifting, cell adhesion molecules, endothelin-1*

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ФУНКЦИОНАЛЬНАЯ АКТИВНОСТЬ ЭНДОТЕЛИЯ ПОЛОСТИ РТА У ЛИЦ, СТРАДАЮЩИХ ХРОНИЧЕСКИМ ПАРОДОНТИТОМ, ПРИ ЛЕЧЕНИИ МЕТОДОМ ПЛАЗМОЛИФТИНГА

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РЕЗЮМЕ

Хронический пародонтит как остеоиммунное заболевание полости рта сопровождается изменением функциональной активности эндотелиоцитов. Причём аномальная васкуляризация усугубляет воспаление пародонта, так как способствует трансмиграции большего количества иммунокомпетентных клеток, притоку медиаторов воспаления и цитокинов.

***Целью нашей работы** явилось изучение функциональной активности эндотелия сосудов полости рта у лиц, страдающих хроническим пародонтитом, при лечении методом плазмолифтинга.*

***Материалы и методы**. Под наблюдением находилось 30 пациентов с диагностированным хроническим генерализованным пародонтитом средней степени тяжести (15 мужчин и 15 женщин) в возрасте 35 (32,50; 40,00) лет, с отсутствующей тяжёлой соматической патологией (основная группа). В группу сравнения были включены 20 человек, сопоставимые с основной группой по полу и возрасту, с отсутствием воспалительных заболеваний в полости рта. Всем больным проводилась местная противовоспалительная терапия и санация пародонтальных карманов, коррекция окклюзионных контактов, кюретаж, плазмолифтинг. В ротовой жидкости определяли концентрацию растворимых молекул адгезии ICAM-1 и VCAM-1, эндотелин-1, качественный и количественный состав микрофлоры.*

***Результаты**. После проведённого лечения плазмолифтингом наблюдалось заметное купирование активности воспалительного процесса. У пациентов с хроническим пародонтитом в 100 % случаев обнаружена Porphyromonas gingivalis в титре 5,73 (4,9; 6,7) lg (ГЭ/образец), у 62,5 % – Prevotella intermedia в титре 4,5 (3,0; 5,5) lg (ГЭ/образец). На фоне терапии наблюдалось снижение как встречаемости микроорганизма, так и количества микроорганизмов. Концентрация растворимой формы VCAM-1 в ротовой жидкости больных хроническим пародонтитом превышала уровень контрольной группы в 38,3 раза (p = 0,000001), а ICAM-1 – в 18,1 раза (p = 0,00001). На фоне терапии плазмолифтингом концентрация изучаемых веществ снижалась, но превышала значения контроля в 25,2 и 6,4 раза соответственно. Содержание эндотелина в ротовой жидкости у больных пародонтитом увеличивалось на 40,7 % (p = 0,003), а на фоне терапии снижалось, но не достигало уровня здоровых добровольцев (p = 0,04).*

***Ключевые слова:** дисфункция эндотелия, хронический пародонтит, плазмолифтинг, молекулы клеточной адгезии, эндотелин-1*

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Chronic periodontitis is an osteoimmune oral disease affecting the periodontal apparatus, caused by a disruption of the symbiotic relationship between the oral flora and the immune system of the host, characterized by successive periods of exacerbation followed by periods of remission, causing progressive tooth destruction and loss [1].

Leukocytes recruited into the focus of inflammation cause the development of secondary alteration and modulate the functional activity of endotheliocytes [2, 3].

The physiological function of the vascular endothelium is the dynamic secretion of thrombotic substances, anticoagulants and fibrinolysis factors, vasoactive substances, growth factors, etc., as well as leukocyte chemoattractants, inflammatory cell adhesion molecules, cytokines and reactive oxygen species [4, 5].

Periodontal disease begins with the invasion of oral bacteria into the gingival tissue. Bacterial peptidoglycans in oral biofilms, lipoteichoic acid, and lipopolysaccharides penetrate into deeper tissues due to both a number of destructive proteases of microbial origin and oral cells destroying the epithelial surface and periodontal ligament. These enzymes contribute to deepening of the periodontal pocket and damage to the endothelium of the subepithelial vascular network [6].

For example, *Porphyromonas gingivalis*, by binding to endothelial cells, increases gene expression of various chemokines (e. g., CXCL8, CCL2), adhesion molecules (CD54, CD62E, PECAM-1), ICAM-1/CD54, VCAM-1/CD106, and activates the kallikrein-kinin system [7, 8].

Increased vascularization with neoangiogenesis in healthy tissues gives an advantage in pathogen elimination. However, in presence of chronic periodontitis, abnormal vascularization is likely to exacerbate periodontal inflammation as it promotes the transmigration of a larger number of immunocompetent cells, the influx of inflammatory mediators and cytokines.

Plasmolifting as a method of therapy for oral diseases was first used in 1997 [9]. The therapeutic effect of platelet-rich plasma injected into the area of dental papillae is primarily due to the degranulation of platelet α-granules containing b-thromboglobulin and platelet-nonspecific proteins (fibronectin, fibrinogen), blood clotting factors, fibrinolysin, immunoglobulins and synthesized growth factors [10].

The aim of our work was to assess the functional activity of the endothelium of the vessels of the oral cavity in persons with chronic periodontitis in the treatment of plasmolifting.

MATERIALS AND METHODS

The study was conducted from February 2021 to February 2022 on the basis of the Chita State Medical Academy of the Ministry of Health of Russia (Chita). Under observation were 30 patients (15 men and 15 women) diagnosed with chronic generalized periodontitis of moderate severity at the age of 35 (32.50; 40.00) years, with no severe somatic pathology (main group). The comparison group included 20 people, comparable to the main group by gender and age, with no inflammatory diseases in the oral cavity. All study participants signed a voluntary informed consent to participate in the study (Approval No. 107 of the Local Ethics Committee of the Chita State Medical Academy dated 27.01.2021).

The dental status of the subjects was assessed in accordance with the clinical recommendations (treatment protocols) approved by the Russian Dental Association (2013), as amended and supplemented [11] using standard methods: interview, examination, determination of index indicators of the state of hard dental tissues and periodontal tissues, and X-ray. All patients underwent local anti-inflammatory therapy and sanitation of periodontal pockets, correction of occlusal contacts, curettage, plasma lifting.

For plasmolifting, platelet-rich autoplasm was obtained: blood was collected into specialized Plasmolifting™ tubes, centrifuged at 1300 rpm for 10 min. The supernatant was injected with an insulin needle into the area of the dento-gingival papillae 0.1–0.2 ml and the area of the muco-gingival junction 0.3–0.5 ml. Each patient underwent the procedure 5 times: the first visit – injections were performed in two segments of the upper jaw (in the 1st and 2nd segments); the second – 3 days later in the lower jaw (in the 3rd and 4th segments); the third – after 7 days; the fourth – after 30 days and in the fifth visit, after 6 months, injections were performed in all four segments.

Oral fluid was collected from healthy human volunteers ($n = 20$) and patients with generalized moderate periodontitis ($n = 30$) after an overnight fast. The collected saliva was centrifuged at 400 g and 4 °C for 10 min to remove cells. The cell-free supernatant was then collected and centrifuged again at 1500 g and 4 °C for 20 min to remove the remaining cells and cellular detritus. Cell-free saliva samples were stored on ice until use. Saliva sampling in patients with chronic periodontitis was performed twice: before the therapy and after achieving remission of the disease.

The concentration of soluble adhesion molecules ICAM-1 and VCAM-1 was determined in oral fluid using Human Vascular Inflammation Panel 1 multiplex assay kits (Biolegend, USA). Endothelin-1 levels were performed by ELISA using the Endotelin kit (1–21) (Biomedica, Austria). Oral fluid was analyzed without dilution. All stages of the study were performed according to the instruction of the kits.

The qualitative and quantitative composition of oral fluid microflora was studied by PCR using the ParodontoScreen kit.

Non-parametric criteria were used in statistical analysis: the Mann-Whitney U test for comparing two independent subgroups, the Wilcoxon test for comparing dependent subgroups, the Spearman's rank correlation coefficient (R) for analysing the correlation between different indica-

tors. Descriptive statistics are represented by median and interquartile range (25th, 75th percentiles).

Statistical processing of the data was performed using the IBM SPSS software package. Differences were considered statistically significant at $p < 0.05$.

RESULTS

The incidence of periodontal inflammatory diseases at outpatient appointments was more than 75 %, of which the prevalence of chronic periodontitis was 63.2 ± 2.5 % of cases. It occurred with equal frequency in individuals of both genders and different social statuses, but its prevalence increased with age.

As reported by the patients, the duration of the disease was 5.2 (4.2; 6.2) years. Oral hygiene was assessed using the OHI-S index. It was revealed that patients of the main group pay insufficient attention to oral hygiene: the index value at the initial examination was 3.02 (2.86; 3.30) points, against 0.38 (0.15; 0.51) in the comparison group. The index decreased after therapy but did not reach control values and was 1.40 (0.90; 1.90).

The results of the study of periodontal tissue condition of the subjects are presented in Table 1. Objective examination of patients with chronic periodontitis revealed gingival hyperemia and swelling, periodontal pockets and bleeding on probing, soft dental plaque and mineralized dental deposits. Tooth mobility in all included in the study was within the physiological range. Resorption of interalveolar septa up to 1/3 was determined radiologically in the main group. After the treatment with plasmolifting there was a noticeable relief of the activity of the inflammatory process, which was expressed in the reduction of PMA, bleeding index, gingival recession, depth of periodontal pocket.

Tannerella forsythia (2.9 (1.6; 4.1) lg (gEq/sample)) and *Treponema denticola* (3.0 (1.1; 4.0) lg (gEq/sample)) were most common in control group. The titre of these microorganisms was 4.73 (4.2; 5.1) and 4.26 (3.8; 5.2) lg (gEq/sample) among patients with chronic periodontitis. In addition, *Porphyromonas gingivalis* was detected in 100 % of cases at a titre of 5.73 (4.9; 6.7) and *Prevotella intermedia* was found in 62.5 % of patients at a titre of 4.5 (3.0; 5.5) lg (gEq/sample). Against the background of therapy, both a decrease in the occurrence and num-

TABLE 1

INDICATORS OF THE STUDY OF THE STATE OF THE ORAL CAVITY IN PATIENTS OF THE MAIN GROUP, ME (25th; 75th PERCENTILES)

Indicators	Control group (n = 20)	Patients with periodontitis (n = 30)	
		initial examination	after plasmolifting
Bleeding index	0.00 (0.00; 0.00)	2.30 (2.10; 2.80) p_1 = 0.00001	0.68 (0.62; 0.73) p_1 = 0.0001 p_2 = 0.0001
PMA	0.00 (0.00; 0.00)	40.6 (30.85; 50.00) p_1 = 0.00001	12.89 (10.21; 13.99) p_1 =0.00001 p_2 =0.0001
Gingival recession, mm	0.00 (0.00; 0.00)	1.39 (1.26; 1.53) p_1 =0.00001	0.75 (0.63; 0.88) p_1 =0.00001 p_2 =0.361
Plaque index	0.00 (0.00; 0.00)	3.50 (1.98; 3.85) p_1 =0.00001	2.42 (2.30; 2.50) p_1 =0.00001 p_2 =0.0001
Periodontal pocket depth, mm	2.00 (2.00; 3.00)	6.10 (4.40; 6.60) p_1 =0.00001	3.7 (3.2; 4.2) p_1 =0.002 p_2 =0.0001

Note. p_1 – level of statistical significance of differences compared to the control group; p_2 – compared to the group of patients receiving plasmolifting.

ber of microorganisms was observed. For example, *Porphyromonas gingivalis* was found only in 30 % of the subjects, and its number decreased by 2.5 times ($p = 0.003$).

The level of soluble adhesion molecules and endothelin-1 was determined in order to confirm the involvement of vascular endothelium in the pathological process.

Cell adhesion molecules (CAMs) are cell surface proteins involved in the binding of cells to each other, to endothelial cells or to the extracellular matrix. VCAM-1 (CD106) is predominantly expressed on the membrane of endothelial cells. However, in case of severe or chronic inflammation it is also expressed on the surface of other cells including tissue macrophages, dendritic cells, bone marrow fibroblasts, myoblasts, oocytes, Kupffer cells, Sertoli cells and cancer cells [12]. This protein is a major regulator of leukocyte adhesion and transendothelial migration through interaction with α4β1 integrin [13].

Soluble intercellular adhesion molecule-1 (sICAM-1) is a circulating form of ICAM-1 that is constitutively expressed or induced on the cell surface of various tissues. It serves as a counter-receptor for lymphocyte function-associated antigen (LFA-1). The interaction between ICAM-1 present on endothelial cells promotes leukocyte adhesion and their migration across the endothelium, while its soluble form blocks this process [14].

Two mechanisms for the formation of soluble forms of adhesion molecules have been suggested: proteolytic cleavage of the molecule [15], which characterizes ICAM-1 expression on cells, and transcription of the matrix RNA encoding their formation [16].

We found a 38.3-fold increase in the concentration of the soluble form of VCAM-1 in oral fluid and an 18.1-fold increase was noted in the concentration of ICAM-1 (Table 2). Against the background of plasmolifting therapy, the level of the studied substances decreased, but exceeded the control values by 25.2 and 6.4 times, respectively.

High levels of soluble forms of adhesion molecules indicate an actively ongoing inflammatory process despite a clinically achievable state of remission.

The next stage of the work was to assess the level of developed endothelial dysfunction by endothelin-1 concentration. It is synthesized mainly by endotheliocytes in response to damaging stimuli and is immediately secreted into the external environment [17]. Endothelin-1 is classically considered a potent vasoconstrictor peptide. However, in addition to its effects on vascular smooth muscle cells, this substance is increasingly recognized as a pro-inflammatory cytokine. Endothelin-1 causes platelet aggregation and is involved in increased expression of leukocyte adhesion molecules, synthesis of inflammatory mediators, and mechanisms contributing to vascular dysfunction [18]. We found an increase of endothelin content in oral fluid among patients with periodontitis by 40.7 % ($p = 0.003$), a decrease in its concentration in treated patients to 16.55 % ($p = 0.04$).

The homeostatic dynamics of the oral cavity are constantly changing depending on the composition of the microflora. The oral dissemination of pathogens is hindered by the immune system, which, in addition to destroying pathogens, is responsible for repairing damaged tissues. Tissue alteration and vascular changes become the determining factors in the focus of the host-pathogen relationship. We have determined that even when clinical remission is achieved, an active inflammatory process continues in the periodontal tissues. This is confirmed by the presence of correlations. Thus, papillary-marginal-alveolar index has a high positive correlation with soluble form of ICAM-1 ($r = 0.764$), VCAM-1 ($r = 0.825$) and endothelin-1 ($r = 0.729$).

TABLE 2

THE LEVEL OF SOLUBLE FORMS OF INTERCELLULAR ADHESION MOLECULES IN PATIENTS WITH CHRONIC PERIODONTITIS, ME (25th; 75th PERCENTILES)

Parameters	Control group ($n = 20$)	Patients with periodontitis ($n = 30$)	
		initial examination	after plasmolifting
ICAM-1, pg/ml	0.99 (0.45; 1.34)	17.89 (14.99; 22.97) $p_1 = 0.000001$	6.34 (2.36; 10.82) $p_1 = 0.00001$ $p_2 = 0.01$
VCAM-1, pg/ml	0.30 (0.20; 0.40)	11.48 (8.99; 16.89) $p_1 = 0.000002$	7.56 (5.36; 9.54) $p_1 = 0.000001$ $p_2 = 0.000001$

Note. p_1 – level of statistical significance of differences compared to the control group; p_2 – compared to the group of patients receiving plasmolifting.

Autoplasm contains a large number of activated platelets, which, adhering to the exposed collagen of the damaged vessel, improve its barrier function, reduce thrombogenicity and enhance the growth of smooth muscle cells surrounding the endothelium [19]. All this leads to normalization of endotheliocyte function and is manifested by a decrease in the levels of both adhesion molecules and endothelin-1.

Based on the above, it can be concluded that the use of plasmolifting in chronic periodontitis is a pathogenetically justified method of therapy.

Thus, patients suffering from chronic periodontitis have increased levels of soluble forms of adhesion molecules and endothelin-1, which reflects the ongoing inflammatory process in periodontal tissues. In this case, the course of chronic periodontitis is accompanied by the development of endothelial dysfunction, manifested by an increase in the concentration of endothelin-1. The use of plasmolifting method leads to a decrease in the levels of adhesive and vasoactive molecules, reduction of bacterial load, and promotes clinical remission of the pathological process, which may be promising for further research in periodontology.

Conflict of interest

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

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PHARMACOLOGY AND PHARMACY

OBTAINING EMULSIONS OF FURANOCOUMARINS FROM SOSNOWSKY'S HOGWEED AND *IN VITRO* ASSESSMENT OF THEIR PHOTOTOXIC EFFECT

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ABSTRACT

Background. *Furanocoumarin-based drugs are used for photochemotherapy of various diseases. Sosnovsky's hogweed can be an available source of furanocoumarins for the development of drugs.*

The aim of the study. *To obtain stable emulsions containing furanocoumarins from Sosnowski's hogweed and to evaluate their photocytotoxicity.*

Materials and methods. *To obtain the emulsions, furanocoumarins were extracted with chloroform from the sap of the aerial part of the Sosnowski's hogweed. The chloroform extract was clarified by silica gel gradient column chromatography. The extractive fraction containing furanocoumarins was analyzed by high performance liquid chromatography with ultraviolet (UV) detection.*

An extract with a high content of 8-methoxypsoralen was used to prepare two types of emulsions. The extract was dissolved in peach oil and emulsified in water with tween-80 (emulsion No. 1) and in an aqueous glycerin solution with lecithin (emulsion No. 2).

The emulsions were tested for dark and photo-induced toxicity for human lung fibroblasts. The dose of UV radiation for the photoactivation of furanocoumarins was 9 J/cm^2. A solution of chlorine e6 was used as a comparison photosensitizer.

Results. *The obtained emulsions contained 1 mg/ml 8-methoxypsoralen. Both emulsions were homogeneous at macro- and microscopic visualization, remained stable when stored under various temperature conditions for 14 days. Emulsion No. 2 did not show dark toxicity and caused a statistically significant inhibition of cell viability under UV irradiation at a concentration of 12.5–31.3 μg/mL. Emulsion No. 1 had a toxic effect on cells regardless of UV irradiation due to the content of tween-80. According to fluorescent microscopy, the phototoxic effect of emulsion No. 2 was manifested mainly due to apoptosis, in contrast to the effect of chlorine e6, in which there were more pronounced signs of cell necrosis.*

Conclusion. *The developed experimental emulsions of furanocoumarins from Sosnovski's hogweed are an example of promising medicinal photosensitizers of plant origin for phototherapy of various dermatological and oncological diseases.*

Key words: *emulsion, furanocoumarins, Sosnowski's hogweed, UV radiation, tween-80, lecithin, apoptosis*

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ПОЛУЧЕНИЕ ЭМУЛЬСИОННЫХ ФОРМ ФУРАНОКУМАРИНОВ БОРЩЕВИКА СОСНОВСКОГО И ОЦЕНКА ИХ ФОТОТОКСИЧЕСКОГО ДЕЙСТВИЯ *IN VITRO*

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РЕЗЮМЕ

Обоснование. *Для фотохимиотерапии различных заболеваний используются средства на основе фуранокумаринов. Доступным источником фуранокумаринов для создания лекарственных препаратов может быть борщевик Сосновского.*

Цель исследования. *Получить стабильные эмульсии, содержащие фуранокумарины, из борщевика Сосновского и оценить их фотоцитотоксичность.*

Материалы и методы. *Фуранокумарины для получения эмульсий экстрагировали хлороформом из сока надземной части борщевика Сосновского. Хлороформный экстракт очищали с помощью градиентной колоночной хроматографии на силикагеле. Экстрактивную фракцию, содержащую фуранокумарины, анализировали с помощью высокоэффективной жидкостной хроматографии с ультрафиолетовым (УФ) детектированием.*

Экстракт с высоким содержанием 8-метоксипсоралена использовали для получения двух видов эмульсий. Экстракт растворяли в персиковом масле и эмульгировали в воде твином-80 (эмульсия № 1) и в водно-глицериновом растворе лецитином (эмульсия № 2).

Эмульсии тестировали на темновую и фотоиндуцированную токсичность для фибробластов лёгких человека. Доза УФ-излучения для фотоактивации фуранокумаринов составила 9 Дж/см^2. В качестве фотосенсибилизатора сравнения использовали раствор хлорина е6.

Результаты. *Полученные эмульсии содержали 1 мг/мл 8-метоксипсоралена. Обе эмульсии были гомогенными при макро- и микроскопической визуализации, сохраняли стабильность при хранении в различных температурных условиях в течение 14 дней. Эмульсия № 2 не проявляла темновой токсичности и вызывала статистически значимое угнетение жизнеспособности клеток при УФ-облучении и концентрации 12,5–31,3 мкг/мл. Эмульсия № 1 оказывала токсическое действие на клетки независимо от УФ-облучения из-за содержания в составе твина-80. По данным флуоресцентной микроскопии, фототоксическое действие эмульсии № 2 проявлялось главным образом за счёт апоптоза, в отличие от действия хлорина е6, при котором имелись более выраженные признаки некроза клеток.*

Заключение. *Разработанные экспериментальные эмульсионные формы фуранокумаринов борщевика Сосновского являют собой пример перспективных лекарственных фотосенсибилизаторов растительного происхождения для фототерапии различных заболеваний в сфере дерматологии и онкологии.*

Ключевые слова*: эмульсия, фуранокумарины, борщевик Сосновского, УФ-излучение, твин-80, лецитин, апоптоз*

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OBJECTIVES

One of the substances widely used in medicine for photochemotherapy are furanocoumarins, among which psoralen derivatives (5-methoxypsoralen (5-MOP), 8-methoxypsoralen (8-MOP), etc.) have clinically proven effect [1]. Sosnowsky's hogweed stands out among the plants containing high concentrations of psoralen and its derivatives [2]. In some regions of the globe, including Russia, Sosnowsky's hogweed, as well as other species of giant hogweeds, is recognized as a dangerous phytoinvasive species and is widely eradicated [3]. As Sosnowsky's hogweed is widespread and rapidly growing phytomass, its plant raw material is very accessible for harvesting and pharmaceutical use.

Currently, the plant *Ammi majus* is the source of furanocoumarins for the production of Ammifurin, photosensitizing drug. Its fruits contain about 2 % furanocoumarins. Ammi preparations are used as antipsoriatic agents for PUVA (psoralen and ultraviolet A) therapy [4].

Ammi majus is inferior to Sosnowsky's hogweed in phytomass for the production of furanocoumarins. Ammi furanocoumarins are localized predominantly in the fruits of the plant and are mixed with a large number of hydrophobic related substances [4]. Ammi majus can be found wild only in warm climatic conditions [5, 6] and in other regions it requires special cultivation conditions.

Furanocoumarins are very lipophilic substances, but they must be in an aqueous environment to realize their photobiological potential [7]. In various studies, furanocoumarins are tested as alcoholic solutions or dimethyl sulfoxide (DMSO) solutions [8, 9], where furanocoumarins remain stable at high concentrations (more than 1 mg/ml) provided at least 80 % DMSO is present in the solvent. The problem of solubility of furanocoumarins in water can be solved by creating an emulsion form [10]. Obtaining an emulsion form of furanocoumarins may alter or disappear the native photosensitizing effect induced by the plant itself [11]. Until now, furanocoumarin emulsions based on medicinal plant raw materials have not been developed and used. In this regard, it becomes relevant to control the specific activity of active substances, in our case furanocoumarins.

An effective dosage form of furanocoumarins with available raw materials for its manufacture can be widely used as an agent for PUVA therapy or as an antineoplastic agent against skin neoplasia: for example, microemulsion for transcutaneous delivery of furanocoumarins containing various emulsifiers (isopropyl myristate, tween-80, span-80, octanediol) [10]. Microemulsions of 8-methoxypsoralen are intended for PUVA therapy as topical photosensitizers. However, the correlation between dark and photoinduced toxicity is unfairly little considered in such studies.

There is a view that a simple photosensitizer emulsion is not suitable for emulsion droplets without polymer coatings, and the photosensitizing effect of simple and polymer-associated emulsions has not been compared [11].

The use of nanoemulsions of furanocoumarins, where essential oil is used as the oil phase, has been proposed for local administration. It has been found that despite the finely dispersed composition (about 30 nm) of the resulting emulsion, it is also well retained in the skin [12]. However, we cannot, for example, talk about the parenteral administration in case of such emulsions.

Researchers offering emulsion formulations for skin application have a fairly wide choice of solvents, as furanocoumarins can dissolve in many essential and fatty oils. However, if the photosensitizer is to be administered parenterally, the range of oils available for use is sharply narrowed. The need to administer the photosensitizer intravascularly may be motivated by a more significant effect, as opposed to superficial application, which has been repeatedly shown by various researchers [13, 14]. Parenteral formulation of the photosensitizer is also necessary for intratumoral administration during therapy of unresectable tumors [15, 16] or delivery of activating radiation via optical fiber into the tumor tissue [17].

The aim of this study was to obtain stable emulsions containing furanocoumarins isolated from Sosnowsky's hogweed and to evaluate their photocytotoxicity.

MATERIALS AND METHODS

The source of plant raw material was the aerial part of *Heracleum sosnowskyi* Manden. The plant was defined according to *The Giant Hogweed Best Practice Manual. Guidelines for the Management and Control of an Invasive Weed in Europe* (2005). The furanocoumarins fraction was extracted from the sap of the aerial part. The extraction, purification and high-performance liquid chromatography (HPLC) process was carried out according to the previously described methodology [18], the scheme of which is shown in Figure 1.

Two emulsions with different emulsifying agents were prepared from the extractive fraction containing furanocoumarins. For the manufacturing of emulsions, the dried extract containing 100 mg of 8-MOP was dissolved on heating in 10 ml of peach oil (Mirrolla, Russia). Further work was carried out with the heated oil extract solution for emulsion manufacturing.

An aqueous 2.5 % solution of polysorbate 80 (Tween-80) (Sigma-Aldrich, USA) was used as a stabilizer for the first method of furanocoumarins emulsion (emulsion No. 1) manufacturing. Under vigorous stirring and constant temperature of 25 °C, 1 ml (0.916 g) of an oil solution of furanocoumarins of Sosnowsky's hogweed with a concentration of 8-MOP 6.125 mg/ml was added dropwise to 5 ml of polysorbate solution. The prepared mixture was stirred for 10 minutes and then exposed to ultrasound (50 W) for 10 minutes. A total of 3 repetitive emulsification cycles were performed. The quality of the prepared emulsion was controlled by light microscopy in three stages: 1) immediately after preparation;

2) after storage in a refrigerator for 96 h at 4 °C; 3) after storage at 20–22 °C for 14 days.

The second variant of furanocoumarins emulsion (emulsion No. 2). The aqueous phase was made by adding 0.1315 g of glycerol (Sigma-Aldrich, USA) to 5 ml of deionized water; the mixture was stirred on a magnetic stirrer at 70–75 °C. At the same time, an oil phase was prepared from 0.1 g of lecithin (phosphatidylcholine, EPCS 10 8018-1/130, Lipoid, Germany) and 0.916 g of an oil solution of hogweed furanocoumarins obtained according to the method described above. The mixture was stirred at 90 °C until a homogeneous consistency is obtained. The oil phase was slowly added to the aqueous phase at a temperature of 70–75 °C while stirring vigorously. The resulting emulsion was stirred at 70–75 °C for 10 minutes. The mixture was then homogenized using ultrasound (200 W – 1 min; 2 s/2 s cycle) until emulsion globules were formed.

The study of emulsion cytotoxicity was carried out on cell culture of human lung fibroblasts (HLF) in the National Research Center for Epidemiology and Microbiology named after Honorary Academician N.F. Gamaleya of the Ministry of Health of Russia. During the exponential growth phase, cells were dispersed into a 96-well plate at a concentration of 5,000 cells/well and incubated for 24 h under standard conditions on DMEM medium supplemented with 10 % FBS (fetal bovine serum) and antibiotics (penicillin-streptomycin) at 5 % CO_2 and 37 °C.

Phototoxic reactions of furanocoumarins were induced using an ultraviolet (UV) lamp (365 nm, 40 W; Camelion LH26-FS/BLB/E27, China), which was placed at a fixed distance of 20 cm from the target cells to obtain a constant radiation intensity of 35 mW/cm^2. Radiation intensity was measured using a ThorLabs PM100D radiometer (Germany). The photocytotoxic effects of the emulsions were determined at concentrations of 8-MOP in the well of the plate: 125, 62.5, 31.3, 15.6, 7.8, 3.9, 2 and 1 μg/ml. Cells were irradiated with UV light four hours [19] after emulsions were applied.

Chlorin e6, a photosensitizer from the group of porphyrins, was used to control photodynamic stress. Chlorin e6 solution (50 μg/ml) was prepared in DMSO (50 mg/ml) and stored in a dark place at 4 °C. For the induction of photodynamic action with chlorin e6, concentrations in the well of the plate were used: 6.3–0.05 μg/ml [20, 21]. The cells were irradiated with 660 nm LED lamp irradiation.

The radiation dose was selected empirically based on the results of a control experiment without application of the test agents. 1 hours after irradiation, cells were trypsinized and transferred to a 96-well plate with medium. After 24 hours of incubation, cell viability was assessed using the MTT assay [22]. Tween-80 and lecithin were administered separately to assess their contribution to cytotoxic effects. The phototoxic effect of each photosensitizer was monitored in relation to cells

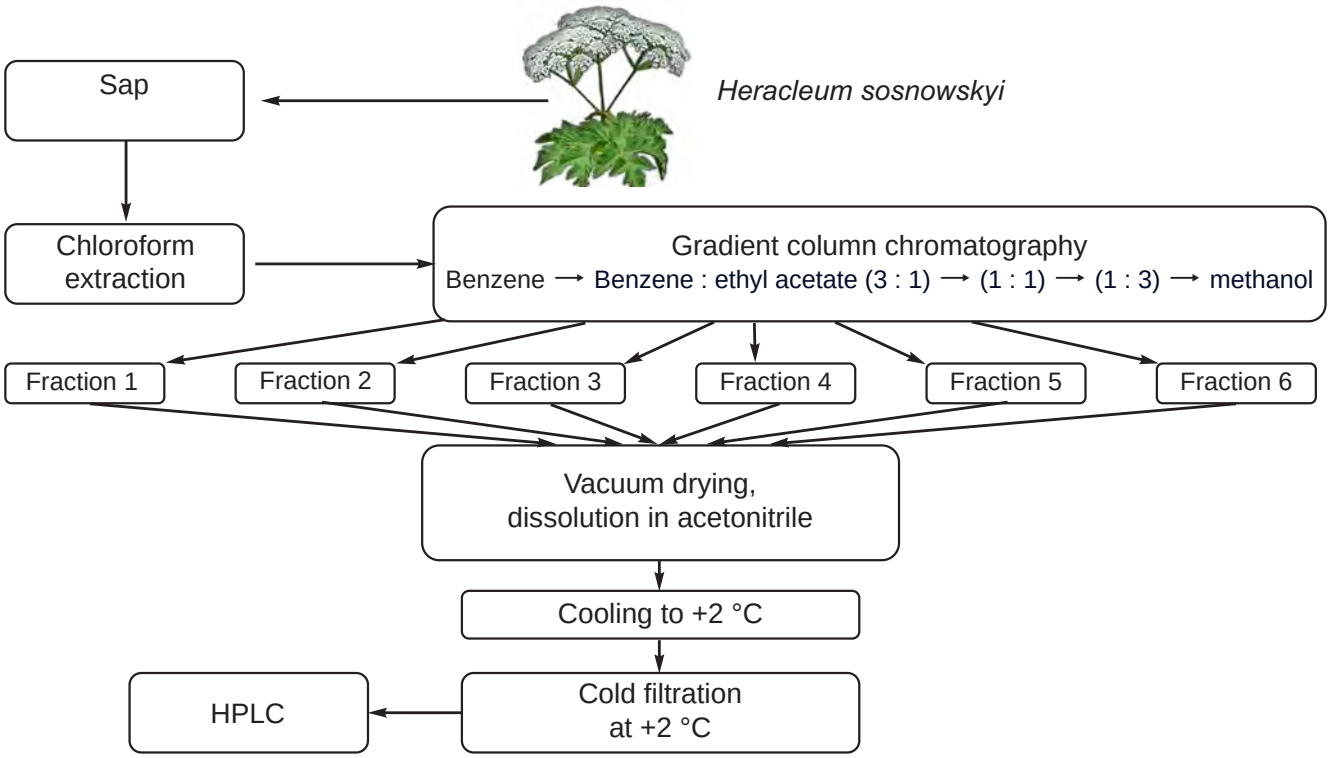

FIG. 1.

Scheme for isolating the furanocoumarin fraction from the Sosnowsky's hogweed sap. High-efficiency liquid chromatography of extractive fractions was carried out in isocratic mode (chromatography system by Gilson (France); Kromasil C18 column 4.5 mm × 5 μm × 250 mm). Mobile phase: water/acetonitrile (1:1). Flow rate 0.8 ml/min. Detection at wavelength 250 nm. Software "Millichrome" (Russia). Analytical standards: 5-methoxypsoralen, 8-methoxypsoralen (Sigma-Aldrich, USA)

that were placed in a dark place after administration of the test substances. Cell viability in all experimental groups was evaluated relative to a control series of wells (negative control) in which no test component was placed and no irradiation was performed.

After 24 hours of incubation, the medium was replaced with 5 % MTT solution (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl). MTT was reduced to formazan crystals, which was evaluated using an inverted microscope (Micromed, Russia). The medium was then removed and 150 µl of DMSO was added and stirred for 20 min at 37 °C. Optical density was measured on Varioskan Lux Reader (Thermo Fisher Scientific, USA) at a wavelength of 570 nm versus 650 nm reference wavelength. Optical density (OD) results were displayed in % OD (sample) / OD (control).

Data are presented as mean ± standard error of mean (SEM) for > 3 independent experiments or half maximal inhibitory concentration (IC_{50}) values and their 95 % confidence intervals obtained by nonlinear regression. Differences between experimental groups were compared by statistical data processing using Student's t-test and ANOVA test. The critical level of statistical significance of the differences was 5 % ($p < 0.05$). All statistical analyses were performed using SPSS Statistics 10.0 software (IBM Corp., USA).

RESULTS

A fraction with high content of furanocoumarins (8- and 5-methoxypsoralen) was found using HPLC of the extract. The appearance of the chromatograms is shown in Figure 2.

The retention time of the substances of the Sosnowsky's hogweed extract corresponded to the retention time of the analytical standards 8- and 5-MOP. According to the calibration curve (peak area versus concentration) constructed using a solution of 5- and 8-MOP in acetonitrile, the concentration of 8-MOP in the original sap was 506 mg/l, and that of 5-MOP was 23 mg/l.

Based on the predominance of 8-MOP in the sap of Sosnowsky's hogweed, this furanocoumarin was taken as the dosage active substance in the emulsions.

When the concentration of 8-MOP in peach oil was 6.125 mg/ml at room temperature (20 °C), no needle crystals of furanocoumarins were formed and a stable oil solution was obtained. However, when the solution was placed in a refrigerator at 4 °C, needle crystal precipitation occurred. The concentration in the 8-MOP oil solution that did not result in crystals at 20 °C and 4 °C was 3 mg/ml. The experimentally obtained solubility limit of extractive furanocoumarins in oil is similar to that obtained by B. Baroli et al. for chemical substances [10].

The quantities of emulsion ingredients are listed in Table 1. Both emulsions contained 8-MOP at a concentration of 1 mg/ml.

The obtained emulsions were homogeneous visually and under microscopy. The emulsion samples remained stable during a storage period of 14 days or more with the temperature range of 4–20 °C.

UV radiation had a statistically significant inhibitory effect on HLF cell development at a dose of 22 J/cm^2. Thus, the ultimate safe dose of this radiation was 18 J/cm^2 (Fig. 3a). However, this dose had a large error in the mean, and we adopted a UV dose of 9 J/cm^2 as an indifferent dose for this cell type. LED wavelength radiation (660 nm) did not cause a statistically significant decrease in cell viability up to a dose of 30 J/cm^2 (Fig. 3a) and was used at this power to photoactivate chlorin e6.

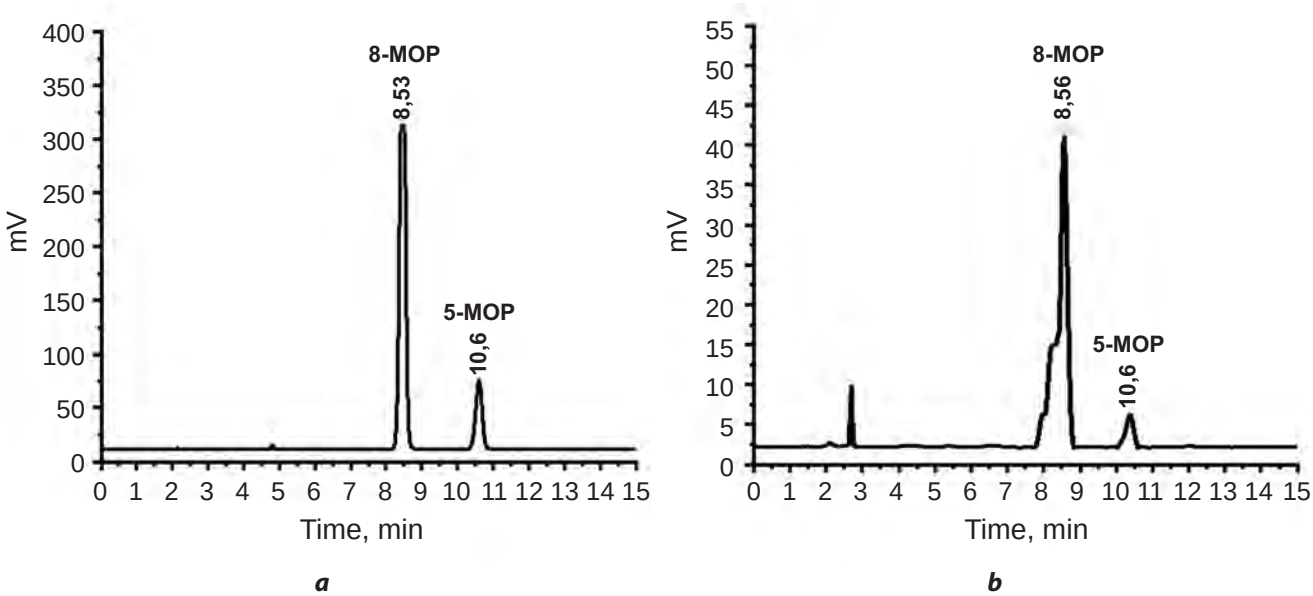

FIG. 2.

*Chromatograms of a solution of furanocoumarins (8- and 5-methoxypsoralen) in acetonitrile (**a**) and of the extract of Sosnowsky's hogweed sap containing high concentrations of the same furanocoumarins (**b**)*

TABLE 1

COMPOSITION OF OBTAINED EMULSIONS OF FURANOCOUMARIN FROM SOSNOWSKY'S HOGWEED

Composition of emulsion sample No. 1 (w/w %)			
Water	Peach oil	8-MOP	Tween-80
82.7	15.15	0.1	2.05
Composition of emulsion sample No. 2 (w/w %)			
Water	Glycerin	Peach oil	8-MOP	Lecithin
81.3	2.12	14.89	0.1	1.63

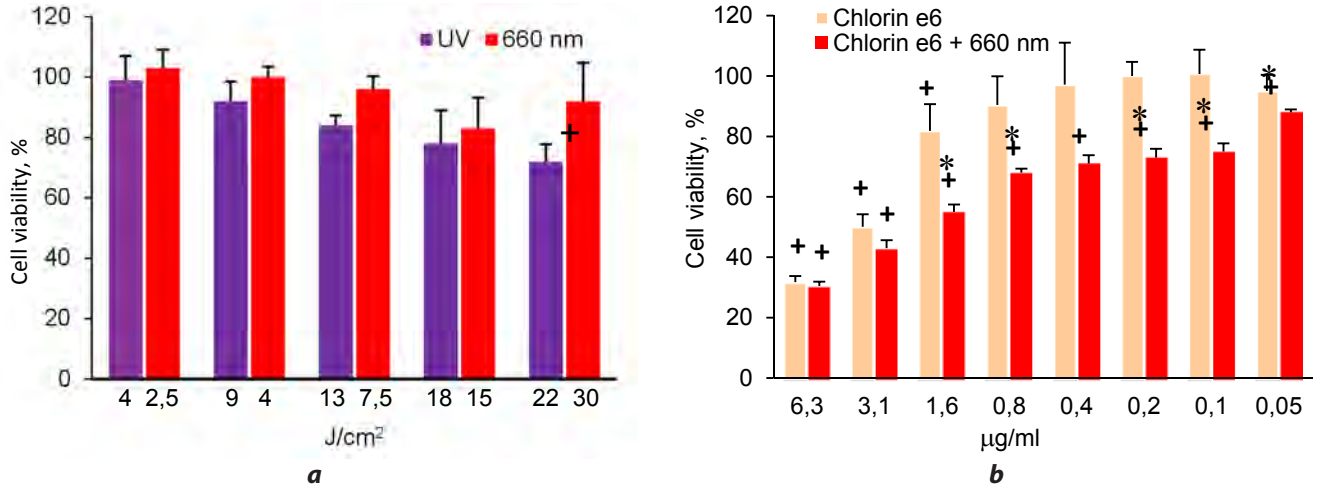

FIG. 3.

*Change in viability of HLF cells under different doses of UV (365 nm) and red (660 nm) radiation (**a**); photodynamic effect of chlorin e6 on HLF cells (**b**): + – statistically significant differences with values of the control group, without application of test substances and irradiation; * – statistically significant differences with values without irradiation at the same concentration of chlorin e6*

MTT assay in the group using the photodynamic photosensitizer chlorin e6 showed statistically significant changes in HLF cell viability due to the photosensitizing effect. At concentrations of 1.6–6.3 µg/ml, chlorin e6 had a significant toxicity against HLF cells, as at these concentrations cell viability was statistically significantly reduced relative to the negative control group in both cultivation regimes (Fig. 3b). Starting from chlorin concentration e6 1.6 µg/ml, the photosensitizing effect of chlorin began to appear, as in this case cell viability was statistically significantly lower ($p < 0.01$) than in the dark regime (Fig. 3b). At lower concentrations of chlorin e6, its phototoxicity was still present at all dilutions (0.05–0.8 µg/ml), as evidenced by the low cell viability (at 68–75 %), which had statistically significant differences from that in the group of cells that received chlorin at the same concentrations but were not exposed to LED irradiation (Fig. 3b). At a chlorin e6 concentration of 0.4 µg/ml and irradiation, no statistically significant differences with the dark regime were noted. However, relative to intact cells, lower viability was noted at $p < 0.001$ (Fig. 3b).

Figure 4a shows that emulsion No. 1 has a cytotoxic effect on cells at fairly low concentrations. It is shown that there is no significant difference in cell survival without and after UV irradiation. Up to a concentration of 2 µg/ml in 8-MOP medium, emulsion No. 1 shows statistically significant cytotoxicity. However, if the intrinsic toxicity of tween-80 is considered (Fig. 4b), it can be seen that it repeats in general the toxicity of emulsion No.1.

Emulsion No. 2 without UV irradiation had no toxic effect on cells. In contrast to emulsion No. 1, lecithin was used as an emulsifier in emulsion No. 2, which did not show its own cytotoxicity at the concentrations studied. Lecithin, examined in isolation from other emulsion components, did not cause statistically significant changes in HLF culture viability under both dark and UV irradiation conditions (Fig. 4b).

When emulsion No. 2 is used in combination with UV, a clear phototoxic effect can be observed against cells at 8-MOP concentrations of 125, 62.5 and 31.3 μg/ml. At these concentrations, emulsion No. 2 reduced cell viability to 38 ± 9, 25 ± 6 and 41 ± 3 %, respectively, versus 86 ± 6 % ($p < 0.01$), 110 ± 1 % ($p < 0.001$) and 87 ± 3 % ($p < 0.001$) at the same concentrations but under dark conditions (Fig. 4b).

Relative to intact cells of the negative control, cells that received emulsion No. 2 in photomode also had statistically significantly lower viability values. Thus, at 8-MOP concentrations of 31.3, 62.5 and 125 μg/ml and UV irradiation, cell viability was statistically significantly lower than in the control at the statistical significance level of 0.017, 0.0048 and 0.018, respectively (Fig. 4a).

At lower concentrations (1–15.6 μg/ml), emulsion No. 2 did not inhibit cell viability under both dark and UV conditions. Therefore, it can be concluded that the concentration of 8-MOP 31.3 μg/ml for this type of cells appeared to be the limit for realization of phototoxic action of emulsion No. 2.

Fluorescence microscopy showed that in a large percentage of cases, cells lost viability due to apoptosis (Fig. 5). Apoptotic cells at early and late stages were detected in all test groups. Without cell culture intervention, the percentage of cells in apoptosis was 7 ± 3 %. After UV irradiation (9 J/cm^2) it increased to 15 ± 3.5 %, which was not statistically significant. A distinctive feature of cells with signs of apoptosis in the group with emulsion No. 2 and chlorin e6 after photoactivation was the presence of vesicles on the cell membrane. Almost all cells in these groups had this trait after irradiation (Fig. 5). The highest number of apoptotic cells could be noted in the group with emulsion No. 2 after UV irradiation, where 84 ± 6 % of all cells showed signs of apoptosis. Necrotic cells with karyorrhexis and monotonous red and brown cytoplasm were found most extensively in the groups with emulsion No. 1 and tween-80 in 25 % and 28 % of cases, respectively.

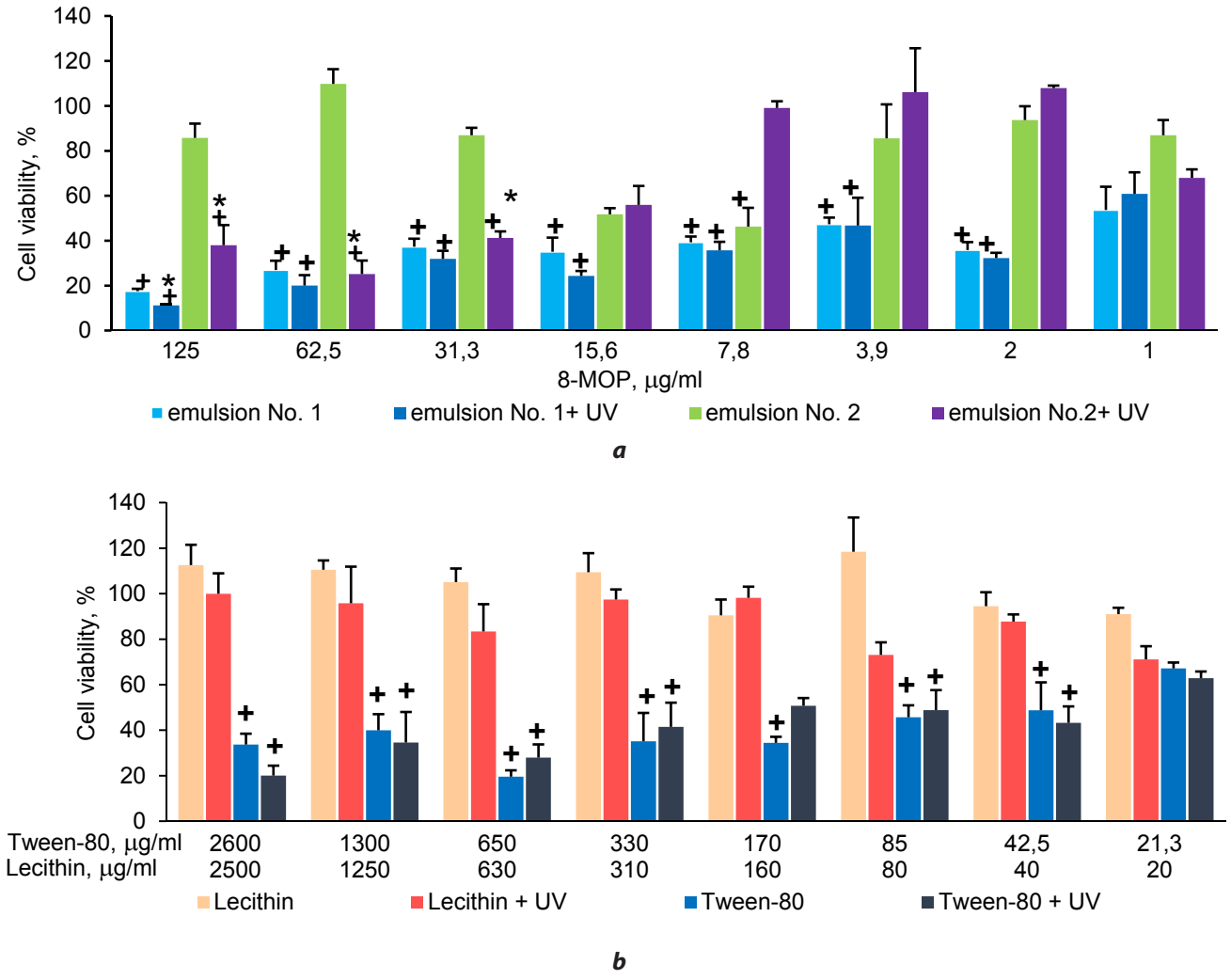

FIG. 4.

*Viability of HLF cells according to the MTT test under the influence of emulsion furanocoumarins from Sosnowsky's hogweed (**a**) and of used emulsifiers (**b**) (dark and photo-induced toxicity): + – statistically significant differences from the values in the control group, without the test substances introduction and irradiation; * – statistically significant differences from the values without UV irradiation and at the same concentration of test substances*

TABLE 2

RATIO OF DARK AND PHOTOINDUCED CONCENTRATION (MG/ML) OF HALF MAXIMAL INHIBITION ($IC_{50\ DARK}/IC_{50\ UV}/660$) FOR 8-MOP IN EMULSIONS, EMULSIFIERS AND CHLORIN E6

Emulsion No. 1 (8-MOP)	Tween-80	Emulsion No. 2 (8-MOP)	Lecithin	Chlorin e6
1.2/10.5	51/69.5	–/36.8	–/–	4/2

At the same time, the number of cells with signs of apoptosis and necrosis did not statistically significantly increase under UV irradiation (Fig. 5). The increase in cells showing signs of apoptosis and necrosis when exposed to UV irradiation on cells treated with emulsion No. 2 was similar to that of chlorin e6, where after red light irradiation an average of 55 ± 2 % of cells were in a state of apoptosis and 14 ± 1 % were in a state of necrosis. In the group treated with lecithin at a concentration of 2.5 mg/ml, vesicles on the cell membrane were extremely rare, and the morphological pattern of this group did not change after UV irradiation.

Injection of lecithin into the medium with cells resulted in 24 ± 2 % of cells showing signs of apoptosis (Fig. 5). However, UV irradiation did little to change cell morphology upon lecithin application with 40 ± 8% of cells in a state of apoptosis.

The concentration of half-maximal inhibition of 8-MOP in emulsion No. 1 did not decrease but increased upon UV irradiation of cells (Table 2), which was apparently due to the prevailing toxicity of tween-80 for this emulsion, which prevented the detection of the photosensitizing effect of 8-MOP *in vitro*.

Emulsion No. 2 was responsive to UV irradiation and markedly reduced cell viability. In the absence of UV, emulsion No. 2 was found to be non-toxic to cells and IC_{50} in a dark place was not reached for this emulsion (Table 2).

DISCUSSION

Therefore, emulsion No. 2 has a photocytotoxic effect due to the furanocoumarins of Sosnowky's hogweed contained in it and is not toxic to human cells in the absence of UV radiation. Emulsion No. 1 has its own cytotoxicity due to its tween-80 content. Tween-80 has previously been shown to have membranotoxic properties [23]. However, the toxicity of tween-80 does not negate the presence of photoinduced toxicity in emulsion No. 1, as the *in vitro* toxicity of tween-80 may override it. The development of a stable emulsion dosage form in our study was aimed at the prospective use of furanocoumarins *in vivo* to create an affordable and easy to manufacture dosage form for photochemotherapy [24]. The emulsion form studied overcomes the difficulties associated with the hydrophobicity of furanocoumarin molecules and effectively stabilizes them as a liquid heterogeneous system [25]. Lecithin used to create emulsion No. 2 showed itself as a photo-independent component, which is consistent with the literature [26], and therefore did not interfere with the phototoxic effect of furanocoumarins.

Despite the fact that Sosnowsky's hogweed is a dangerous invasive plant growing in many regions of the world. Nevertheless, its physiological features and chemical composition make it a unique and accessible source of raw materials for the production of highly active photosensitizers, which may become an alternative to phthalocyanines against various diseases, including neoplasms, requiring the use of photoactive materials [16]. As our study showed, furanocoumarins of Sosnowsky's hogweed have a phototoxic effect on isolated cells, unlike the standard photosensitizer chlorin e6 – mainly due to the activation of apoptosis (Fig. 5). A characteristic morphological sign of cell apoptosis under the influence of furanocoumarins of Sosnowsky's hogweed was the presence of vesicles on the cytoplasmic membrane. This probably complements the mechanism of photocytotoxicity of the hogweed furanocoumarins and suggests their membranotoxic action [27]. The same mechanism of phototoxicity is characteristic of photosensitizers, porphyrin derivatives, which require oxygen and the activator, light, for activation [28]. However, the mechanism of photosensitizing action of furanocoumarins is related to the formation of A-covalent monoadducts and interchain cross-links in DNA between pyrimidine bases under UV radiation [29]. This mechanism is not the classical photodynamic mechanism characteristic of chlorin e6 and other porphyrin-type photosensitizers.

CONCLUSION

The developed experimental emulsions of furanocoumarins from Sosnowsky's hogweed are an example of promising medicinal photosensitizers of plant origin for phototherapy of various dermatological and oncological diseases.

Conflict of interest

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

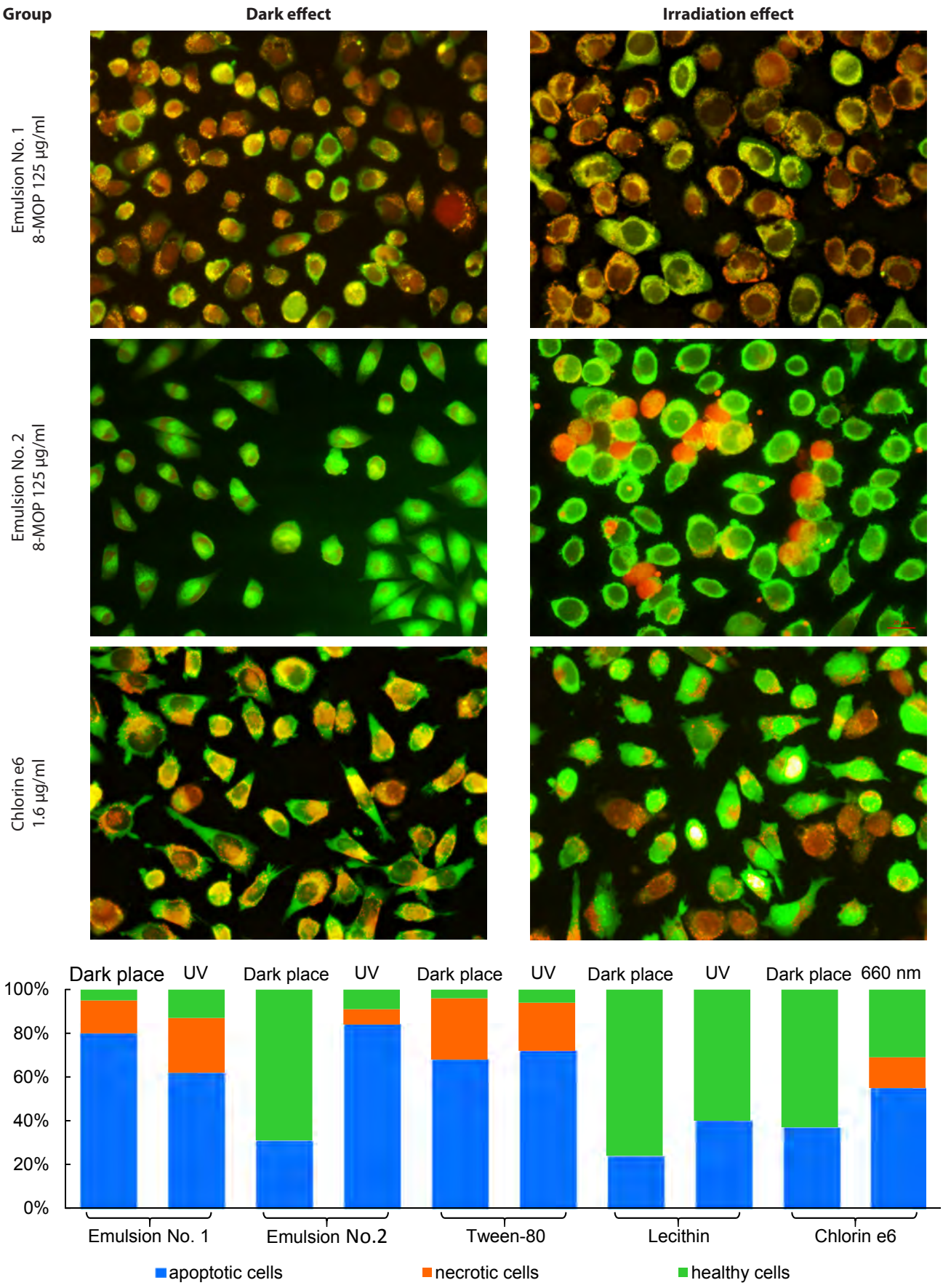

FIG. 5.

Morphology and quantitative evaluation of HLF cells with signs of apoptosis and necrosis under the influence of photosensitizers in the dark and upon photoactivation (explanation in the text)

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SURGERY

OCCLUSION OF RECTAL ARTERIES IN THE TREATMENT OF RECURRENT HEMORRHOIDAL DISEASE AFTER TRANSANAL SURGICAL INTERVENTIONS

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ABSTRACT

The article presents data on variant X-ray anatomy of the superior rectal artery and types of arterial architectonics of the rectum. The causes of recurrent hemorrhoidal disease after traditional transanal surgical interventions are highlighted. The possibilities of endovascular occlusion of rectal arteries in the treatment of relapses of chronic hemorrhoids are shown. Its technical variants are given depending on the number of hemorrhoidal arteries and types of arterial architectonics of the rectum.

Over a 2-year period, clinical results of endovascular occlusion of hemorrhoidal arteries were evaluated in 11 patients with relapse of the disease after various transanal interventions: after latex ligation of internal hemorrhoids – in 5, transanal desarterization of internal hemorrhoids with mucopexia – in 4, hemorrhoidectomy – in 2. The average age of patients was 44.2 ± 12.5 years. Men – 5, women – 6. Superselective occlusion of the target hemorrhoidal branches of the upper rectal artery was performed with EmboGold (Merit Medical) microparticles, 500–700/300–500 mkm in size. Immediate results: cessation of bleeding on the first day – in 4 patients, after 3 days – in 5, after 7 days – in 2. Terms of hospitalization – 1 day, disability – 4.3 ± 0.7 days. Long-term results (from 1 to 2 years) were observed in all 11 patients. The course is relapse-free.

As an illustration, a clinical case is presented – successful treatment of a patient with recurrent hemorrhoidal disease by endovascular occlusion of hemorrhoidal arteries after five traditional transanal surgical interventions: sclerosing, ligation, dearterization of internal hemorrhoids, dearterization of internal hemorrhoids with mucopexia, removal of external hemorrhoids. Within 3 months after endovascular occlusion – complete regression of all symptoms of hemorrhoidal disease. According to the questionnaire (after 1 and 2 years) there is no relapse of the disease, the patient is subjectively satisfied with the endovascular treatment.

Key words: *hemorrhoidal disease, chronic hemorrhoids, recurrence of hemorrhoidal disease, endovascular treatment of hemorrhoids, occlusion of hemorrhoidal arteries, embolization of the superior rectal artery*

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ОККЛЮЗИЯ РЕКТАЛЬНЫХ АРТЕРИЙ В ЛЕЧЕНИИ РЕЦИДИВОВ ГЕМОРРОИДАЛЬНОЙ БОЛЕЗНИ ПОСЛЕ ТРАНСАНАЛЬНЫХ ХИРУРГИЧЕСКИХ ВМЕШАТЕЛЬСТВ

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РЕЗЮМЕ

В статье представлены данные о вариантной рентгеноанатомии верхней ректальной артерии и типах артериальной архитектоники прямой кишки. Освещены причины рецидивов геморроидальной болезни после традиционных трансанальных хирургических вмешательств. Показаны возможности эндоваскулярной окклюзии ректальных артерий в лечении рецидивов хронического геморроя. Приведены её технические варианты в зависимости от количества геморроидальных артерий и типов артериальной архитектоники прямой кишки.

За двухлетний период оценены клинические результаты эндоваскулярной окклюзии геморроидальных артерий у 11 пациентов с рецидивом заболевания после различных трансанальных вмешательств: после латексного лигирования внутренних геморроидальных узлов – у 5, трансанальной дезартеризации внутренних геморроидальных узлов с мукопексией – у 4, геморроидэктомии – у 2. Средний возраст пациентов составил 44,2 ± 12,5 года. Мужчин – 5, женщин – 6. Суперселективную окклюзию целевых геморроидальных ветвей верхней ректальной артерии выполняли микрочастицами EmboGold (Merit Medical), размерами 500–700/300–500 мкм. Непосредственные результаты: прекращение кровотечений в первые сутки – у 4 пациентов, через 3 суток – у 5, через 7 суток – у 2. Сроки госпитализации – 1 сутки, нетрудоспособности – 4,3 ± 0,7 дня. Отдалённые результаты (от 1 года до 2 лет) прослежены у всех 11 больных. Течение – безрецидивное.

В качестве иллюстрации приведён клинический случай успешного лечения пациента с рецидивом геморроидальной болезни путём эндоваскулярной окклюзии геморроидальных артерий после пяти традиционных трансанальных оперативных вмешательств: склерозирования, лигирования, дезартеризации внутренних геморроидальных узлов, дезартеризации внутренних геморроидальных узлов с мукопексией, удаления наружных геморроидальных узлов. В течение 3 месяцев после эндоваскулярной окклюзии – полный регресс всех симптомов геморроидальной болезни. По данным анкетирования (через 1 и 2 года) – рецидива заболевания нет, субъективное удовлетворение проведённым эндоваскулярным лечением.

Ключевые слова: *геморроидальная болезнь, хронический геморрой, рецидив геморроидальной болезни, эндоваскулярное лечение геморроя, окклюзия геморроидальных артерий, эмболизация верхней ректальной артерии*

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INTRODUCTION

The choice of hemorrhoid treatment method depends on the form (acute/chronic), stage of the disease (I–IV) and individual clinical picture. By now, coloproctologists have conservative therapy and various surgical interventions (minimally invasive/hemorrhoidectomy) in their arsenal.

If conservative therapy is ineffective in stage I–III hemorrhoidal disease (HD) without clinically apparent external component, preference is given to minimally invasive surgical treatment methods: sclerotherapy; ligation of internal hemorrhoids with latex rings; transanal desarterization of internal hemorrhoids (IH) under the control of ultrasound navigation ± mucopexia; laser vaporization. In stage IV, the gold standard is hemorrhoidectomy [1].

All known minimally invasive treatments for HD can be categorized as symptomatic and pathogenetic. The latter includes Doppler-guided transanal IH desarterization ± mucopexia. Another pathogenetically substantiated method is endovascular occlusion of hemorrhoidal arteries (HA), which allows to eliminate arterial-venous imbalance [2–4].

There is no doubt that any surgical method of HD treatment, even pathogenetic, is fraught with recurrences in a certain percentage of cases [1, 4]. This raises a number of questions: 1. What underlies HD recurrences after any surgical interventions? 2. How can the risk of recurrence after transanal minimally invasive interventions and hemorrhoidectomy (where repeated invasions through the anus significantly increase the risk of anal canal stenosis/anorectal incontinence) be leveled? 3. Is it reasonable to use endovascular occlusion of rectal arteries for recurrences as the operation of choice?

To date, there is little evidence in the literature to answer the questions posed above. This prompted us to present a vision of the problem of disease recurrence based on our own scientific and clinical studies [4, 5].

THE AIM

To determine the role and place of rectal artery occlusion in recurrent hemorrhoidal disease after traditional transanal surgical interventions.

MATERIALS AND METHODS

In the period from 2020 to 2022, 11 patients with disease recurrence were treated by the method of endovascular occlusion of hemorrhoidal arteries: after latex ligation of internal hemorrhoids – 5, transanal disarterization of hemorrhoidal arteries with mucopexia – 4, hemorrhoidectomy – 2. The recurrences were recorded: within the first year – in 3, from 1 to 2 years – in 8 patients. The patients' mean age was 44.2 ± 12.5 years. Men – 5, women – 6.

Informed consent for this method of hemorrhoidal disease treatment was taken from all patients enrolled in the study.

The technology of endovascular treatment of hemorrhoidal disease was considered and approved by the Ethics Committee of the Prof. V.F. Voino-Yasenetsky Krasnoyarsk State Medical University in 2000 (Minutes No. 7 dated 13.05.2000).

Endovascular intervention was performed through a femoral access on the right by Seldinger. We used: femoral sheath introducer, 5F (Merit Medical, USA), Simmons/Cobra 4, 5F catheters (Boston Scientific, USA), Maestro microcatheter (Merit Medical, USA) – 2.0–2.8F, hydrophilic microwire (Boston Scientific, USA) – 0.035/0.014», contrast – Omnipak. During the diagnostic stage we determined: the number of hemorrhoidal arteries (target vessels), absence/presence of collateral connection of the SRA branches with the middle rectal arteries (MRA). Further, we performed superselective occlusion of all target vessels depending on the X-ray and anatomical variant of SRA division and type of arterial architectonics of the rectum: with EmboGold microparticles, 300–500/500–700 μm (Merit Medical, USA). Immediate results were evaluated in all patients at up to 1 year and long-term results at 1 to 2 years.

RESULTS AND DISCUSSION

The results of our angiographic studies in HD showed that the blood supply of internal hemorrhoids is carried out predominantly by branches of the superior rectal artery (73.1 %). Increased arterial inflow to the hemorrhoids on the background of triggering factors is caused, among other things, by the expansion of the SRA diameter, its distal (hemorrhoidal) branches, predominance of the trifurcation type of branching (69.2 %). A collateral connection of the distal branches of the superior rectal artery (SRA) with the middle rectal arteries (MRA) was detected in 28.3 % of cases [4]. The SRA distal branches were traced in the number of three to the distal rectum (IH localization zone) only in 13.5 % of cases. Four branches were identified in 17.7 % of patients, 5 branches – in 42.2 %, 6 branches – in 16.4 %, and 7 branches – in 10.2 %. Angiographic findings about the variant number of hemorrhoidal arteries are in full agreement with the known anatomical data [1] and ultrasound navigation (Dopplerometry) results [1, 4].

As per angiography data, two variants of SRA branching were found: main type (89.8 % of cases) (Fig. 1a, b) and loose type (10.2 %) (Fig. 1c). Along with that, there was either no collateral connection of distal SRA branches with middle rectal arteries (71.7 %) or its presence: left-sided – in 21.4 % of cases (Fig. 1d), right-sided – in 3.1 % (Fig. 1e), bilateral – in 3.8 % (Fig. 1f).

Based on our identified features of arterial architectonics of the rectum [4] and on our own observations, in 2018, V. Vidal offered a new angiologic classification of arterial architectonics of the rectum, in which two types were distinguished. Type I – absence of collaterals be-

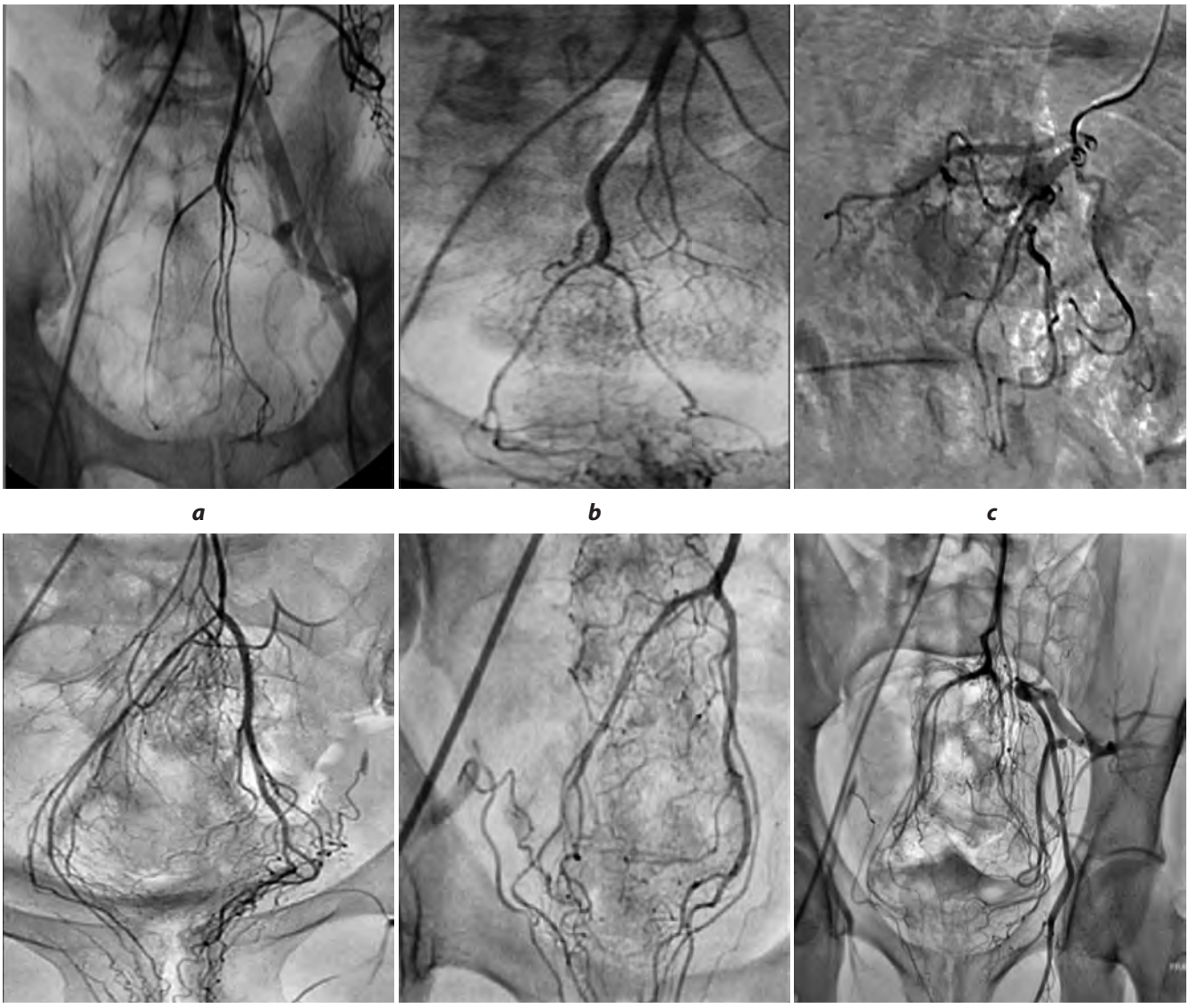


FIG. 1.

Variants of arterial architectonics of the rectum according to angiography. ***a***, ***b*** *– the main type of SRA branching. Absence of collateral connection of hemorrhoidal branches of the SRA with the MRA;* ***c*** *– loose type of SRA branching without a collateral connection with MRA;* ***d*** *– collateral connection of hemorrhoidal branches of the SRA with the MRA (left);* ***e*** *– collateral connection of hemorrhoidal branches of SRA with MRA (right); f – collateral connection of hemorrhoidal branches of SRA with MRA (bilateral)*

tween branches of the superior rectal artery and the middle rectal arteries. Type II – presence of collateral connection [5].

As follows from the above, underestimation of the data on the variant number of hemorrhoidal branches of the SRA and collateral blood supply of the internal hemorrhoids through the middle rectal arteries determines the risk of HD recurrence and may worsen the results of both known transanal minimally invasive interventions and hemorrhoidectomy, as well as endovascular treatment.

Based on these features of blood supply to internal hemorrhoids, different variants of hemorrhoidal artery occlusion are currently used clinically using two main types of microemboli: metal spirals and synthetic particles. In type I arterial architectonics of the rectum only superselective occlusion of hemorrhoidal branches of the SRA with metal spirals is used [6]. In type II, the middle rectal arteries are occluded with metallic spirals simultaneously [6].

Due to the high cost of spirals (in Russia), impossibility of their reinstallation during dislocation, risk of nontarget embolization, we use synthetic microemboli EmboGold, 300–500/500–700 μm (Merit Medical, USA), both for primary treatment of bleeding hemorrhoids and in case of disease recurrence after traditional transanal interventions depending on the X-ray and anatomical variant of SRA division and type of arterial architectonics of the rectum.

In case of disease recurrence after traditional transanal interventions and type I architectonics, superselective occlusion of all target hemorrhoidal arteries

in the SRA basin with EmboGold microparticles (500–700 μm) was performed in 8 patients. In type II – similarly microemboli were used, only of smaller size (300–500 μm) – in 3 patients. They were also injected superselectively into all hemorrhoidal branches of the SRA, but maximally distally – below the collateral anastomoses with the MRA. This technical solution did not require additional endovascular manipulations in the middle rectal artery basin and technically simplified the endovascular intervention.

The mean intervention time was 40.2 ± 9.7 min. Systemic and local complications have not been reported. Pelvic pain syndrome was absent in 8 patients with type I and in 1 patient with type II, both during and after HA occlusion. No pharmacologic therapy was required. In type II (2 patients), there were registered unexpressed pains (2–3 points as per the VAS) in the anus area for up to 3 days. They were effectively treated with oral analgesics.

Immediate results: cessation of bleeding on the first day – in 4 patients, after 3 days – in 5, after 7 days – in 2. Terms of hospitalization – 1 day, disability – 4.3 ± 0.7 days. Long-term results (from 1 to 2 years) were observed in all 11 patients. The course is relapse-free [7].

As an illustration, we present a clinical case of a patient with HD recurrence after repeated transanal surgical interventions.

Patient S., 35 years old. He came to our clinic in August 2020 with complaints of fullness sensation, foreign body sensation in the anus, enlargement of residual hemorrhoids in the upright position, systematic moderate pain in the anus, occasional traces of scarlet blood on toilet paper.

The duration of hemorrhoidal disease is ~10 years. The patient underwent 5 surgical interventions (between 2012–2017) (all by transanal access): 1 – sclerosing, 2 – ligation, 3 – disarterization (HAL), 4 – disarterization with mucopexy of internal hemorrhoidal nodes (HAL-RAR), 5 – removal of external hemorrhoids. Persistent therapeutic effect was not achieved – clinical relapse of the disease. Inability to lead an active lifestyle, predominantly bed rest, job loss, depression. Medical report of the coloproctologist at the place of residence – the whole range of surgical methods of HD treatment is exhausted, conservative therapy is indicated (Detralex 1000 mg 1 time/day, 2-month courses, with intervals of half a year). Conservative therapy is ineffective.

At the time of hospitalization – the condition is satisfactory. General physical status – no abnormalities. Locally (Fig. 2a, b): on examination of the anus area – postoperative deformation of the anal canal, external hemorrhoids are not defined, there are hypertrophied residual internal hemorrhoids with dimensions of 0.6 × 0.9 cm (at 7, 9 and 10 o'clock). At 3 o'clock, the dimensions are 1.2 × 1.5 cm.

Endoscopy (anal canal, rectum, up to 18 cm) showed no organic changes in the rectum mucosa. Dilated hemorrhoidal arteries (up to 0.15–0.2 cm) at 3, 7, 9 and 10 o'clock (4 hemorrhoidal collectors) are visualized in the submucosal layer of the inferior ampullary rectum, and residual hemorrhoidal tissue is visualized accordingly.

DS: Hemorrhoidal disease. Recurrent chronic internal hemorrhoids, stage I–II. Condition after surgical treatment (5 transanal interventions, 2012–2017). Hypertrophied residual internal hemorrhoids (at 3, 7, 9 and 10 o'clock).

The decision of a consilium consisting of a coloproctologist and an endovascular surgeon: endovascular treatment of disease recurrence is indicated.

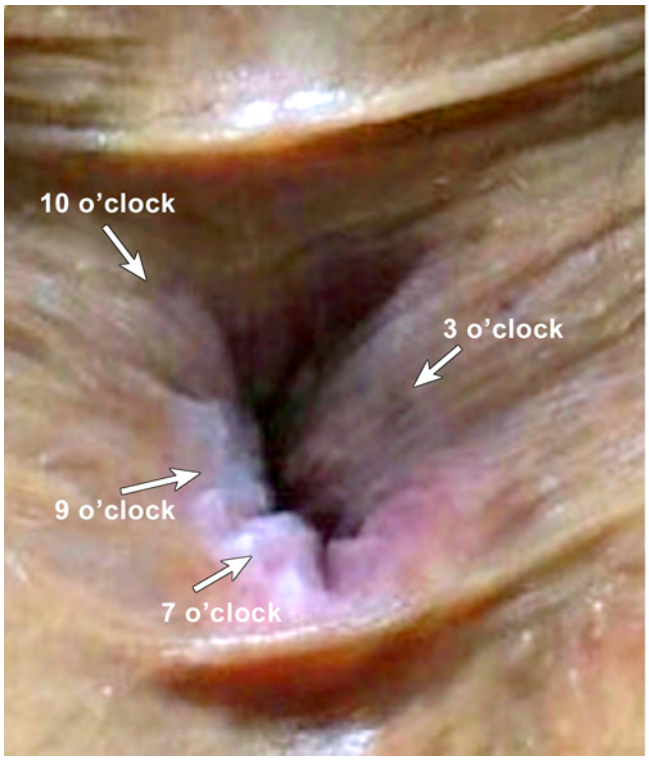
Surgery (27.08.2020): endovascular occlusion of hemorrhoidal arteries in the SRA basin with EmboGold microparticles (500-700 μm). Without colon preparation, under local anesthesia (Lidocaine, 2 ml) by transfemoral Seldinger access on the right side, using a 5F femoral sheath introducer (Merit Medical, USA), a Simmons 4F catheter (Boston Scientific, USA), a 0.014" hydrophilic microwire (Boston Scientific, USA), a Maestro 2.0F microcatheter (Merit Medical, USA), contrast (Omnipak), the diagnostic angiographic stage of the intervention was performed (Fig. 2c). The main type of SRA branching, significant expansion of its diameter, absence of collateral connection with middle rectal arteries (type I of angioarchitectonics of the rectum), 4 hemorrhoidal arteries (also significantly increased in diameter), contrasting of residual IH at 3, 7, 9 and 10 o'clock were established. Complete concordance of clinical data with angiographic findings was confirmed.

Next, superselective occlusion of all (4) hemorrhoidal arteries with EmboGold microparticles, 500–700 μm (Merit Medical, USA) was performed sequentially. The intervention was completed in the absence of contrast of the distal segments of hemorrhoidal arteries and IH (Fig. 2d). The intervention time – 35 min. The patient's well-being during the intervention – satisfactory. No systemic vascular reactions, pelvic and anal pain syndrome, local complications (at the site of femoral artery puncture) were registered. No pharmacologic therapy was required after the intervention. The patient was discharged the next day under the supervision of a coloproctologist at the place of residence. On the 3rd day – 5-hour flight to the place of residence, tolerated well. During the next 3 months – complete regression of all symptoms of hemorrhoidal disease. The patient returned to an active lifestyle and work life. According to the questionnaire (after 1 and 2 years) there is no relapse of the disease, the patient is subjectively satisfied with the endovascular treatment.

CONCLUSION

In recurrences of hemorrhoidal disease after transanal minimally invasive interventions (sclerotherapy, latex ligation, transanal disarterization) and hemorrhoidectomy (when repeated invasions through the anus significantly increase the risk of late complications), it is reasonable to use endovascular occlusion of hemorrhoidal arteries as the operation of choice.

Underestimation of data on the number of hemorrhoidal arteries and the presence of collateral blood supply of in-



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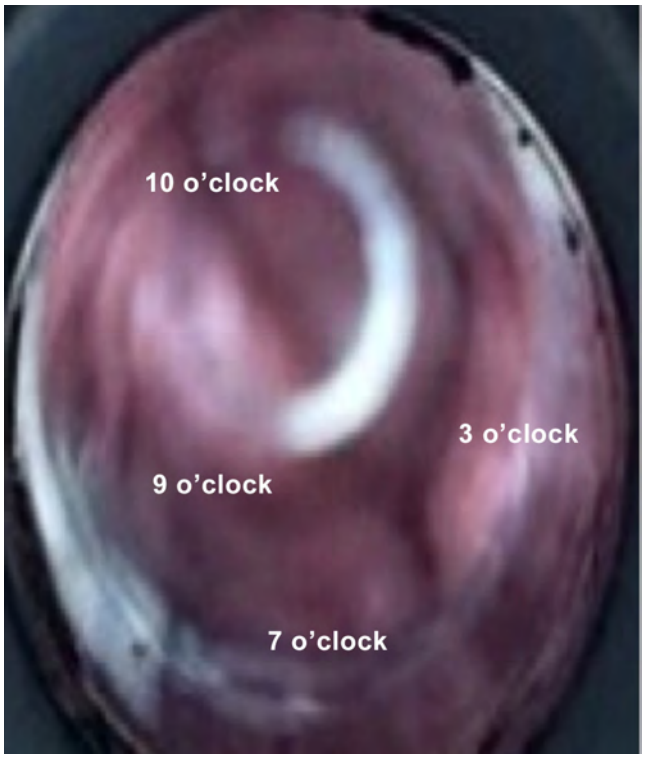

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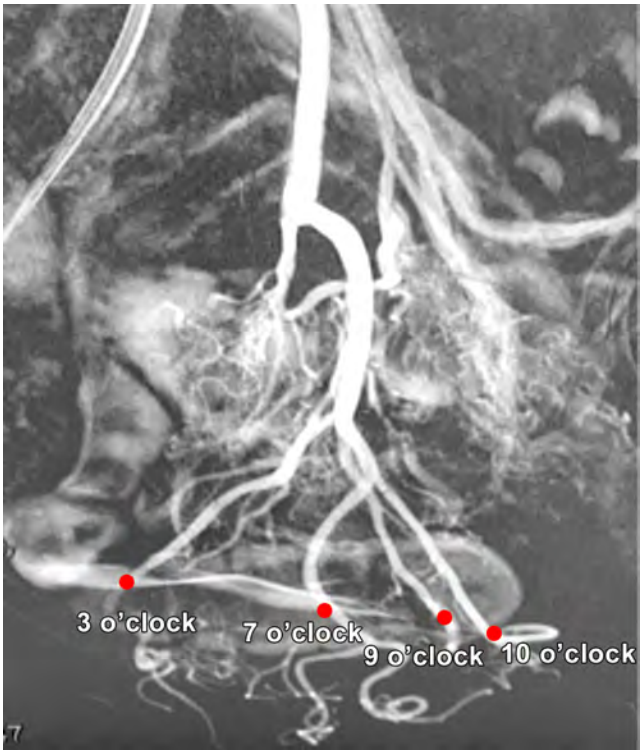

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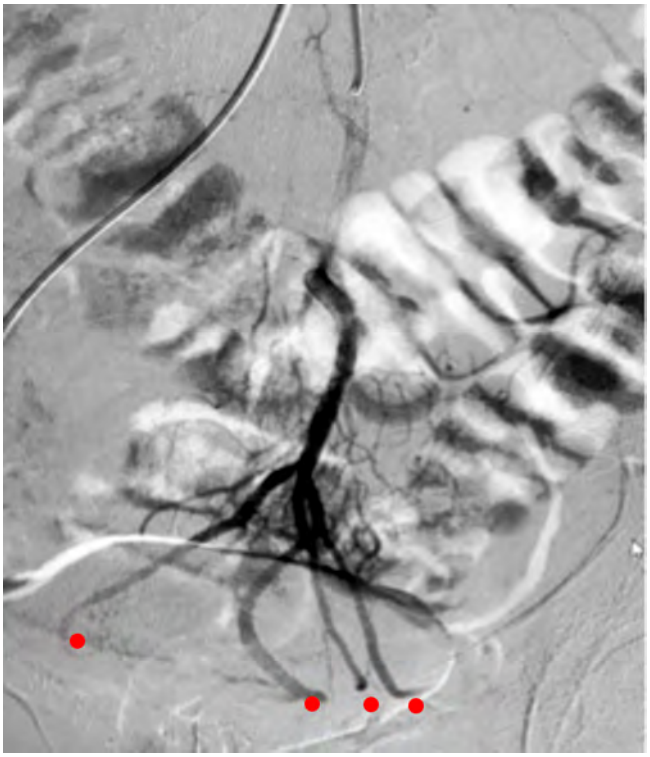

d

FIG. 2.

Patient S., 35 years. Recurrent chronic hemorrhoids, stages I–II (after 5 transanal minimally invasive interventions). ***a*** *– angiography (diagnostic stage). The main branch type is a SRA. Absence of collateral connection with MRA. Four hemorrhoidal arteries. IH contrast at 3, 7, 9 and 10 o'clock;* ***b*** *– external visual inspection of the anus. Postoperative deformity of the anal canal. Hypertrophied IH at 3, 7, 9 and 10 o'clock;* ***c*** *– anoscopy. Hypertrophied IH at 3, 7, 9 and 10 o'clock;* ***d*** *– angiography (therapeutic stage). The immediate result of occlusion of hemorrhoidal arteries (lack of contrast of the IH)*

ternal hemorrhoids through the middle rectal arteries can worsen long-term results (cause recurrences of the disease), both after traditional transanal interventions and after endovascular treatment.

Conflict of interest

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

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ASSESSMENT OF THE RISK OF CARDIOVASCULAR COMPLICATIONS IN CANCER SURGERY

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ABSTRACT

Cardiac complications of non-cardiac surgeries are an actual and unresolved interdisciplinary problem of clinical medicine today. The incidence of cardiovascular events after non-cardiac surgery is higher than in the general population and does not tend to decrease. The risk of cardiac complications in cancer surgery is the highest. Evidence-based approaches to risk assessment and prevention of cardiovascular events in surgical patients with malignant neoplasms have not been developed. In current clinical guidelines on the prevention, prognosis and treatment of cardiac complications of non-cardiac surgeries, the aspects of this problem in surgical oncology are not considered separately.

The aim of this review *was to analyze the current sources of literature on the prediction of cardiovascular complications in surgical treatment of cancer patients. The distinctive features of cancer surgery and additional factors causing an increased risk of adverse cardiac outcomes in patients with malignant neoplasms are described. The article presents the results of large cohort studies on the search for reliable predictors of cardiac complications in non-cardiac surgery and on the development of stratification scales and algorithms for preoperative risk assessment. Particular attention is paid to the possibilities and prospects of using these predictive tools in the surgical treatment of cancer. The surgical risks of interventions for malignant neoplasms are described, as well as methods for calculating cardiac risk and functional status assessment that have been validated in oncological patients cohorts. The data of recent studies on the role of serum biomarkers of myocardial damage and increased cardiovascular risk (cardiac troponins and brain natriuretic peptide) in predicting postoperative cardiac events in non-cardiac surgery are presented. Further prospects for the inclusion of biomarkers in risk stratification systems in patients with malignant neoplasms are discussed.*

Key words: *neoplasms, surgical oncology, myocardial infarction, postoperative complications, risk assessment, natriuretic peptides, troponin*

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ОЦЕНКА РИСКА СЕРДЕЧНО-СОСУДИСТЫХ ОСЛОЖНЕНИЙ В ОНКОХИРУРГИИ

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РЕЗЮМЕ

Кардиальные осложнения внесердечных операций – актуальная и нерешённая на сегодняшний день междисциплинарная проблема клинической медицины. Частота развития сердечно-сосудистых событий после внесердечных хирургических вмешательств выше, чем в общей популяции, и не имеет тенденции к снижению. К наиболее высокому относится риск кардиальных осложнений в онкохирургии. Научно обоснованные подходы к оценке риска и профилактике кардиоваскулярных событий у хирургических пациентов со злокачественными новообразованиями не разработаны. В актуальных клинических руководствах по профилактике, прогнозированию и лечению кардиальных осложнений внесердечных операций аспекты данной проблемы в хирургической онкологии отдельно не рассматриваются.

***Целью данного обзора** явился анализ современной литературы по теме прогнозирования сердечно-сосудистых осложнений хирургического лечения онкологических больных. Описаны отличительные особенности онкохирургических вмешательств и дополнительные факторы, обусловливающие повышение риска неблагоприятных кардиальных исходов у больных злокачественными новообразованиями. В работе приведены результаты крупных когортных исследований по поиску надёжных предикторов кардиальных осложнений во внесердечной хирургии, разработке шкал стратификации и алгоритмов предоперационной оценки риска. Особое внимание уделено возможностям и перспективам использования данных инструментов прогнозирования при хирургическом лечении рака. Описаны хирургические риски вмешательств по поводу злокачественных новообразований, валидированные в когортах онкологических пациентов методы расчёта кардиального риска и оценки функционального статуса. Представлены данные работ последних лет о роли сывороточных биомаркеров повреждения миокарда и повышенного сердечно-сосудистого риска (прежде всего сердечных тропонинов и мозгового натрийуретического пептида) в прогнозировании послеоперационных кардиальных событий во внесердечной хирургии. Обсуждаются дальнейшие перспективы включения биомаркеров в системы стратификации риска у пациентов со злокачественными новообразованиями.*

Ключевые слова: *злокачественные новообразования, онкохирургия, инфаркт миокарда, послеоперационные осложнения, оценка риска, натрийуретические пептиды, тропонин*

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The incidence of cardiovascular complications (CVD) of non-cardiac surgery reaches 5 % [1, 2]. Of the 300 million major non-cardiac surgeries performed annually worldwide, more than one million are complicated by postoperative myocardial infarction (MI), and 750,000 are fatal from cardiac causes within one month of the intervention [3, 4].

Malignant neoplasms significantly increase the risk of perioperative CVD. For example, in surgical treatment of lung cancer, the incidence of all events in high-risk groups reaches 11.9 % [5], and the incidence of MI reaches 9.8 % [6]. In a large cohort of 280,000 cancer patients, any arterial thrombosis developed in lung, gastric, and pancreatic cancer patients – 8.3 %, 6.5 %, and 5.9 % of cases, respectively – over 6 months of follow-up. The relative risk of events was 2.2 times higher than in patients without malignant neoplasms [7]. This is due to a variety of factors: large surgical volume, low functional status of patients due to predominantly elderly age, "frailty", cancer cachexia, sarcopenia, nutritional status disorders and anemia; comorbidity with cardiovascular diseases and chronic obstructive pulmonary disease (COPD); combination with other high cardiovascular risk factors (arterial hypertension, smoking, diabetes mellitus, dyslipidemia); increased thrombogenic risk and systemic pro-inflammatory response characteristic for malignant neoplasms; exposure to neoadjuvant radiation and drug anticancer therapy [8–11]. In advanced cancer stages, the risks of cardiovascular events increase significantly, from 2.3 % for stage 0 to 7.7 % for stage IV [7]. In this regard, disseminated cancer is one of the criteria for the ACS NSQIP (American College of Surgeons' National Surgical Quality Improvement Program) perioperative risk assessment [12].

Implementation of clinical guidelines and algorithms, risk stratification systems in clinical practice allows to improve the quality of medical care, including in non-cardiac surgery [13, 14]. Current guidelines on the prevention, prognosis, and treatment of CVD of non-cardiac surgery lack recommendations with class and level of evidence for cardiovascular risk assessment in oncosurgery [15–17]. Despite the growing interest in this topic, the scale of the problem of cardiac complications and their prognosis is still underestimated by clinicians.

THE AIM OF THIS REVIEW

To analyze the data of current literature on prediction of cardiovascular complications during surgical treatment of cancer patients.

The review was performed using the Russian Science Citation Index, PubMed, and ClinicalTrials databases for the period from 2007 to 2022. The search was performed using the following keywords: neoplasms; surgical oncology; myocardial infarction; heart diseases; postoperative complications; risk assessment; biomarkers; natriuretic peptides; troponin.

SURGICAL RISK ASSESSMENT

The risk magnitude of cardiovascular complications in non-cardiac surgery is determined by three interrelated groups of factors: 1) factors related to the type and volume of surgical intervention (surgical risk); 2) predictors of cardiovascular risk (concomitant heart disease, cardiovascular risk factors, biomarkers); 3) functional status of the patient (Fig. 1).

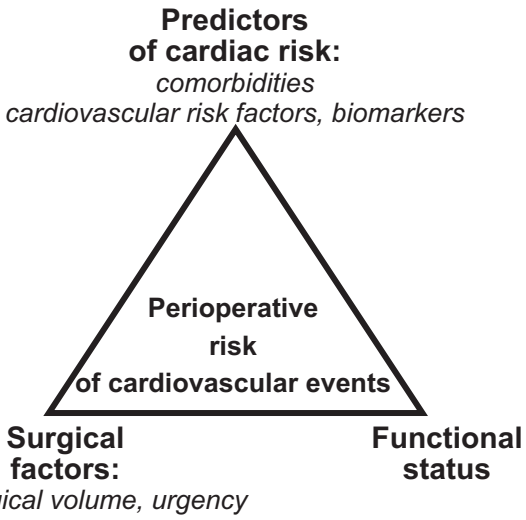

FIG. 1.

Risk factors for perioperative cardiovascular complications of non-cardiac surgery

This is the stepwise approach to perioperative cardiovascular risk assessment proposed in the current clinical guidelines. According to the 2014 ACC/AHA (American College of Cardiology/American Heart Association) Guideline on Perioperative Cardiovascular Evaluation and Management of Patients Undergoing Non-cardiac Surgery, the diagnostic and therapeutic management of a patient is determined by the following sequentially assessed factors: urgency of surgery; presence of signs of acute coronary syndrome; estimated risk of serious adverse cardiovascular events calculated using risk scales/calculators; and functional capacity of the body. The highest risk of CVD is predicted when the calculated risk of serious adverse cardiovascular events and low functional status are elevated, which requires an in-depth cardiologic examination and/or a change in surgical approach [16].

The above-mentioned algorithm is designed to assess the cardiac risk of any non-cardiac surgery. Due to the lack of specialized algorithms validated on cohorts of cancer patients, this approach has been applied in oncosurgery.

When assessing surgical factors in patients with malignant neoplasms, it should be borne in mind that interventions for the most common tumors are classified as high surgical risk due to their extensiveness and are associated with a maximum probability of 30-day cardiovascular mortality or myocardial infarction (MI) (> 5 %) regardless

of cardiovascular factors and functional status of the patient. These include open esophageal and gastric surgeries, pneumonectomies, pancreatoduodenal resections, liver resections, cystectomies, and adrenalectomies [17]. In the 2018 GRICS II (The Goal-Directed Resuscitation in Cancer Surgery) randomized clinical trial to optimize postoperative management of cancer patients, additional criteria for high surgical risk were duration of intervention > 90 min and patient's postoperative stay in the intensive care unit [18]. Medium surgical risk (1–5 %) is associated with interventions on the head and neck, pelvic organs, and minor thoracic surgeries. In such interventions, the outcome of surgery depends largely on the circulatory system and comorbid conditions. Low risk (< 1 %) is associated with surgeries, including oncosurgeries, on skin and subcutaneous tissue, breast, thyroid, minor gynecologic, and urologic (transurethral resection of the prostate).

Surgical risk will increase when the scope of surgery is expanded, such as total resection of the affected organ, anastomosis, and lymph node dissection [19]. In patients with lung cancer, right-sided pneumonectomy had a threefold higher risk of CVD than left-sided pneumonectomy (odds ratio (OR) of developing MI or acute myocardial ischemia was 3.2 with 95 % confidence interval (95% CI): 1.6–6.3) [20]. Conversely, the use of minimally invasive approaches in oncosurgery, such as video-assisted thoracoscopic surgery or robot-assisted surgery, can reduce the incidence of postoperative complications without worsening oncologic outcomes [21, 22].

Surgical risk factors, such as the specific type of surgery and urgency of intervention, are included in modern integral perioperative risk assessment systems, such as the ACS NSQIP Calculator [12]. The presence of high surgical risk procedure is also considered in the calculation of the Revised Cardiac Risk Index (RCRI) [23].

CARDIOVASCULAR RISK ASSESSMENT

Specific scales for assessing the risk of perioperative cardiovascular complications in oncosurgery have not been developed. At the same time, the prognostic significance of previously known risk stratification systems was studied in cohorts of cancer patients. In general non-cardiac surgery, the most widely used index today is the RCRI or "Lee index", which is a revised and simplified version of the first CRI (Cardiac Risk Index) or L. Goldman index created for this purpose. The RCRI index was developed from a single-center cohort study (4,315 patients, 92 events) and determines the risk of MI, pulmonary edema, ventricular fibrillation, cardiac arrest or complete atrioventricular block, and mortality within 30 days after non-cardiac interventions. It includes 6 predictors: high-risk surgery (for abdominal aortic aneurysm, on peripheral vessels, thoracotomy, major abdominal surgery); congestive heart failure; coronary heart disease (CHD); pre-existing acute cerebrovascular accident (CVA); insulin-dependent diabetes mellitus; chronic kidney disease (serum creatinine > 2.0 mg/dL (177 μmol/L) or glomerular filtration rate < 50 ml/min/1.73 m^2), – each of which is assigned one point. With a total score of 0 points, the risk corresponds to 0.4 %, 1 point to 0.9 %, 2 points to 7 %, and 3 points or more to 11 % [23].

However, the prognostic accuracy of the RCRI scale in oncosurgery has proven to be insufficient. In a study by R. Wotton et al. (2013), the RCRI index was calculated in 703 patients (including 640 patients with lung cancer) who underwent lung resection. Cardiac complications in the form of MI, pulmonary edema, cardiac arrest and atrial fibrillation within 30 days after surgery were detected in 34 (4.8 %) patients. According to the results of ROC analysis, the ability of RCRI to correctly predict postoperative cardiac complications was low (area under ROC curve (AUC, area under curve) – 0.59) [24]. In a retrospective case-control study including 163 patients with non-small cell lung cancer (NSCLC) (of whom 33 had postoperative MI, 130 without cardiac complications), RCRI index was not associated with the development of postoperative MI (OR = 1.0 with 95% CI: 0.5–2.2) [25]. In a prospective observational study of 82 patients undergoing pneumonectomy for lung cancer, the associations of RCRI with 6-month overall postoperative mortality were examined. According to univariate regression analysis, the relative risk of mortality increased 2.8-fold with a 95% CI [1.2–6.4] for each point increase in RCRI. However, in multivariate regression, this relationship became statistically insignificant [26].

After 15 years of using the RCRI index in clinical practice, in 2014, European and American experts recommended a new risk stratification system developed by P.K. Gupta et al. (2011) [27]. The new scoring system, NSQIP MICA (The National Surgical Quality Improvement Program Myocardial Infarction & Cardiac Arrest), was created as part of the U.S. National Surgical Quality Improvement Program based on data analysis of more than 450,000 surgical patients from 250 centers and 2,772 events. Five major risk predictors were identified: type of surgery; functional status of the patient; blood creatinine level > 130 μmol/L; ASA (American Society of Anesthesiologists) class; and age. The risk of a predicted event – cardiac arrest or acute MI within 30 days after surgery – is expressed as %. If the index value is < 1 %, the risk is considered low; if the value is ≥ 1 %, the risk is considered elevated. Despite its widespread use in clinical practice, the NSQIP MICA Calculator has not been comprehensively tested outside of the NSQIP registry. No external validation was performed on cohorts of cancer patients.

The idea of creating a universal calculator to calculate the risk of any complications of non-cardiac surgery, including cardiac complications, was realized in 2013. A set of 21 predictors including all major components of perioperative risk was determined: characteristics of surgical intervention, cardiovascular and other clinical factors, and functional status of the patient. Thirteen adverse outcomes were predicted within 30 days of the interven-

tion, including death from any cause and cardiac complications (cardiac arrest or MI) with a high degree of concordance between predicted and occurred events (c-statistic for cardiac outcomes 0.895) [12].

It should be noted that the ACS NSQIP Calculator includes disseminated cancer as one of the predictors for the first time, suggesting that the index can be used in cancer surgery. However, its external validation in several areas of oncosurgery has demonstrated mixed results. In particular, for gynecologic oncologic laparotomy, the scale predicted cardiac complications, deaths, and renal failure well, but failed to accurately predict most other complications [28]. After surgery for gastrointestinal neuroendocrine tumors (GI NETs), the calculator estimated the risks of cardiac complications, pneumonia, and urinary tract infection with acceptable accuracy (AUC > 0.70), but poorly predicted other complications, such as surgical site infection, reintervention, and hospitalization [29]. The ACS NSQIP index, in contrast to RCRI, was an independent predictor of postoperative MI in lung cancer patients undergoing thoracotomy. In this case-control study, 33 patients with MI and 130 patients without cardiac complications were included in the control group. Based on the results of univariate regression analysis, the OR for the development of MI for cardiac risk by ACS NSQIP was 2.2 [1.6–3.2] and for total risk by ACS NSQIP was 1.1 [1.06–1.2]. When the ACS NSQIP cardiac risk was included in multivariate regression, the predictive value of the odds ratio score increased to 3.86 [1.36–10.9] [25].

The Oncology NSQIP National Cancer Center Collaborative attempted to increase the predictive accuracy of the NSQIP ACS by adding oncology-related variables (prior surgery, XRT, or chemotherapy in the same area later and earlier than 90 from the time of index procedure). The sample size was 8,425, 3,166 (37.6 %) of whom underwent colon resection, 2,269 (26.9 %) underwent pancreatectomy, and 1,529 (18.2 %) underwent hepatectomy. The probability of developing a death or serious complication was calculated; cardiovascular outcomes were not counted separately. Univariate analysis showed a 34 % increase in the unadjusted odds ratio for the development of the endpoint in patients with more than 90 days of prior chemotherapy and a 44 % increase in patients with prior X-ray therapy. However, the added variables were not included in the predictive models in the multivariate regression analysis [30].

Attempts are being made to create specialized risk scales for specific tumor localizations, primarily lung cancer. The EuroLung1 and EuroLung2 scales and their simplified versions were developed to assess the risk of car-

TABLE 1

THE RISK ASSESSMENT OF CARDIAC COMPLICATIONS IN THORACIC SURGERY ACCORDING TO THE THRCRI SCALE

Criteria	Points
Prior CHD	1.5
Prior cerebrovascular disease	1.5
Serum creatinine > 2 mg/dL (177 μmol/L)	1
Pneumenectomy	1.5
Class A: 0 points. Risk of cardiac complications – 1.5 % (low)	
Class B: 1-1.5 points. Risk of cardiac complications – 5.8 % (moderate)	
Class C: 2-2.5 points. Risk of cardiac complications – 19 % (high)	
Class D: > 2.5 points. Risk of cardiac complications – 23 % (very high)	

diopulmonary complications (EuroLung1) and 30-day mortality (EuroLung2) after lung resections by analyzing the outcomes of 47,960 operations from the European Society of Thoracic Surgeons (ESTS) Database. They include spirometry, type and volume of surgical intervention, in addition to regular general cardiovascular risk factors (age, male gender, CHD and cerebrovascular disease, chronic kidney disease). Patients are stratified into 6 risk categories based on expected mortality or incidence of cardiopulmonary complications [31, 32]. However, external validation of the original and modified EuroLung2 scale on a sample of 6,600 patients did not confirm its ability to correctly predict 30- and 90-day mortality after lung resection [33]. No external validation of the EuroLung1 scale was performed.

The Thoracic Revised Cardiac Risk Index (ThRCRI) scale was developed based on the RCRI scale in a cohort of 1,629 patients with non-small cell lung cancer, 1,426 of whom underwent lobectomy and 270 of whom underwent pneumonectomy. This model has a reduced number of predictors compared to the RCRI scale. The scale ranks the risk of cardiac complications in thoracic patients from 1.5 % (at 0 points) to 23 % (at > 2.5 points) (Table 1) [34].

The ThRCRI is further validated on two external samples. The first consisted of 2,621 patients, 2,431 of whom underwent lobectomy and 190 underwent pneumonectomy. The incidence of major cardiac events was 0.9 %, 4.2 %, 8 %, and 18 % ($p < 0.0001$) in risk classes A, B, C, and D, respectively [35]. The second sample included 1,255 patients, 85 % of whom underwent lobectomy and 15 % of whom underwent pneumonectomy. The observed event rate was 2.4 % ($n = 30$). The calculated risk in both the test sample as a whole and in each of the risk categories was close to the observed [36]. Later, the ability of the ThRCRI scale to correctly predict not only the risk of perioperative cardiac complications, but also the long-term survival of patients, as well as mortality from cardiac causes [37] was proved. An algorithm for preoperative examination of patients with non-small cell lung cancer using this index has been developed [38]. According to the algorithm, it is necessary to calculate ThRCRI in the first step. If ThRCRI < 2, no further cardiovascular examination is required. A ThRCRI value ≥ 2 indicates increased risk and suggests examination by a cardiologist with additional non-invasive diagnostic tests according to the 2014 ACC/AHA general algorithm described above. [16]. All lung cancer patients scheduled for lung resection, irrespective of ThRCRI value, should have tests to assess external respiratory function and functional pulmonary reserve. Some attempts to validate the ThRCRI were unsuccessful: in a sample of 703 patients undergoing lung resection, the ThRCRI, as well as the RCRI, showed poor predictive efficacy as assessed by ROC analysis (AUC = 0.57) [24]. However, it is the only one of the cardiac risk stratification systems included in the current guidelines for perioperative risk assessment in lung cancer patients [39].

THE ROLE OF BIOMARKERS OF MYOCARDIAL INJURY IN RISK ASSESSMENT OF PERIOPERATIVE CARDIAC COMPLICATIONS

The biomarker concept of risk assessment is a promising and rapidly developing field in cardiology. In recent years, robust evidence has been obtained for the predictive value of preoperative levels of brain natriuretic peptide (BNP) or the N-terminal fragment of its precursor (NT-proBNP) and postoperative cardiac troponin levels in assessing the risk of overall mortality and cardiac complications in a general population of patients undergoing non-cardiac surgery [40, 41]. In a cohort of 4,632 cancer patients at long-term (up to 22 years) prospective follow-up, the biomarkers of myocardial injury troponin T and NT-proBNP were statistically significantly directly correlated with the incidence of cardiac events and mortality. Elevation of NT-proBNP > 900 pg/mL increased the relative risk (RR) of mortality by 2.95-fold (95% CI: 2.28–3.82; $p < 0.001$), and elevation of troponin T > 0.05 μg/L by 2.08-fold (95% CI: 1.83–2.34; $p < 0.001$) [42]. In the work of I.M. Shestopalova et al. (2008) an independent prognostic value of preoperative NT-proBNP in the development of fatal outcomes in cancer patients with different volumes of thoracoabdominal surgeries was revealed [43]. Among 82 patients with non-small cell lung cancer who underwent pneumonectomy, preoperative NT-proBNP level was an independent predictor of 6-month overall mortality after pneumonectomy (OR = 1.2 with 95% CI: 1.08–1.22 for every 100 pg/mL increase). Troponin I increment above the 99th percentile 24–48 hours after surgery was also associated with endpoint development in univariate regression (OR = 3.68 with 95% CI: 1.99–13.58), but lost its predictive value in multivariate regression analysis [26].

According to the 2017 Canadian Cardiovascular Society Guidelines on Perioperative Cardiac Risk Assessment and Management for Patients Who Undergo Noncardiac Surgery, it is recommended that BNP or NT-proBNP be measured before surgery in patients aged ≥ 65 or ≥ 45 years with cardiovascular disease or RCRI ≥ 1. When BNP ≥ 92 mg/L or NT-proBNP ≥ 300 mg/L, troponin levels should be measured daily for 48–72 hours postoperatively to screen for asymptomatic myocardial injury (strong recommendation, medium-quality evidence) [17]. The 2022 ESC Guidelines on cardiovascular assessment and management of patients undergoing non-cardiac surgery require that individuals with cardiovascular disease or its risk factors (including age of 65 years and older) or symptoms should have high-sensitivity troponin T or I measured in the blood before high- and intermediate-risk non-cardiac surgery and 24 and 48 hours afterward (I B). In addition, assessment of BNP or NT-proBNP levels before surgery is envisioned (Iia B) [15].

Thus, the determination of biomarkers of myocardial injury may be an independent criterion for cardiovascular risk assessment of non-cardiac surgery. According to a Cochrane meta-analysis of 51 studies, the inclusion of NT-proBNP, troponin and their combination

in the RCRI scale as an additional predictor improved the prediction accuracy of cardiac complications of non-cardiac surgery (c-statistic increase of 0.08, 0.14 and 0.12, respectively) [44]. It remains unclear whether the inclusion of biomarkers in risk stratification systems for patients with malignant neoplasms improves prediction accuracy, which determines the need for specifically designed studies on this issue.

FUNCTIONAL STATUS ASSESSMENT

General approaches to assessing functional status in patients with malignant neoplasms do not differ from those in surgical patients without neoplasms. For this purpose, the Duke Activity Status Index (DASI) questionnaire is most commonly used in oncosurgery [19, 45]. After assessment of functional status expressed in metabolic equivalents (MET), further patient management is determined according to the ACC/AHA 2014 general algorithm. [16].

Cardiopulmonary exercise testing (CPET) provides the most accurate assessment of functional status. This technique allows to determine not only tolerance to physical exercise, but also a number of important additional parameters: peak oxygen consumption (VO_2max), anaerobic threshold, ventilatory equivalent for carbon dioxide (VE/VCO_2). A number of small-sample studies have confirmed the significance of these CPET parameters in predicting postoperative mortality and cardiac events in patients with gastroesophageal cancer [46], colorectal cancer [47], and liver and pancreatic cancer [48]. At the same time, a large multicenter cohort study evaluating the predictive value of various functional indices and biomarkers (n = 1,404, including 43 % of patients with malignant neoplasms) showed that neither VO_2max nor anaerobic threshold was associated with the development of postoperative MI, myocardial damage, or death [49]. Similar results were obtained in a large prospective study of 1,725 patients, including cancer patients, undergoing major thoracic and abdominal surgery [50]. An important factor limiting the use of CPET in real clinical practice is the high cost of the method and its unavailability in most Russian surgical and oncology clinics. The 2022 European Society of Cardiology (ESC) and 2017 Canadian Cardiovascular Society Guidelines on Cardiovascular Assessment and Management of Patients Undergoing Non-Cardiac Surgery do not include CPET for risk stratification of postoperative complications and mortality. According to the 2014 ACC/AHA guidelines, performing CPET may be considered (IIb B) in patients undergoing high surgical risk procedure in whom functional status is unknown [16].

Increased use of CPET may be considered in patients with lung cancer when assessing the risk of cardiac events and mortality after lung resections or pneumonectomy. This is due to the fact that CPET indices reflect not only coronary but also pulmonary functional reserve and are associated with the prognosis of both cardiac and pulmonary postoperative complications [51]. There is now substantial evidence for the significance of VO_2max and VE/VCO_2 in the prognosis of cardiopulmonary complications of lung surgery. The prognostic value of CPET increases as pulmonary function deteriorates postoperatively [52, 53]. Therefore, the test appropriateness is established based on an assessment of the prognosis of pulmonary function after lung resection. The current American College of Chest Physicians guidelines "Physiologic evaluation of the patient with lung cancer being considered for resectional surgery" (2013) [39] recommend CPET with VO_2max determination for patients with lung cancer and predicted postoperative forced expiratory volume in 1 s (ppFEV1) < 30 % or predicted postoperative diffusing capacity of the lungs for carbon monoxide (DLCO) < 30 % (1 B).

Per the algorithm of M. Salati (2016) concerning preoperative examination of patients with non-small cell lung cancer, the indication for CPET is ppFEV1 < 60 % or predicted postoperative diffusing capacity of the lungs for carbon monoxide < 60 %. Next, VO_2max and VE/VCO_2 are estimated based on the CPET results. When VO_2max < 10 ml/kg/min or VE/VCO_2 > 35, the risk of cardiopulmonary complications after surgery and death is considered high, therefore minimally invasive surgery should be used or other therapies should be considered. If VO_2max is between 10-20 ml/kg/min, risk is assessed by VE/VCO_2. The risk is considered low when ppFEV1 > 60 % or VO_2max > 20 ml/kg/min [38].

"Low-tech" function tests (stair climbing test, 6-minute walk test (6-MWT), incremental shuttle walk test (ISWT)) can be used as a first screening test with exercise. In oncosurgery, their appropriateness is discussed primarily in lung cancer. In a stair climbing test in patients with impending lung resection, climbing less than 12 m (< 3 flights of stairs) was associated with a 2-fold and 13-fold increase in the incidence of postoperative complications and mortality, respectively, compared with climbing more than 22 m (< 1 % mortality) [54]. The 6-minute walk test is widely recognized, well reproducible, and the easiest to perform. The published data demonstrate the clear benefit of the test in perioperative risk stratification of lung resection. Preoperative 6-MWT distance ≥ 400 m was associated with a lower complication rate after lobectomy for lung cancer [55]. Conversely, 6-MWT distance < 525 m was associated with decreased overall patient survival [56]. The incremental shuttle walk test can also be used, especially in patients with concomitant chronic obstructive pulmonary disease. ISWT distance < 400 m correlates with VO_2max < 15 ml/kg/min and a 10% incidence of cardiopulmonary complications. Distance < 250 m in higher-risk patients was associated with a 2.5-fold increase in the rate of serious cardiopulmonary complications (44 % vs. 18 %; p = 0.04) [57]. Patients with ISWT distance < 250 m were 3 times more likely to develop complications after colorectal surgeries (specificity was 0.88, sensitivity – 0.58) [58]. According to a meta-analysis of 7 studies including 1,418 pa-

tients with colorectal cancer, walking distance < 250 m was associated with longer hospital stay after surgery, and no prognostic value could be identified [47]. According to the 2013 ACCP guidelines "Physiologic Evaluation of the Patient with Lung Cancer Being Considered for Resectional Surgery", patients with lung cancer are indicated to perform a stair climbing test or an incremental shuttle walk test when the PpFEV1 or predicted postoperative lung diffusing capacity for carbon monoxide (DLCO) is between 30 % and 60 % (1 C). If the test results show ISWT distance < 400 m or stair climbing height < 22 m, CPET is indicated [39]. Thus, "low-tech" functional tests can be used as an alternative to CPET in patients with a small decline in pulmonary function assessed previously by respiratory tests. Later published algorithms for perioperative evaluation of patients with lung cancer and other malignant neoplasms do not include these functional tests [45, 59].

CONCLUSION

Most cavitary surgeries for malignant neoplasms are categorized as high surgical risk with a probability of 30-day cardiovascular mortality or MI > 5 %.

No specialized guidelines have been developed to assess the risk of cardiac complications in oncosurgery. Preoperative evaluation of cancer patients follows step-by-step algorithms outlined in current guidelines for cardiac risk assessment of non-cardiac surgeries (e. g., the 2014 ACC/AHA Guidelines). The use of the universal ACS NSQIP Surgical Risk Calculator, which has been validated on cohorts of cancer patients, is preferred for quantifying the risk of CVD in patients with malignant neoplasms. In surgical treatment of lung cancer, the ThRCRI scale specifically designed for this category of patients should be used. ThRCRI values ≥ 2 indicate an increased cardiac risk and the need to expand the examination scope. The RCRI index in cancer patients has lower prognostic accuracy. The NSQIP MICA index has not been validated on cancer cohorts.

The significance of preoperative brain natriuretic peptide levels and postoperative cardiac troponins in predicting cardiac complications has been confirmed in cancer patients. If these biomarkers are increased (e. g., NT-proBNP > 300 mg/l preoperatively), careful postoperative screening for acute myocardial injury is required according to the CCS Heart Failure Comprehensive Guidelines (2017).

The Duke Activity Status Index (DASI) questionnaire is used to assess preoperative functional status in oncologic surgery. Low functional status (< 4 METs) suggests a pharmacological stress testing. In high-risk lung cancer patients (ThRCRI ≥ 2 or cardiovascular disease requiring drug therapy or newly diagnosed or inability to climb two flights of stairs), the use of cardiopulmonary exercise testing is being considered to determine functional status and further management. Values of ventilatory equivalent for carbon dioxide > 35 and/or peak oxygen consumption < 10 ml/kg/min indicate a high risk of cardiopulmonary complications, necessitating a change in treatment policy.

In asymptomatic patients with lung cancer with risk factors and a slight decrease in pulmonary function as a screening test, the diagnostic efficacy of "low-tech" functional tests (stair climbing test, 6-minute walk test, Incremental shuttle walk test) has been proved. A cardiopulmonary exercise testing is indicated if ISWT distance < 400 m or stair climbing height < 22 m.

Conflict of interest

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

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OUTCOMES OF SURGERY FOR HIGH TRANSSPHINCTERIC ANAL FISTULAS: PROSPECTIVE RANDOMIZED TRIAL

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ABSTRACT

Background. *Reliable data on the efficacy and safety of fistulectomy with primary sphincter repair for the treatment of high transsphincteric anal fistulas are deficient.*
The aim. *To compare the efficacy and safety of fistulectomy with advancement muco-muscular flap (F) and fistulectomy with primary sphincter reconstruction (SR) for the treatment of high anorectal fistulas.*
Methods. *A cohort of 92 consecutive patients with transsphincteric anal fistula involving 1/3 to 2/3 of the sphincteric complex were included in prospective randomized study. The primary endpoint was the recurrence rate. The duration of surgery, blood loss, pain intensity, postoperative complications, the duration of wound healing, incontinence, quality of life were registered.*
Results. *Forty-six patients were randomized in each group. A statistically significant difference was obtained for operative time (Group "F" – 45 (20–160) min, Group "SR" – 33 (10–55) min). The blood loss was 3 (1–20) and 2 (1–10) ml in Groups "F" and "SR", respectively ($p = 0.482$). The return to work in Groups "SR" and "F" occurred after 7 (2–14) and 8 (4–20) days, respectively ($p = 0.005$). The pain syndrome was significantly greater in Group "F" ($p < 0.05$) on days 1 and 7. Recurrence rate was in 23.9 % (11 cases) in Group "F" and in 6.5 % (3 cases) in Group "SR" ($p = 0.042$). Incontinence was in 7 (15.2 %) people in Group "F", in 10 patients (21.7 %) – in Group "SR" ($p = 0.591$). There was no statistically significant difference in postoperative complications.*
Conclusion. *Findings can expand the indications for the treatment of high transsphincteric anorectal fistulas involving from 1/3 to 2/3 of the sphincter complex without statistically significant risk for functional results.*

Key words: *fecal incontinence, fistula, surgical flaps, recurrence, magnetic resonance imaging*

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РЕЗУЛЬТАТЫ ЛЕЧЕНИЯ ПАЦИЕНТОВ С ВЫСОКИМИ ТРАНССФИНКТЕРНЫМИ АНОРЕКТАЛЬНЫМИ СВИЩАМИ: ПРОСПЕКТИВНОЕ РАНДОМИЗИРОВАННОЕ ИССЛЕДОВАНИЕ

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РЕЗЮМЕ

Обоснование. *Существует недостаток убедительных данных об эффективности и безопасности иссечения свища в просвет с ушиванием сфинктера для лечения высоких транссфинктерных аноректальных свищей.*

Цель исследования. *Сравнение эффективности двух методик – иссечение свища с пластикой слизисто-мышечным лоскутом (Л) и иссечение свища в просвет с ушиванием дефекта сфинктерного комплекса (УС) – для лечения высоких аноректальных свищей.*

Методы. *В проспективное рандомизированное исследование последовательно были включены 92 пациента с транссфинктерным свищом при вовлечении от 1/3 до 2/3 запирательного аппарата. Первичной конечной точкой являлась частота рецидивов заболевания. В ходе исследования проанализированы длительность операции, объём кровопотери, болевой синдром, частота и характер послеоперационных осложнений, длительность заживления раны, инконтиненция, качество жизни.*

Результаты. *Количество пациентов в каждой группе составило 46 человек. Статистически значимая разница получена для длительности операции (группа «Л» – 45 (20–160) мин; группа «УС» – 33 (10–55) мин), при этом объём кровопотери оценивался в 3 (1–20) и 2 (1–10) мл в группах «Л» и «УС» соответственно (p = 0,482). Возвращение к труду в группах «УС» и «Л» наступило через 7 (2–14) и 8 (4–20) дней соответственно (p = 0,005). Болевой синдром был статистически значимо больше в группе «Л» (p < 0,05) на 1-е и 7-е сутки. В группе «Л» рецидивы выявлены в 11 (23,9 %), в группе «УС» – в 3 (6,5 %) случаях (p = 0,042). Инконтиненция в группе «Л» наблюдалась у 7 (15,2 %) человек, в группе «УС» – у 10 (21,7 %) пациентов (p = 0,591). Не получено статистически значимой разницы в отношении послеоперационных осложнений.*

Заключение. *Полученные результаты могут расширить показания для лечения высоких аноректальных свищей при вовлечении от 1/3 до 2/3 сфинктерного комплекса без значимого риска для функциональных результатов.*

Ключевые слова: *анальная инконтиненция, свищ, перемещённый лоскут, рецидив, магнитно-резонансная томография*

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OBJECTIVES

The goal of surgical treatment of anorectal fistulas of any complexity is to prevent recurrence of the disease while preserving fecal continence [1]. Excision of the fistulous passage through the sphincter complex (transsphincteric fistula), its external and internal opening and restoration of sphincter integrity are characterized by the lowest recurrence rates [2, 3]. The higher the level of the location of the internal opening of the fistula, the larger the part of the sphincter that must be crossed and repaired. There is a direct correlation between the volume of the transected part of the rectal closing apparatus and the probability and severity of subsequent incontinence. In this case, the lower third of the anal canal is the boundary beyond which the risk of developing incontinence becomes real.

For higher fistulas, various sphincter-preserving procedures are currently performed. They can be divided into excisional and obliterative procedures. Excisional procedure is aimed at excision of the fistulous passage and its branches with closure of the internal opening using various flaps. Obliteration procedure is aimed at suturing the internal opening of the fistula, which is accompanied by physical, chemical and biological methods aimed at its healing and closure without excision [4–7]. The advantage of the sphincter-preserving surgery is a low rate of incontinence (0–10 %) [8]; the disadvantage is a high rate of recurrence, which varies depending on the procedure, frequency, duration of follow-up and can range from 25 to 100 % [4].

This prospective trial compared two excisional treatments of high transsphincteric anorectal fistulas.

HYPOTHESIS OF THE TRIAL

The hypothesis of the trial was the assumption that fistulectomy with sphincter reconstruction (SR), when 1/3 to 2/3 of the closing apparatus is involved, is accompanied by a lower number of recurrences and does not increase the incontinence rate compared to fistulectomy without sphincter transection and repair of the internal opening using a muco-muscular flap (F).

MATERIALS AND METHODS

The trial was conducted at the I.M. Sechenov First Moscow State Medical University (Sechenov University) and was approved by the local ethics committee (Record No. 18-01 dated 30.09.2017).

Inclusion criteria:

1) transsphincteric rectal fistula, involving 1/3 to 2/3 of the rectal closing apparatus according to magnetic resonance imaging (MRI) of the pelvic organs (Fig. 1);

2) the age of the trial participant is older than 18 years of age;

3) signed informed voluntary consent to participate in the trial.

Exclusion criteria:

1) transsphincteric, involving less than 1/3 and more than 2/3 of the sphincter complex; intersphincteric; extrasphincteric rectal fistula;

2) recurrent fistula (recurrence of the disease after previous surgical treatment for a rectal fistula with an internal opening of the same localization);

3) incontinence 1–20 points (according to the CCFFIS (Cleveland Clinic Florida Fecal Incontinence Score) scale) [9];

4) inflammatory bowel diseases confirmed by endoscopic and morphological methods (ulcerative colitis, Crohn's disease);

5) anterior anorectal fistula in women;

6) acute purulent paraproctitis;

7) inability to perform MRI of the pelvic organs (presence of metal implants, claustrophobia, etc.);

8) patient's refusal to participate in the trial.

Placement of drainage ligature was not an obligatory stage of treatment, but its presence did not serve as a contraindication to the inclusion of patients in the trial. In case of ligature placement, surgery was performed 8 weeks after that.

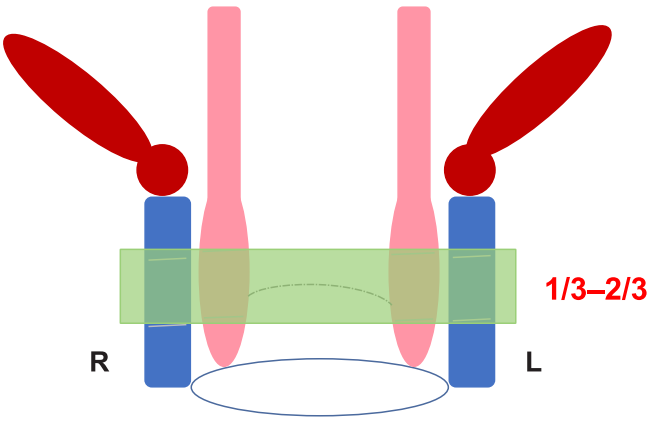


FIG. 1.
Schematic drawing of rectal obturator in patients included in the trial

The primary endpoint of the trial was the recurrence rate of the disease.

One condition or a combination of several conditions was considered recurrence:

1) recurrence of clinical picture not earlier than 2 months after surgical intervention in the form of development of acute inflammation and/or discharge from the wound after its complete healing;

2) chronic non-healing wound (absence of complete epithelialization for more than 3 months);

3) fistulous passage and/or residual cavities according to control MRI of the pelvic organs with intravenous contrast.

The secondary endpoints chosen were:

1) duration of the surgery;
2) volume of blood loss;
3) pain syndrome;
4) frequency and nature of postoperative complications;
5) duration of wound healing;
6) Incontinence from 1 to 20 points (CCFFIS scale);
7) quality of life (SF-36 (Short Form 36) scale) [10].

The following postoperative complications were evaluated: bleeding, flap retraction, postoperative wound pyogenesis, and suture failure on the muscle complex.

The design of the randomized single-center trial is shown in Figure 2.

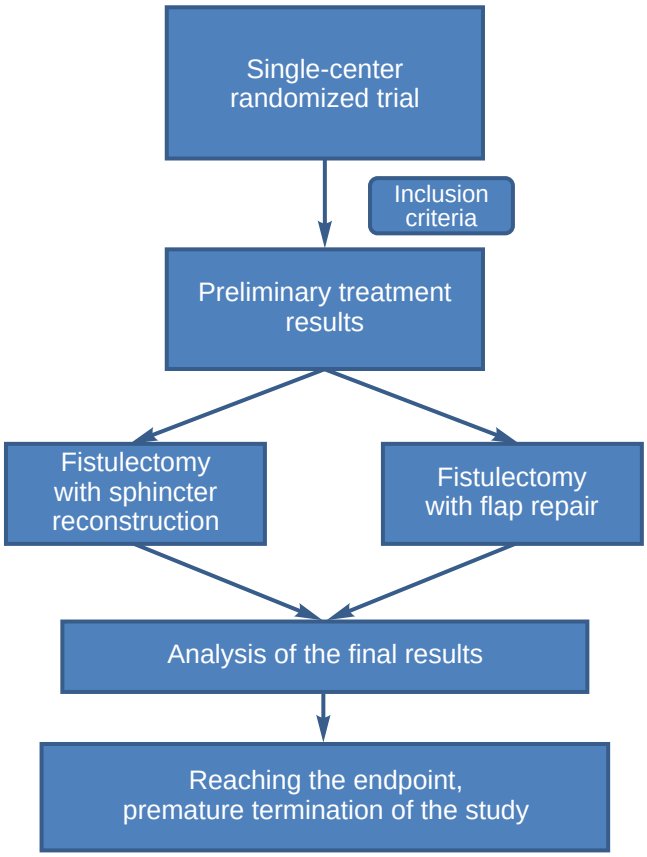


FIG. 2.
Design of a single-center prospective randomized trial

Sample size calculation

The number of patients required to compare the results of fistulectomy with primary sphincter suturing and the use of a mobilized muco-muscle flap was determined using Lehr's formula. The value of a minimum clinically significant difference in recurrence rate of 15 % is based on available data from the literature and our own retrospective experience. Therefore, considering a given power of the trial of 80 % and a type 1 error of 5 %, and to achieve the expected difference, the minimum sample size was 158 patients (79 patients in each group). Interim data analyses were performed when 50 % of the planned number of subjects were enrolled.

Randomization

After meeting the inclusion/non-inclusion criteria, patients were randomly allocated to groups by cluster randomization using Random Allocation Software. Therefore, a sample of 158 units including 2 groups of patients was formed. Patients of Group 1 underwent fistulectomy with sphincter reconstruction, and Group 2 underwent fistulectomy with repair of the internal opening using a muco-muscular flap of the rectal wall. The probability of a patient being placed in one group or another was 50 %.

Given the hypothesis testing nature of the trial, an interim analysis of the results was conducted when 50 % of the planned sample was reached.

In an interim analysis of 92 patients, a statistically significant difference in the number of disease recurrences was obtained, reaching the expected primary endpoint of the trial prematurely and stopping the trial for ethical reasons.

Statistical data processing

The data were analyzed using the SPSS Statistics software package version 22.0 (IBM Corp., USA) after testing for normality of distribution using the Kolmogorov–Smirnov test. The following parameters were used in the analysis: Student's t-test, Mann – Whitney U test, Pearson's chi-squared test, Fisher's exact test. A p value < 0.05 was considered statistically significant.

Preoperative examination

Visual inspection of the perianal area and digital rectal examination were performed. All patients underwent full colonoscopy.

It was also mandatory to perform pelvic MRI with IV contrast on a tomograph with a 3 Tesla magnetic field.

All studies included the following sequence: T1-weighted image (WI), T2-WI with short- and long-axis to the anal canal, T2-WI with suppression of signal from adipose tissue to anal canal, T1-WI with suppression of signal from adipose tissue with intravenous injection of contrast agent into the cubital vein. The record included the following characteristics: localization of the internal and external fistula openings, extent, topographic-anatomical characteristics (direction of the passage, location of the passage in relation to the external sphincter, leakage), assessment of inflammatory changes (infiltrate, abscesses, sphincter involvement).

Continence function was also assessed using the CCFFIS scale and patient's quality of life using

the SF-36 Questionnaire (MH (Mental Health) – mental health assessment; PH (Physical Health) – physical health assessment).

Postoperative patient management

During the postoperative period, antibacterial therapy with broad-spectrum drugs (ciprofloxacin, metronidazole) was performed.

Pain syndrome correction was carried out depending on its severity according to the Visual Analogue Scale (VAS). Monotherapy with non-steroidal anti-inflammatory drugs or combination with narcotic analgesics was used in case of pain intensity more than 6 points. The intensity of pain syndrome was recorded 1, 7 and 28 days after surgery.

There was no stool retention, and the first defecation was performed after the urge to defecate using microclysis. Wound care consisted of daily dressings with treatment with aqueous antiseptic solution, followed by application of hydrophilic ointments.

Quality of life was assessed 14, 28 days and 12 months after surgery using the SF-36 Questionnaire. The level of incontinence was assessed using the CCFFIS scale 28 days and then 12 months after surgery.

Surgical technique

Preoperative preparation

The surgical intervention was performed in a modified lithotomy position using subarachnoid anesthesia.

During intraoperative revision, the fistulous passage was stained through the external fistulous opening with a dye solution in order to identify the internal fistulous opening. A bulbous-end probe was inserted into the fistulous passage to further assess the nature of the passage, the degree of involvement of the sphincter complex, and secondary leakage. If inclusion criteria were met, the patient was randomized to one of the groups.

Fistulectomy with repair of the internal opening using a muco-muscular flap (F)

The external fistulous opening was grasped with an Allis clamp and an electrocoagulator was used to make a skin incision in its projection. The fistulous passage was then sequentially isolated in a single block without dissecting the tissues overlying the fistula.

The next step was mobilization of a lip-shaped muco-muscular flap of the rectal wall (the ratio of the flap width to its length was at least 2:1). The flap was formed according to the size of the sphincter defect after previously performed fistulectomy.

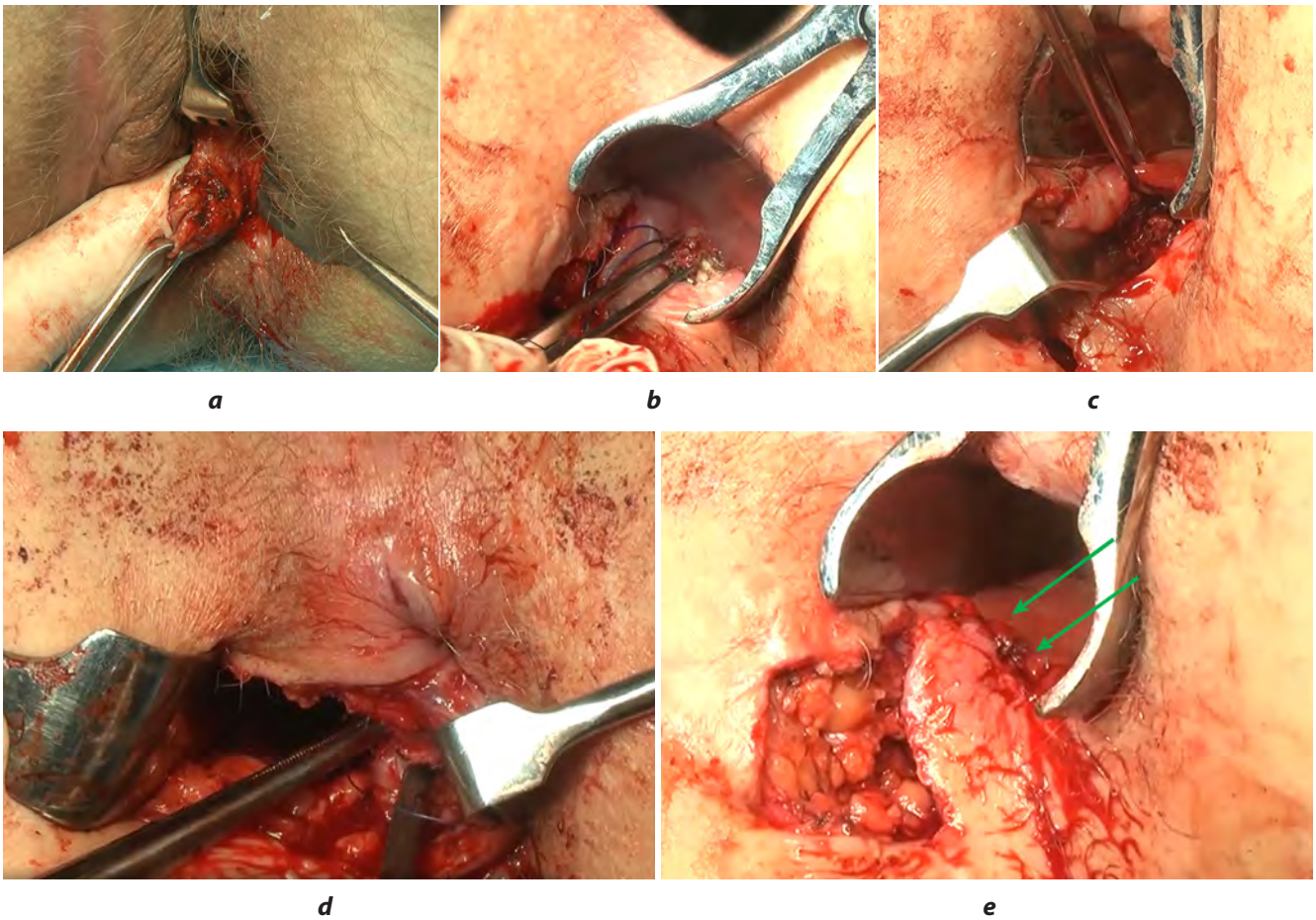


FIG. 3.

*Steps of surgery in Group "F": **a** – fistulectomy; **b** – excision of the internal opening; **c** – mobilization of the muco-muscular flap; **d** – suturing of the sphincteric defect; **e** – fixation of the flap to the anoderm*

The flap was fixed to the distal edge of the defect without tension with separate knotted sutures using Vicryl/Polysorb 3/0. The muscle skeleton defect was sutured with separate knotted sutures on the wound side (Vicryl/Polysorb 2/0). The external perianal wound was not sutured (Fig. 3).

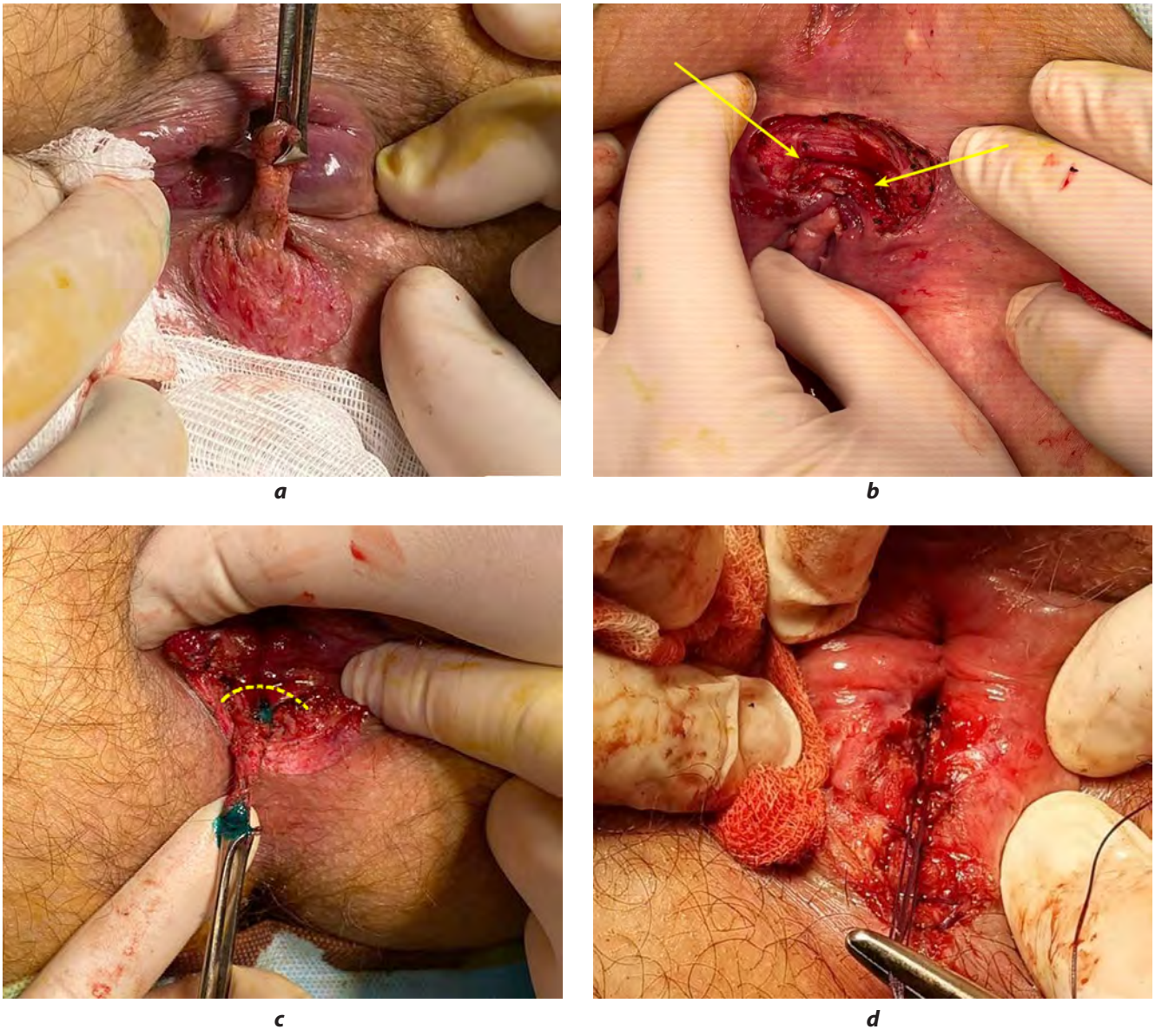
Technique of fistulectomy with sphincter reconstruction (SR)

The external fistulous opening was grasped with an Allis clamp and an electrocoagulator was used to make a skin incision in its projection. The incision was then continued along the course of the main fistulous tract towards the anal canal lumen. The passage was isolated from the surrounding tissues while preserving the integrity of its lumen to remove it en bloc and prevent fragmentation.

After complete removal of the passage, the intersphincteric space, the bed of the affected gland, becomes accessible for visual inspection.

In the projection of the anal canal mucosa, excision was performed with grasping the internal opening.

Sequential suturing of the sphincter defect was performed using individual knotted sutures (Vicryl/Polysorb 2/0). The edges of the sphincter and subcutaneous adipose tissue were aligned with each other to form a flat wound surface without recesses. Suturing of the anal canal mucosa, anoderm and skin over the wound was not performed (Fig. 4).



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

FIG. 4.

*Steps of surgery in Group "SR": **a** – visualization of the posterior wall of the fistula during excision with en-block; **b** – intersphincter space after fistulectomy (indicated by arrows); **c** – the border of internal opening excision (highlighted with a dotted line), the internal opening is painted; **d** – final appearance (separate sutures on the muscle complex)*

RESULTS

The clinical characteristics of the patients are summarized in Table 1.

The groups were comparable in terms of sex, age and duration of ligature placement. In Group "F", drainage ligature was placed statistically significantly more frequently than in Group "SR" ($p = 0.036$). This difference was probably a result of the small sample size.

According to the MRI data, the number and nature of leakage in Groups "F" and "SR" are not statistically significantly different.

When patients' quality of life was assessed using the SF-36 preoperative questionnaire, no statistically significant differences were found in both groups, neither in PH (SF-36) nor in MH values (SF-36).

The duration of surgical intervention in Groups "F" and "SR" was 45 (20–160) and 33 (10–55) min, respective-

TABLE 1
CHARACTERISTICS OF PATIENTS

Characteristics	Group "F" (*n* = 46)	Group "SR" (*n* = 46)	*p*
Age, years	40 ± 10	40 ± 11	0.884
Male, *n* (%)	32 (69.6 %)	34 (73.9 %)	0.817
Female, *n* (%)	14 (30.4 %)	12 (26.1 %)	
Presence of ligature, *n* (%)	26 (56.2 %)	15 (32.6 %)	0.036
Duration of ligature placement before surgery, days	62 (24–397)	81 (18–545)	0.117
Localization of the internal fistulous opening:			
posterior, *n* (%)	27 (58.7 %)	25 (54.4 %)	0.785
anterior, *n* (%)	16 (34.8 %)	17 (37 %)	
lateral, *n* (%)	3 (6.5 %)	4 (8.6 %)	
Presence of leakage			
yes, *n* (%)	11 (23.9 %)	5 (10.9 %)	0.170
no, *n* (%)	35 (76.1 %)	41 (89.1 %)	
Leakage localization			
ischiorectal	3 (6.5 %)	4 (8.7 %)	0.209
intersphincteric	3 (6.5 %)	2 (4.3 %)	
subleavatory	4 (8.7 %)	0	
SF-36 (MH)	53.1 (53.1–54.0)	53.1 (46.2–54.0)	0.151
SF-36 (PH)	54.2 (53.5–57.6)	54.8 (535.–57.6)	0.914

ly, and statistically significant differences between groups were obtained ($p = 0.004$).

The volume of blood loss was 3 (1–20) and 2 (1–10) ml in the "F" and "SR" groups, respectively ($p = 0.482$).

At statistical analysis of VAS 1 and 7 days after the operation there is a statistically significantly greater pain syndrome in the group with repair of the rectal wall using a mucous-muscular flap in comparison with primary fistulectomy with sphincter reconstruction ($p < 0,05$), but after 28 days this difference ceases to be significantly statistically significant ($p = 0,733$).

The following complications were recorded in the early postoperative period: bleeding from the wound, which required re-hospitalization of one patient in the group. One patient in this group was found to have wound infection, which required additional drainage and debridement with antiseptic solutions. In addition, there were two cases of flap retraction that did not require repeated surgical intervention, and conservative treatment consisted of transanal irrigation of the failure area with antiseptic solutions. Two cases of partial suture line disruption on the muscle complex with subsequent wound healing by secondary tension were noted in Group "SR".

The groups were comparable in terms of the number and nature of complications.

The healing time of the external perianal wound in Group "F" was 30.10 ± 1.99 days and in Group "SR" was 26.73 ± 2.55 days and had no statistically significant differences ($p = 0.311$).

During the postoperative period, impaired continence function was noted among 7 (15.2 %) patients in Group "F" and in 10 (21.7 %) patients in Group "SR" ($p = 0.591$) (Fig. 5).

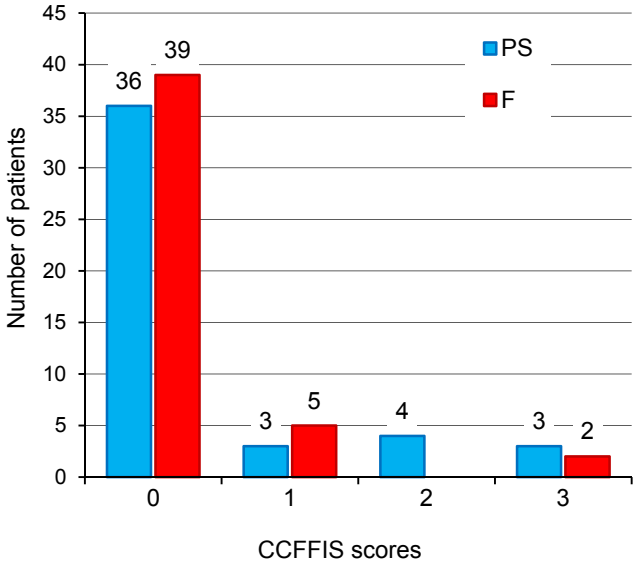


FIG. 5.
Assessment of postoperative incontinence with CCFFI scores

No statistically significant differences were obtained when patients' quality of life was assessed 14, 28 days and 12 months after surgery. A statistically significant difference was obtained only for the physical health assessment (SF-36 (PH)) at day 14 ($p = 0.009$), as shown in Figure 6.

The number of disease recurrences in Group "F" was 11 (23.9 %) cases, in Group "SR" – 3 (65 %), and statistically significant difference in the studied groups is determined ($p = 0.042$).

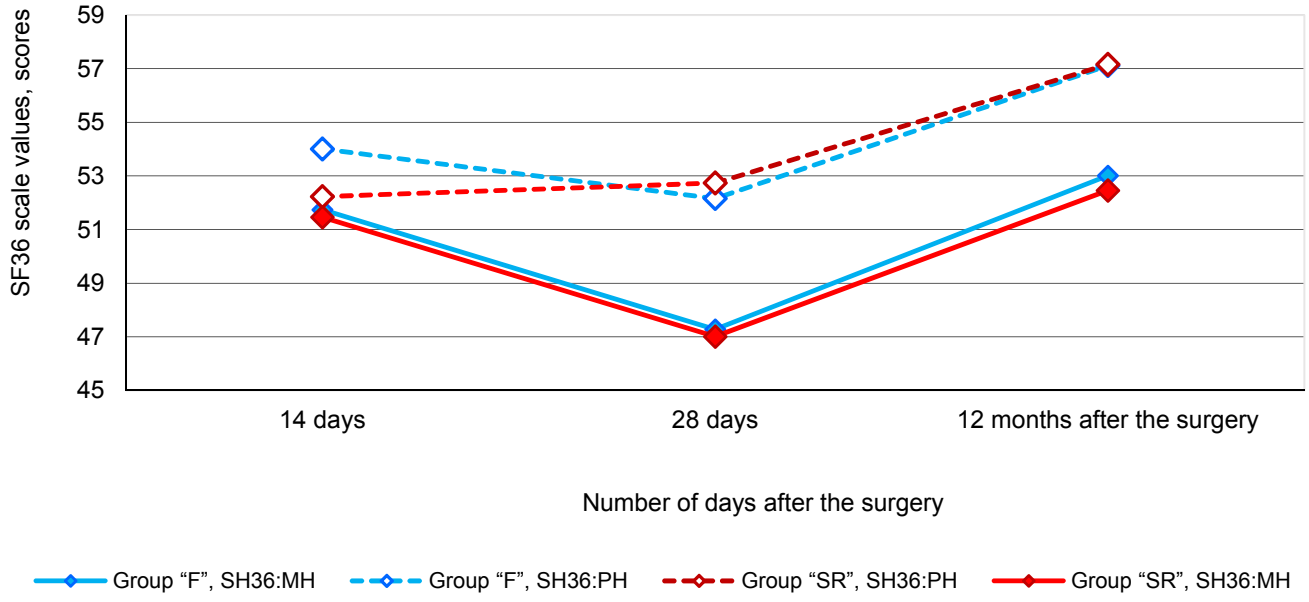


FIG. 6.
Postoperative quality of life. SF-36 (MH) – mental status, SF-36 (PH) – physical status. Patients in Group "SR" recovered faster than those in Group "F" (7 (2–14) and 8 (4–20) days, respectively ($p = 0.005$)).

RESULTS DISCUSSION

Finding the optimal treatment for transsphincteric rectal fistula is a challenge for colorectal surgeons due to a number of aspects, the main ones being preservation of the continence function and prevention of recurrence in the postoperative period.

The position regarding rectal fistulas occupying up to 1/3 of the sphincter complex is now well established, in which fistula dissection or fistulectomy into the intestinal lumen is recommended. The incidence of impaired continence function after such interventions is 0–45 % [11] with a high success rate of 92 % to 97 %.

Various sphincter-preserving techniques have been recommended for transsphincteric fistulas occupying more than 1/3 of the sphincter complex [12, 13]. The advantage of this approach is the low rate of incontinence, the disadvantage is the high recurrence rate even with a highly skilled reference center surgeon [7, 13]. However, the efficacy of the procedure in relation to healing ranges from 65.6 % to 83.7 % [14] for cryptoglandular fistulas.

The absence of a clear position for such a difficult category of patients, when we are talking about the involvement in the pathological process of the area of the sphincter complex (1/3–2/3) provides conditions for further search for an appropriate method of treatment.

In practice, digital and probe examinations were more often used to determine the involvement of the closing apparatus, which was rather subjective. The emergence of more accurate methods for determining the height of the fistula location (transrectal ultrasound (TRUS), MRI of the pelvic organs) has raised the question of the possibility of a continence-safe change in the scope of the use of fistulectomy with sphincter reconstruction.

The experience of the clinic and reports in the literature indicated that the formation of a «safe» scope of fistulectomy with subsequent reconstruction of the closing apparatus took place in an era when it was impossible to confidently determine the height of the passage location and the volume of the sphincter part below this level.

Nevertheless, fistulectomy with primary sphincter reconstruction is now routinely used by surgeons to treat simple low-grade anorectal fistulas with a high recovery rate and without significant compromotation of sphincter function [2, 15, 16]. Fistulectomy in different modifications for high-level fistulas is used only by a number of authors with satisfactory functional results and a relatively low recurrence rate of 12 % [17].

In order to improve functional results, it was proposed in the middle of the 20th century to combine the excision stage with subsequent suturing of the sphincter or suturing of the wound edges to the fundus. However, a number of authors have argued against any kind of plastic surgery in fistulectomy because of the risk of wound infection, sphincter edge dehiscence and subsequent incontinence.

Our trial compared two excisional treatments for high transsphincteric anorectal fistulas.

During the course of the work, a statistically significant difference in disease recurrence between Group “F” and Group “SR” was obtained when assessing the intermediate outcomes, thus confirming the hypothesis and stopping the trial prematurely for ethical reasons. It was possible to prove the safety of fistulectomy with sphincter reconstruction from the point of view of functional results, which expands the indications for use in patients with involvement of the closing apparatus more than 1/3 (less than 2/3).

When analyzing the results, it was found that the duration of surgery was statistically significantly longer in patients with muco-muscular flap formation. This fact is probably due to the fact that fistulectomy without separation of the sphincter fibers is technically more difficult. In addition, the duration of the intervention is also increased by shaping the flap according to certain requirements (sufficient mobility, adequate blood supply).

There are also statistically significant differences in the intensity of pain syndrome: patients in Group “F” have statistically significantly higher VAS scores on day 1 and day 7.

The determined difference in quality of life in patients on day 14 is noted only when analyzing physical health. Statistically significant differences in the form of lower SF-36 (PH) indicators in patients in Group “F” may be due to pain syndrome, size of the wound defect and reduced ability to work.

Incontinence was diagnosed in patients of both groups, but without statistically significant difference ($p = 0.59$). The degree of incontinence, according to the CCFFIS scale, did not exceed 3 points in all patients, which corresponds to minimally severe manifestations of incontinence. According to the meta-analysis by I. Balciscueta et al., the incidence of incontinence increases with increasing flap thickness [16].

However, we did not obtain a statistically significant difference in functional results in the compared groups, but the rate of disease recurrence was statistically significantly higher in the group with flap repair surgery.

The main limitation of this trial is its single-center nature. The surgeries were performed in a reference center with participation of highly qualified surgeons. The presented methods performed by coloproctologists at other clinics may lead to worse results due to their position on a learning curve. Further multicenter studies involving surgeons specializing in treating fistulas are essential.

Also, the trial did not include patients with recurrent fistulas and incontinence, so for this category of patients, fistulectomy with sphincter reconstruction should be recommended with caution.

Another limitation is the fact that after preliminary analyses of patient data, due to a statistically significant difference in recurrence rates, the trial was stopped for ethical reasons.

CONCLUSIONS

Fistulectomy with primary reconstruction of the sphincter complex for the treatment of high transsphincteric fistulas is safe from the point of view of the development of incontinence, accompanied by a lower number of recurrences in comparison with flap repair surgery. The findings may expand the indications for the treatment of high anorectal fistulas when 1/3 to 2/3 of the sphincter complex is involved without significant risk to functional outcomes. Preoperative diagnostics of high accuracy, in particular pelvic MRI with IV contrast, is required to plan surgical treatment from the point of view of the involvement of closing apparatus in the pathological process and the presence of secondary retractions.

Conflict of interest

The authors declare the absence of a conflict of interest regarding this work.

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EXPERIMENTAL RESEARCHES

INFLUENCE OF OBESITY ON THE TONE OF BRONCHIAL SMOOTH MUSCLES IN RATS

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ABSTRACT

Background. *Overweight and obesity are key factors for the occurrence of many morphofunctional disorders in organs and tissues, including bronchopulmonary system.*
The aim. *To study the influence of metabolic disorders that occur against the background of obesity on the state of the airways tone in rats.*
Materials and methods. *Obesity in male Wistar rats was induced using a high-fat and high-carbohydrate diet. In animals, body weight and fat mass were measured, and the heart-lung complex was extracted. In blood serum, the levels of glucose, insulin, leptin, triglycerides, and cholesterol were assessed. Bronchoalveolar lavage fluid was obtained by an open method, in which the concentration of protein, interleukin (IL) 6 and IL-10 was determined. The contractile activity of the isolated bronchial smooth muscle segments was studied using mechanographic method. The effect of acetylcholine (10^{-7}–10^{-4} M), indomethacin (10^{-5} M), and forskolin (10^{-7}–10^{-5} M) on the changes in the tone of airway smooth muscles was assessed.*
Results. *High-fat and high-carbohydrate diet caused an increase in body weight, visceral obesity, hyperglycemia, insulin resistance, leptinemia, dyslipidemia in rats of the experimental group. In the bronchoalveolar lavage fluid of experimental animals, an increase in the content of protein and IL-6 was found, which positively correlated with the level of leptin and the fat mass. In obese rats, the contractile responses of bronchial smooth muscle segments increased in response to the effect of the cholinergic agent acetylcholine. The bronchoconstrictor effect of acetylcholine was reduced by the cyclooxygenase inhibitor indomethacin. In turn, the adenylate cyclase activator forskolin caused relaxation of the airway segments smooth muscles in rats of both groups, which was more pronounced in the experimental group.*
Conclusion. *The obtained results indicate that the change in the reactivity of the respiratory tract can be the cause of bronchospastic conditions in obesity and of the inflammatory reaction in the respiratory system induced by obesity.*

Key words: *bronchi, smooth muscles, obesity, inflammation, insulin resistance*

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ВЛИЯНИЕ ОЖИРЕНИЯ НА ТОНУС ГЛАДКИХ МЫШЦ БРОНХОВ КРЫС

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РЕЗЮМЕ

Обоснование. *Избыточная масса тела и ожирение являются ключевыми факторами для возникновения множества морфофункциональных нарушений в различных органах и тканях, в том числе в бронхолёгочной системе.*
Цель исследования. *Изучить влияние метаболических нарушений, возникающих на фоне ожирения, на состояние тонуса воздухоносных путей крыс.*
Материалы и методы. *Ожирение у крыс-самцов Wistar индуцировали с использованием высокожировой и высокоуглеводной диеты (ВЖВУД). У животных измеряли массу тела и жировой ткани, извлекали комплекс сердце-лёгкие. В сыворотке крови оценивали содержание глюкозы, инсулина, лептина, триглицеридов, холестерола. Открытым способом получали бронхоальвеолярную лаважную жидкость, в которой определяли концентрацию белка, интерлейкина (IL) 6 и IL-10. Сократительную активность изолированных гладкомышечных сегментов бронхов изучали механографическим методом. Оценивали влияние ацетилхолина (10^{-7}–10^{-4} М), индометацина (10^{-5} М), форсколина (10^{-7}–10^{-5} М) на изменение тонуса гладких мышц воздухоносных путей.*
Результаты. *ВЖВУД приводила к увеличению массы тела, висцеральному ожирению, гипергликемии, инсулинорезистентности, лептинемии, дислипидемии у крыс опытной группы. В бронхоальвеолярной лаважной жидкости экспериментальных животных обнаружено повышение содержания белка и IL-6, которое положительно коррелировало с уровнем лептина и массой жировой ткани. У крыс с ожирением происходило усиление сократительных ответов гладкомышечных сегментов бронхов в ответ на действие холиномиметика ацетилхолина. Бронхоконстрикторное действие ацетилхолина снижалось при воздействии ингибитора циклооксигеназы индометацина. В свою очередь, активатор аденилатциклазы форсколин вызывал расслабление гладких мышц сегментов воздухоносных путей крыс обеих групп, более выраженное в опытной группе.*
Заключение. *Полученные результаты свидетельствуют о том, что изменение реактивности дыхательных путей может являться причиной бронхоспастических состояний при ожирении и индуцируемой им воспалительной реакции в респираторной системе.*

Ключевые слова: *бронхи, гладкие мышцы, ожирение, воспаление, инсулинорезистентность*

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INTRODUCTION

Chronic non-communicable respiratory diseases represent an urgent medical and social problem due to high morbidity and early disability of patients [1, 2]. Overweight and diet-induced obesity are considered by researchers as risk factors for the development of bronchopulmonary pathology [3, 4]. Current evidence suggests a close relationship between chronic systemic inflammation induced by nutrients, metabolites, and bioactive substances of adipose tissue cellular elements and the development of increased airway reactivity [1, 5]. Several clinical studies have reported that patients with bronchial asthma and obesity have higher exacerbation rates, impaired response to corticosteroid treatment, and poor quality of life [6, 7].

The regulatory mechanisms underlying such abnormalities are a matter of debate, but have been shown to be directly related to dysfunction of contractile activity of smooth muscle cells of the airway wall [8, 9]. A recent study has demonstrated that airway smooth muscle cells in obesity are characterized not only by increased generation of contractions due to calcium-mediated mechanism, but also by a change in their bioenergetic profile accompanied by an increase in the rate of glycolysis [10]. Along with this, it has been noted that excessive adipose tissue accumulation and associated metabolic disorders can alter cellular composition and contribute to airway remodeling through various molecular mechanisms that form the basis of systemic inflammation [11, 12].

Since the pathological processes in the bronchopulmonary system is formed slowly, and clinical manifestations of respiratory failure are significantly delayed, it is difficult to study the pathogenesis of respiratory dysfunction in obese patients. Therefore, it is of particular interest to use biological models to evaluate the role of diet-induced obesity in the development and progression of the pathological process in the organs of the respiratory system [12, 13].

In this regard, the aim of the work was to study the influence of metabolic disorders that occur against the background of obesity on the state of the airways tone in rats.

MATERIALS AND METHODS

The experiment was performed on 18-week-old male Wistar rats (n = 18) that were on a high-fat and high-carbohydrate diet for 3 months [14]. Animals of the control group (n = 15) received standard laboratory diet during this period. When working with experimental animals, we adhered to the principles of humanity set forth in the European Community Directive (86/609/EEC) and the Declaration of Helsinki; the study was approved by the Ethics Committee of the Siberian State Medical University (protocol No. 8201 dated 27.03.2020).

At the end of the experiment, the animals were CO_2-euthanized. Blood was drawn from the heart, which was then centrifuged for 10 min at 2,000 g to obtain serum. Visceral adipose tissue, heart-lung complex were extracted. To determine the specific gravity of adipose tissue, samples were weighed on analytical balance (Pioneer PX224; OHAUS, PRC).

Bronchoalveolar lavage was performed by the open method on the isolated heart-lung complex [15]. Protein concentration in lavage fluid was determined spectrophotometrically (BCA Protein Assay Kit; Sigma-Aldrich, USA) and cytokines interleukin (IL) 6 and IL-10 by ELISA (Bender MedSystems GmbH kits, Austria). The concentration of glucose (Glucose-TR; Chronolab, Spain), triglycerides, cholesterol (Triglycerides, Cholesterol kits, respectively; Chronolab, Spain) was determined in serum by a colorimetric method, insulin (Insulin Rat ELISA Kit; Thermo Fisher Scientific, USA) and leptin (Rat Leptin ELISA Kit; ELK Biotechnology, PRC) – by ELISA. The HOMA-IR (Homeostasis Model Assessment of Insulin Resistance) index was calculated as serum insulin × serum glucose / 22.5.

The mechanical tension of isolated smooth muscle segments of rat airways (up to the 2nd order) was recorded using a mechanographic method (Myobath II; WPI, Germany). The resulting preparations were incubated in aerated chambers (95 % O_2, 5 % CO_2) filled with Krebs physiological solution (37 °C, pH = 7.35–7.40). Segment contracture was induced with potassium chloride (30 mM) or acetylcholine (10^{-5} M), the amplitude of contractile responses to which was taken as 100 %. The effects of acetylcholine (10^{-7}–10^{-4} M), indomethacin (10^{-5} M), and forskolin (10^{-7}–10^{-5} M) (all Sigma-Aldrich, USA) on contractile responses of airway segments were studied.

Data analysis of the study results was performed in SPSS Statistics 23 program (IBM Corp., USA). The obtained data are presented as mean (M) and standard deviation (± SD), median (Me) and 25th and 75th percentiles (Q_{25}; Q_{75}). Student's t-test or Mann – Whitney U test was used to analyze differences between samples. Differences were considered statistically significant at $p < 0.05$. The Spearman's Rank Correlation Coefficient was determined to assess the relationship between the indicators.

RESULTS

Animals administrated for 12 weeks on a special high-fat and high-carbohydrate diet had an increase in body weight, specific gravity of visceral adipose tissue. This high-fat and high-carbohydrate diet promoted the increase of glucose, insulin and leptin levels in the serum of rats belonging to the experimental group (Table 1). The value of insulin resistance index HOMA-IR in animals receiving high-fat and high-carbohydrate diets was statistically significantly higher than in the control group (Table 1). The rats of the experimental group also showed

TABLE 1

PHYSIOLOGICAL AND BIOCHEMICAL PARAMETERS IN RATS FROM CONTROL AND EXPERIMENTAL GROUPS (M ± SD)

Parameters	Control group (n = 15)	Experimental group (n = 18)
Body weight, g	433.3 ± 39.4	489.1 ± 47.9 (p = 0.01)
Specific gravity of visceral adipose tissue, g	2.2 ± 0.2	4.3 ± 0.6 (p < 0.001)
Fasting glucose, mM	4.7 ± 0.5	6.6 ± 0.4 (p < 0.001)
Insulin, pM	11.2 ± 0.8	24.2 ± 5.6 (p = 0.001)
HOMA-IR	0.4 ± 0.1	1.3 ± 0.4 (p = 0.004)
Leptin, ng/ml	3.1 ± 0.3	4.5 ± 0.1 (p = 0.01)
Cholesterol, mM	1.7 ± 0.2	2.3 ± 0.3 (p = 0.001)
Triglycerides, mM	0.7 ± 0.2	1.7 ± 0.5 (p = 0.001)

Note. p – statistically significant differences with the control group.

a statistically significant increase in the blood content of triglycerides and cholesterol compared to control animals (Table 1).

The study of bronchoalveolar lavage fluid revealed a 1.5-fold increase in protein concentration in rats of the experimental group compared to the control group (1.1 ± 0.3 g/l in the experimental group vs. 0.7 ± 0.2 g/l in the control group; p = 0.037). Also, a statistically significant increase in the concentration of IL-6 in lavage fluid (9.7 (9.4; 15.7) pg/ml in the experimental group vs. 5.3 (4.7; 9.2) pg/ml in the control group; p = 0.007) was found in animals of the experimental group, whereas the level of IL-10 did not differ between the investigated groups. There was a positive correlation between serum leptin levels and protein (r = 0.355; p = 0.020) and IL-6 (r = 0.573; p = 0.005) concentrations, as well as adipose tissue mass and IL-6 levels (r = 0.486; p = 0.005) in lavage fluid.

As a result of studying the constrictor reactions of airway smooth muscles, it was found that the action of the non-selective cholinergic receptor agonist acetylcholine (10^{-7}–10^{-4} M) caused a dose-dependent increase in the mechanical stress of bronchial segments of rats belonging to the control and experimental groups (Fig. 1). At the same time, the amplitude of contractile responses of ring segments in animals belonging to the experimental group was higher than in the control group in the concentration range from 5 × 10^{-6} to 5 × 10^{-4} M (p < 0.05). Airway segment pretreatment with the cyclooxygenase inhibitor indomethacin ((10^{-5} M) for 40 min caused a decrease in acetylcholine-induced contraction of segments in both control and experimental groups (Fig. 1). Statistically significant differences were found with the action of acetylcholine at concentrations of 5 × 10^{-6}–10^{-4} M (p < 0.05). The effect of indomethacin caused a greater inhibition of mechanical stress in the segments of the control group. Activation of adenylyl cyclase by addition of forskolin (10^{-7}–10^{-5} M) against the background of pre-contraction of bronchial segments with acetylcholine caused a dose-dependent decrease in the amplitude of the contractile response of segments obtained from animals of both study groups (Fig. 2). Moreover, a more pronounced drop in mechanical stress was observed in the segments of rats belonging to the experimental group.

DISCUSSION

Visceral obesity is a trigger for multiple metabolic disorders mediating neuroimmunoendocrine dysfunction at the systemic level [1, 3]. Current studies show that chronic inflammation associated with adipocyte hypertrophy and impaired secretory status is strongly associated with the development of airway hyperresponsiveness and may be a cause of respiratory pathology [3, 5, 9].

A key link in the pathogenesis of bronchopulmonary pathology is inflammation and remodeling of the airways, which are regulated by various cell types including immune, epithelial, smooth muscle and fibroblast cells. In obesity, immunocompetent cells of the airways

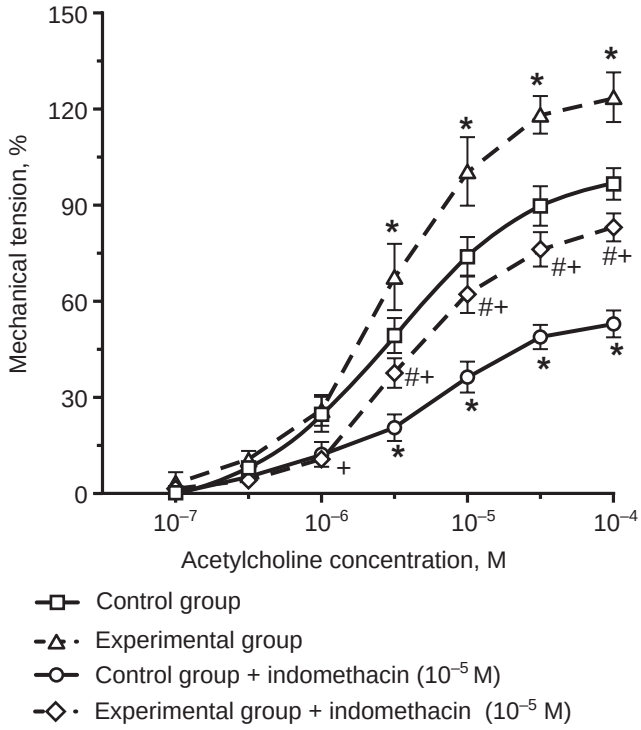

FIG. 1.

*The effect of acetylcholine on the contractile responses of bronchial smooth muscles in rats of control and experimental groups: * – statistically significant differences with the control group at p < 0.05; # – statistically significant differences with the control group + indomethacin (10^{-5} M) at p < 0.05; + – statistically significant differences with experimental group at p < 0,05*

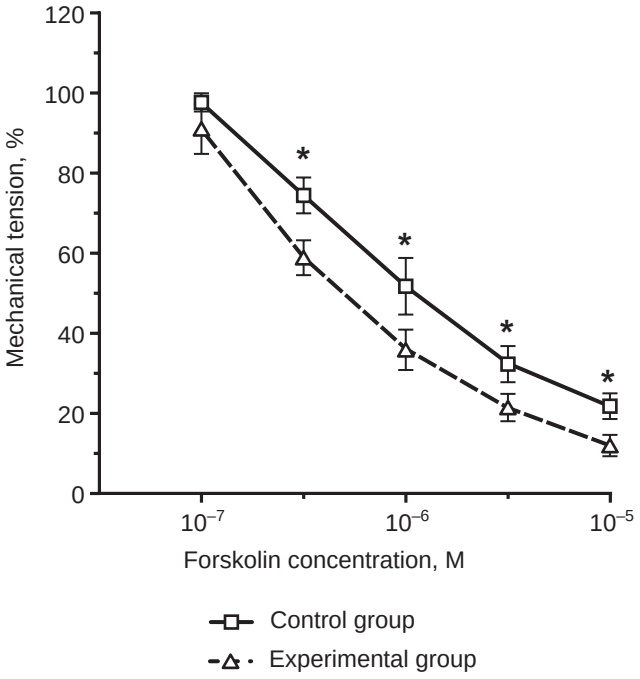

FIG. 2.

*The effect of forskolin on the contractile responses of bronchial smooth muscles in rats of control and experimental groups: * – statistically significant differences with the control group at p < 0,05*

can change their functional phenotype with a predominance of proinflammatory differentiation, which leads to hypersecretion of inflammatory cytokines, thickening of the bronchial wall, subepithelial fibrosis, neovascularization and increased proliferation and hypertrophy of smooth muscle cells [11, 12]. In the studies of A. Kurokawa et al. [8], K. Watanabe et al. [16] showed that the leptin is secreted almost exclusively by adipocytes, can enhance airway hyperresponsiveness by increasing the production of inflammatory mediators and accelerating the differentiation of myofibroblasts. Along with this, leptin increases the expression of intercellular adhesion molecules ICAM-1 in epithelial cells, which promotes the penetration of eosinophils and blood neutrophils into the airway mucosa [17], and various proinflammatory cytokines (IL-6, IL-8, IL-12, IL-12p40, IL-25, IL-33, CCL, etc.) produced by epithelial cells aggravate the inflammatory response of the airways and their hyperresponsiveness [11]. Activated epithelial cells can also enhance airway remodeling by promoting migration of airway smooth muscle cells into the epithelial layer [18]. Smooth muscle cells, in turn, can also maintain a pro-inflammatory status by secreting cytokines such as IL-1, IL-5, IL-6 and IL-8, TGF-β1 and VEGF [19]. The study of A. Matoba et al. [20] showed that an increase in the level of free long chain fatty acids (oleic and linoleic) through the MEK/ERK and PI3K/AKT signaling cascade induces proliferation and hyperplasia of smooth muscle cells of the airways *in vitro*.

To study the airway hyperresponsiveness development mechanisms and inflammation in obesity, the use of animal models is appropriate. We performed experiments on rats maintained for 12 weeks on a high-fat and high-carbohydrate diet. It was found that a high-fat and high-carbohydrate diet leads to changes in physiological and biochemical parameters, which are expressed in the development of diet-induced obesity, hyperglycemia, insulinemia, insulin resistance, leptinemia, dyslipidemia in animals of the experimental group. The obtained results correlate with the data of literature sources, which confirms the effectiveness of a high-fat and high-carbohydrate diet for modeling metabolic disorders, including those caused by increased accumulation of adipose tissue [8, 12]. Thus, biochemical and immunological analysis of bronchoalveolar lavage fluid of experimental animals showed active development of pathological process in the respiratory system. Rats of the experimental group showed an increase in protein and IL-6 concentration, which correlated with leptin levels and adipose tissue mass. In a similar study, mice treat-

ed with a high-fat diet showed increased levels of IL-5, IL-10, and tumor necrosis factor alpha in lavage fluid compared to those of control mice [21]. Thus, it can be concluded that obesity is accompanied by the development of inflammatory response in the bronchopulmonary system of experimental animals.

As it was noted earlier, the processes of airway remodeling are closely related to changes in the functional activity of respiratory epithelial cells and smooth muscles. The epithelium plays a significant role in regulating the contractile activity of airway smooth muscle by secreting various relaxant and constrictor factors, including NO, prostaglandin E_2, and EpDHF [13, 22]. The results of our study suggest that there is an increase in the contractile responses of bronchial smooth muscle cells in response to acetylcholine action during obesity. According to the literature, such cholinomimetic effects may be due to increased release of intracellular calcium from the sarcoplasmic reticulum and subsequent phosphorylation of myosin light chains [9] and/or overexpression of M-cholinergic receptors [23]. There is also evidence that bronchial hyperresponsiveness during obesity and diabetes is due to epithelial damage and impaired prostaglandin production [13, 24]. Pretreatment of airway segments with the cyclooxygenase inhibitor indomethacin leveled the acetylcholine-evoked contraction, but to a lesser extent in rats belonging to the experimental group. The decrease in mechanical tension of smooth muscles of segments against the background of indomethacin supports the hypothesis that it can have an inhibitory effect on phosphodiesterase [25] and thus increase the intracellular concentration of cyclic adenosine monophosphate. In turn, the effect of the adenylate cyclase activator forskolin caused dose-dependent relaxation of smooth muscles of airway segments in rats of both groups, more pronounced – in the experimental group. The obtained results indicate that the change in the reactivity of the respiratory tract can be the cause of bronchospastic conditions in obesity and of the inflammatory reaction in the respiratory system induced by obesity.

CONCLUSION

Overweight and obesity are key factors for a variety of morphofunctional abnormalities in various organs and tissues, including the bronchopulmonary system. This paper shows that high-fat and high-carbohydrate diet-induced obesity promotes the formation of a local inflammatory response and increased airway reactivity in experimental animals. Given the close relationship between obesity and bronchopulmonary dysfunction, an in-depth study of its pathogenesis is necessary in order to improve the methods of prevention and treatment of bronchial obstruction diseases in obese individuals.

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Conflict of interest

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

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NON-ALCOHOLIC FATTY LIVER DISEASE AS A RISK FACTOR FOR ANEMIA OF CHRONIC INFLAMMATION (EXPERIMENTAL RESEARCH)

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ABSTRACT

The aim of the study. *In recent years, non-alcoholic fatty liver disease (NAFLD) has been considered a hepatic manifestation of the metabolic syndrome. The main consequence of NAFLD is chronic hepatic inflammation, which leads to dyslipidemia, inflammation, increased oxidative stress, and endothelial dysfunction. Immune activation in response to interaction with agents of a metabolic nature induces the release of pro-inflammatory cytokines in the liver, which subsequently cause iron homeostasis disorder. This leads to a frequent association of NAFLD with anemia of various etiology. In this regard, we considered it important to assess the severity of the systemic inflammatory response in NAFLD in the experiment in order to diagnose anemia of chronic inflammation.*

Materials and methods. *The study was carried out on 26 male Wistar rats, which were divided into control and experimental groups. In animals of the experimental group, NAFLD was modeled according to the generally accepted method. In order to assess metabolic disorders, we determined the main biochemical parameters, a complete blood count with the calculation of erythrocyte indices, the concentration of the main pro-inflammatory cytokines – interleukin (IL) 1, IL-6.*

Results. *In laboratory rats with NAFLD, a statistically significant increase of intrahepatic enzymes in blood serum was found. The state of the erythrocyte lineage of hematopoiesis in the experimental group progressively worsened and caused the development of anemic syndrome. Synchronously, a statistically significant increase in serum levels of IL-1, IL-6 was recorded, which confirms the correlation of NAFLD with anemia of chronic inflammation.*

Conclusions. *A high concentration of IL-1, IL-6 cytokines in NAFLD inhibits iron absorption in the duodenum, leads to the activation of macrophages, blocking the release of iron processed from aging erythrocytes into plasma.*
Further study of the mechanisms of anemia in NAFLD provides important therapeutic targets in the treatment of both NAFLD and its comorbidities.

Key words: *non-alcoholic fatty liver disease, anemia of chronic inflammation, rats*

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НЕАЛКОГОЛЬНАЯ ЖИРОВАЯ БОЛЕЗНЬ ПЕЧЕНИ КАК ФАКТОР РИСКА АНЕМИИ ХРОНИЧЕСКОГО ВОСПАЛЕНИЯ (ЭКСПЕРИМЕНТАЛЬНОЕ ИССЛЕДОВАНИЕ)

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РЕЗЮМЕ

Цель исследования. *В последние годы неалкогольная жировая болезнь печени (НАЖБП) считается печёночным проявлением метаболического синдрома. Основным последствием НАЖБП является хроническое воспаление печени, которое приводит к дислипидемии, воспалению, усилению окислительного стресса и дисфункции эндотелия. Иммунная активация в ответ на взаимодействие с агентами метаболической природы индуцирует в печени высвобождение провоспалительных цитокинов, которые впоследствии приводят к нарушению гомеостаза железа. Это приводит к частой ассоциации НАЖБП с анемиями различной этиологии. В связи с этим мы посчитали важным оценить выраженность системного воспалительного ответа при НАЖБП в эксперименте с целью диагностики анемии хронического воспаления.*

Материалы и методы. *Исследование проведено на 26 крысах-самцах линии Wistar, которые были разделены на контрольную и экспериментальную группы. У животных экспериментальной группы моделировалась НАЖБП по общепринятой методике. С целью оценки метаболических нарушений определяли основные биохимические показатели, общий анализ крови с подсчётом эритроцитарных индексов, концентрацию основных провоспалительных цитокинов – интерлейкина (ИЛ) 1, ИЛ-6.*

Результаты. *У лабораторных крыс с НАЖБП регистрировалось статистически значимое повышение в сыворотке крови внутрипечёночных ферментов. Состояние эритроцитарного ростка гемопоэза у животных экспериментальной группы прогрессивно ухудшалось, приводя к развитию анемического синдрома. Синхронно регистрировалось статистически значимое повышение в сыворотке уровней ИЛ-1, ИЛ-6, что подтверждает корреляцию НАЖБП с анемией хронического воспаления.*

Выводы. *Высокая концентрация цитокинов ИЛ-1, ИЛ-6 при НАЖБП ингибирует всасывание железа в двенадцатиперстной кишке, приводит к активации макрофагов, блокируя высвобождение железа, переработанного из стареющих эритроцитов в плазму.*

Дальнейшее изучение механизмов развития анемии при НАЖБП предоставляет важные терапевтические мишени в лечении как НАЖБП, так и сопутствующих заболеваний.

Ключевые слова: *неалкогольная жировая болезнь печени, анемия хронического воспаления, крысы*

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Excessive consumption of foods containing fast-digesting carbohydrates, such as fructose and sucrose, leads to the development of metabolic disorders in the liver. Non-alcoholic fatty liver disease (NAFLD) is a striking example of such a disease [1, 2].

A 2016 meta-analysis with a sample size of 8,515,431 people from 22 countries found that 25 % of the world's adult population suffers from NAFLD. Thus, currently, NAFLD is the most common liver disease and one of the main causes of metabolic syndrome [1].

The increasing incidence of NAFLD leads to an increased risk of mortality from associated cardiovascular disease, obesity, type 2 diabetes mellitus and hepatocellular carcinoma [1–3].

The increasing prevalence, especially in recent decades, has made NAFLD the second most common cause of liver transplantation in the United States. The hallmark of NAFLD is primary hepatic steatosis, subsequently exacerbated by non-alcoholic steatohepatitis (NASH), which is characterized by liver inflammation, hepatocyte damage and fibrosis, highlighting the potentially progressive nature of the disease [4–6]. The severity of cirrhosis is the most reliable predictor of long-term clinical outcomes, with marked fibrosis indicating a high risk of hepatocellular carcinoma and death [2, 6]. Metabolic dysfunctions, such as insulin resistance, dyslipidemia, and cardiovascular disease, are directly correlated with hepatic steatosis and appear to be more related to hepatic fat accumulation and NAFLD than to obesity per se [4–6].

The first stage of NAFLD, hepatic steatosis, is the earliest and most common response to excessive ethanol consumption and/or high-calorie and high-carbohydrate diet [4–6]. It is characterized by the accumulation of fat (more than 5 %), mainly triglycerides (TG), in the liver. Excessive lipid accumulation results in multiple parallel blows to the liver: pro-inflammatory action of leptin, release of inflammatory mediators, endoplasmic reticulum stress, Kupffer cell activation, etc. Progressive fatty dystrophy leads, among other things, to mitochondrial dysfunction due to disruption of mitochondrial membrane integrity. The release of free oxygen radicals exacerbates lipid peroxidation, activates liver cell inflammation and apoptosis, which eventually progresses from steatosis to NASH [4–7].

Thus, in addition to lipogenic effects, excessive consumption of fast-digesting fructose triggers inflammatory processes in hepatocytes due to mitochondrial dysfunction and oxidative stress [7].

According to many authors, chronic liver disease is often associated with hematologic abnormalities. Anemias of diverse etiologies occur in approximately 75 % of patients with chronic liver disease [8, 9]. The frequent association of anemia with chronic liver disease provides a rationale for investigating the role of the liver in red blood cell formation and destruction [2, 10], to find out whether the liver itself may indeed be involved in many different mechanisms that contribute to anemia in patients with NAFLD [10].

In this regard, we considered it important to assess the severity of the systemic inflammatory response in NAFLD in the experiment in order to diagnose anemia of chronic inflammation. The study results will provide more information on the pathogenesis and therapeutic strategies for NAFLD.

MATERIALS AND METHODS

The study was conducted on 26 male Wistar rats with body weight of 250–300 g at the time of inclusion in the study in the research laboratory of the Department of Pathological Physiology with a Course of Immunopathology, St. Petersburg State Pediatric Medical University. The animals were obtained from the Nursery for Laboratory Animals of the Branch of the Institute of Biological Chemistry of the Russian Academy of Sciences (IBCh RAS), Pushchino (Moscow Region). Before starting the study, animals were isolated in a special box to undergo a 14-day quarantine.

The study design, standardized operating procedures, and accompanying documentation underwent ethical review by the Local Ethics Committee of the St. Petersburg State Pediatric Medical University (Minutes No. 09/04 dated 11.02.2022).

A total of 2 groups of laboratory animals were formed:

1. "Control" ($n = 13$) – healthy intact rats in which metabolic parameters were investigated to calculate background reference values ("normal values").

2. "NAFLD" ($n = 13$) – rats, which throughout the experiment for 30 days as feed received briquettes containing food components in the following ratios (by weight): 26 % – protein, 10 % – animal fat, 50 % – fructose, 8 % – cellulose, 5 % – minerals, 1 % – vitamins. This diet is standard for experimental modeling of NAFLD [11] and allows to obtain morphological and metabolic changes in hepatocytes of laboratory animals characteristic of this pathology in a fairly short period of time.

Drinking restrictions, hypodynamic conditions were not imposed. The duration of the experiment was 30 days.

Blood was collected from the animals on the 30th day of the experiment by percutaneous puncture of the rat heart into 6 ml Monovette vacuum system (Germany). In the control group, blood collection from all rats was performed on the first day of the experiment.

In order to assess metabolic disorders in experimental animals, the following biochemical parameters were evaluated: biochemical parameters – activity of enzymes alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT).

To detect signs of an impaired red hematopoietic lineage, the hematocrit (HCT), red blood count (RBC), reticulocytes (RTC), hemoglobin level (HGB) were determined

in rats of the tested groups using an Automatic Abbott I Stat Blood Analyzer (Abbott Laboratories, USA) and i-STAT CG8+ cartridges.

The content of the main pro-inflammatory cytokines: interleukin (IL) 1, IL-6 was determined in the blood serum of experimental animals by enzyme immunoassay (EIA) using ELISA diagnostic kits (Cloud-Clone Corp., USA).

To assess morphologic changes and make a final diagnosis, animals were decapitated to collect autopsy material for histologic verification of liver damage in the studied groups of animals. Histologic examination was performed using hematoxylin and eosin staining by light microscopy at ×20 and ×40 magnification.

Statistical processing of the study results was performed using Prism 8 software (GraphPad, USA) and MS Excel 2016 (Microsoft Office Corp., USA). Results are reported as arithmetic mean ± arithmetic mean error (M ± SE). The Kolmogorov – Smirnov test was used to determine the nature of data distribution. The Mann – Whitney U test was used to compare the mean data of independent sample populations (in case of variant distribution other than normal). A statistically significant level of difference was taken as $p < 0.05$ (probability not less than 95 %), which is standard for biomedical experiments.

RESULTS

In the group of animals with NAFLD, the results of biochemical markers of liver damage revealed a statistically significant increase in ALT (46.23 ± 1.19 U/L) and AST (123.3 ± 7.691 U/L) compared to the control (30.96 ± 1.16 U/L, $p = 0.005$ and 101.5 ± 2.404 U/L, $p = 0.005$, respectively). Increased AST and ALT are considered the two most important indicators of liver hepatocyte damage characterizing the development of cytolytic syndrome (Fig. 1).

There was also a statistically significant difference in the level of alkaline phosphorus ($p = 0.005$) in the experimental (22.99 ± 1.092 U/L) and control (14.51 ± 0.81 U/L) groups (Fig. 1).

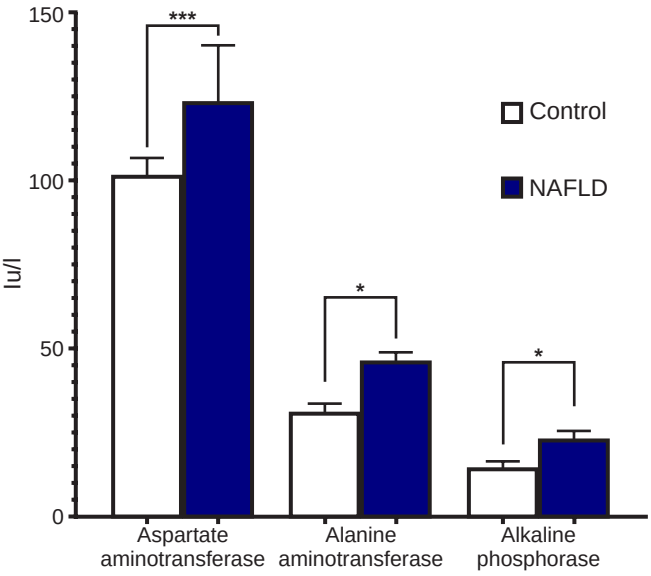
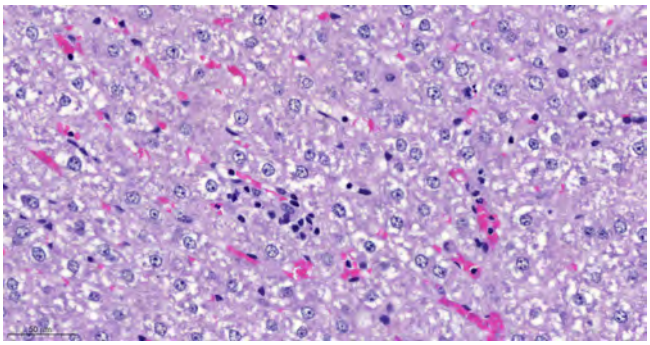


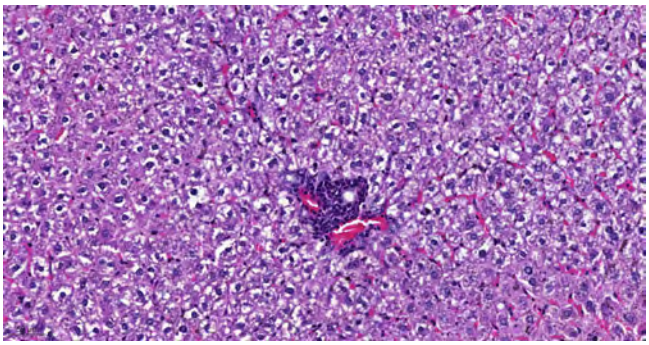
FIG. 1.

*Change in the concentration of hepatic biochemical markers in the blood serum of rats with experimental NAFLD and in control group: * – $p < 0.05$; *** – $p < 0.0003$ in comparison with the intact control group*

The histological study of liver autoptates from animals of control and experimental groups revealed morphological changes of various severity degrees. In rats of the experimental group, marked hyperemia of sinusoids, violation of the beam structure and infiltration by mononuclear cells were registered (Fig. 2a, b).



a



b

FIG. 2.

*Non-alcoholic fatty liver disease (liver autopsy, hematoxylin and eosin staining): lobular lymphocytic infiltration, small droplet fatty degeneration of hepatocytes. Magnification ×40 (**a**), ×20 (**b**)*

In rats' liver autoptates with reproduced NAFLD, large droplet fatty degeneration of hepatocytes was observed: large lipid droplets in the cytoplasm, the nucleus was displaced to the periphery of the cell. Fibrosis of different localization and degree is observed: in some loci – pericellular, pericentral and even in some places bridging portal veins with central veins. All these structural changes indicate a powerful inflammatory process, which may lead to activation of various extrahepatic functions of hepatocytes, such as synthesis of acute phase inflammatory proteins such as ferritin, C-reactive protein, and hepcidin.

Assessment results of erythrocytic hematopoietic lineage in animals with NAFLD confirm the development of anemic syndrome of mild severity by the end of the experiment. A statistically significant decrease in hematocrit (p = 0.016), RBC (p = 0.021) and reticulocytes (p = 0.038) as well as hemoglobin concentration (p = 0.041) per unit of blood volume was registered compared to the control group (Table 1).

TABLE 1

THE STATE OF THE ERYTHROCYTE LINEAGE OF HEMATOPOIESIS IN RATS WITH EXPERIMENTAL NAFLD AND IN THE CONTROL GROUP

Test item	Control group	Experimental group
HCT, %	47.3 ± 0.94	39.1 ± 1.08*
RBC, × 10^{12}/L	7.8 ± 0.18	6,7 ± 0,20*
RTC, %/RBC	15.5 ± 0.85	11.1 ± 1.02*
HGB, g/L	127.3 ± 1.31	95.6 ± 4.77*

Note. * – $p < 0.05$ in comparison with the intact control group

During EIA of concentrations of pro-inflammatory cytokines IL-1 and IL-6 in blood serum of animals belonging to the control and experimental groups, statistically significant differences between these groups were observed, which confirms the important role of cytokines in the response of the liver to pathological effects.

For example, the IL-1 content on the 30th day of the experiment in the group of animals with NAFLD (5.27 ± 0.20 pg/ml) significantly exceeded the initial control (0.84 ± 0.06 pg/ml) (p = 0.011); even more significant differences on the 30th day of the study were observed in the dynamics of IL-6 (12.04 ± 0.4 pg/ml) compared to the animals of the control group (1.54 ± 0.07 pg/ml; p = 0.012) (Fig. 3).

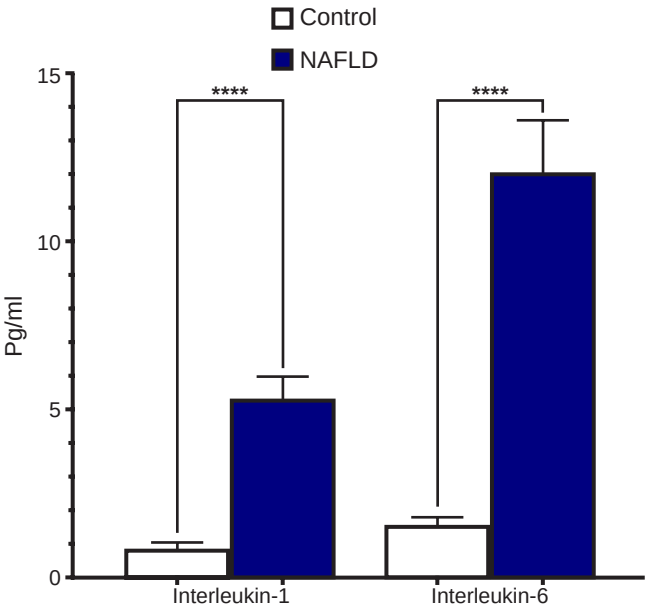


FIG. 3.

*Change in the concentration of pro-inflammatory cytokines in the blood serum of rats with experimental NAFLD and in control group: **** – $p < 0.0001$ compared with intact control*

DISCUSSION

Chronic liver diseases are often associated with hematologic abnormalities. Anemias of various etiologies occur in approximately 75 % of these patients [12].

The underlying causes of anemia associated with chronic liver disease are: gastrointestinal bleeding due to portal hypertension; clotting factor deficiencies [13]; immune-mediated aplasia of red bone marrow [14]; and pharmacological effects of drugs used to treat viral hepatitis [15, 16].

It is well known that one of the leading functions of the liver is protein-synthetic. All blood protein fractions, acute phase proteins, clotting factors, transport proteins, in particular proteins transporting and storing iron (ferritin, transferrin) are formed in the liver [8, 17].

Based on the results of this study, the anemia developed by modeling of NAFLD is hyporegenerative (RTC – 11.1 ± 1.02 % vs. control – 15.5 ± 0.85 % (p = 0.038)), hypochromic (HGB – 95.6 ± 4.77 g/l vs. control – 127.3 ± 1.31 g/l (p = 0.041)), which confirms the fact of iron deficiency development.

Immune activation in response to interaction with agents of microbial, immune, tumor, and metabolic nature induces the release of pro-inflammatory cytokines that subsequently lead to disruption of iron homeostasis. Although it is impossible to fully disentangle the iron-regulatory influences of multiple cytokine networks, IL-6 appears to be the most important, at least in animal models [18]. One of the effects of IL-6 associated with iron metabolism in laboratory animals and humans is its stimulatory effect on increased hepcidin production by hepat-

ocytes [19, 20]. Hepcidin, a major systemic regulatory factor of iron metabolism, binds to the protein ferroportin 1 and induces its lysosomal degradation. This reduces circulating iron concentration by decreasing the release of recycled iron from macrophages and deposited iron in hepatocytes [8, 17, 19, 21].

It is known that most iron is deposited in hepatocytes and macrophages of the reticuloendothelial system as part of ferritin, while hepatocytes obtain iron mainly by absorption of transferrin. Hemosiderin, another iron-storage protein, is formed when ferritin is depleted and is mainly found in cells with iron overload and mobilizes iron irregularly and slowly [10, 18].

Iron sequestration in macrophages also plays a significant role, as recycling of iron from aging erythrocytes by macrophages accounts for > 90 % of the daily iron requirement for hemoglobin synthesis and erythropoiesis [8, 22].

Liver disease is often associated with hematologic abnormalities. A large number of patients with NAFLD have anemia of varying severity, and its pathogenesis is complex. Timely diagnosis and therapy of anemic syndrome in NAFLD can prevent complications of the underlying disease.

CONCLUSIONS

Thus, there is reason to believe that the increased concentration of pro-inflammatory cytokines IL-1, IL-6 in NAFLD inhibits iron absorption in the duodenum, where ferroportin is required for absorption of dietary iron into the bloodstream, and they also act on macrophages to block the release of iron recycled from aging erythrocytes into plasma.

Further study of anemia development mechanisms as one of the links in the pathogenesis of NAFLD may provide important therapeutic targets in the treatment of both NAFLD and comorbidities.

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Conflict of interest

The authors declare no conflict of interest.

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EPIDEMIOLOGY

RETROSPECTIVE ANALYSIS AND MODERN SPATIOTEMPORAL CHARACTERISTICS OF TULAREMIA IN THE TERRITORY OF THE WEST KAZAKHSTAN AND NORTH KAZAKHSTAN REGIONS

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ABSTRACT

Rationale. *An important task is to monitor the incidence of tularemia among the population of Kazakhstan. Natural foci of this infection occupy large areas. In some regions with large numbers of rodents and ectoparasites and low vaccination coverage, human cases of tularemia have been reported.*

The aim of the study. *To carry out retrospective analysis and to study modern spatiotemporal characteristics of tularemia in the West Kazakhstan and North Kazakhstan regions in order to improve the effectiveness of preventive measures.*

Materials and methods. *In our work, we used public records, the results of an epizootological survey of tularemia natural foci and the official data from the Departments of Sanitary and Epidemiological Control of two regions on the human cases of tularemia in 2000–2021. We used descriptive statistics methods, relative and absolute indicators of the tularemia incidence in the population for the analysis. The phenotypic and genetic properties of the strains isolated in 2000–2021 were studied according to the guidelines.*

Results. *A retrospective analysis of the tularemia incidence among the population of the North Kazakhstan and West Kazakhstan regions showed an improvement in the epidemic situation. Over the past 20 years, 4 human cases of tularemia have been registered in the West Kazakhstan region, while the epizootic potential was quite high; more than 300 strains of the tularemia microbe were isolated during the studied period. In the North Kazakhstan region from 2000 to 2021, 11 human cases of tularemia were registered; when studying rodents, mammals and environmental objects, single positive samples for specific tularemia antibodies and antigens were detected; no strains of tularemia microbe were isolated.*

Conclusion. *An analysis of long-term data on the epizootic and epidemic activity of tularemia natural foci, processed using descriptive statistics and GIS technology, made it possible to identify places of long-term persistence of the tularemia agent in the natural focus of the North Kazakhstan and West Kazakhstan regions and to create an electronic map of the territories endemic for tularemia to determine the scope of preventive measures.*

Key words: *tularemia, natural foci, incidence, ectoparasites, rodents*

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РЕТРОСПЕКТИВНЫЙ АНАЛИЗ И СОВРЕМЕННАЯ ПРОСТРАНСТВЕННО-ВРЕМЕННАЯ ХАРАКТЕРИСТИКА ТУЛЯРЕМИИ НА ТЕРРИТОРИИ ЗАПАДНО-КАЗАХСТАНСКОЙ И СЕВЕРО-КАЗАХСТАНСКОЙ ОБЛАСТЕЙ

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РЕЗЮМЕ

Обоснование. *Важной задачей является слежение за заболеваемостью населения Казахстана туляремией. Природные очаги этой инфекции занимают большие территории. В некоторых регионах с высокой численностью грызунов и эктопаразитов и низким охватом вакцинацией регистрируют случаи заболевания людей туляремией.*

Цель исследования. *Ретроспективный анализ и изучение современной пространственно-временной характеристики туляремии в Западно-Казахстанской и Северо-Казахстанской областях для повышения эффективности профилактических мероприятий.*

Материалы и методы. *В работе использованы архивные документы, результаты эпизоотологического обследования природных очагов туляремии, официальные сведения Департаментов санитарно-эпидемиологического контроля двух областей о случаях заболевания людей туляремией в 2000–2021 гг. Для анализа использованы методы описательной статистики, относительные и абсолютные показатели заболеваемости населения туляремией. Фенотипические и генетические свойства штаммов, выделенных в 2000–2021 гг., изучали согласно методическим рекомендациям.*

Результаты. *Ретроспективный анализ заболеваемости людей туляремией в Северо-Казахстанской (СКО) и Западно-Казахстанской (ЗКО) областях показал, что в период с 2000 по 2021 г. наблюдается улучшение эпидемической ситуации. В ЗКО за последние 20 лет было зарегистрировано 4 случая заболевания людей туляремией, при этом эпизоотический потенциал довольно высокий – в рассматриваемый период выделено более 300 штаммов туляремийного микроба. В период с 2000 по 2021 г. в СКО зарегистрировано 11 случаев заболевания людей туляремией; при исследовании грызунов и млекопитающих, объектов внешней среды выявляют единичные положительные пробы на специфические туляремийные антитела и антигены, штаммы туляремийного микроба не выделены.*

Заключение. *Анализ многолетних данных по эпизоотической и эпидемической активности природных очагов туляремии, обработанный с помощью описательной статистики и ГИС-технологии, позволил выявить места длительного сохранения возбудителя туляремии в природном очаге Северо-Казахстанской и Западно-Казахстанской областей и создать электронную карту эндемичных по туляремии территории населённых пунктов для определения объёмов профилактических мероприятий.*

Ключевые слова: *туляремия, природный очаг, заболеваемость, эктопаразиты, грызуны*

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Tularemia has been reported among population in many countries around the world [1]. The tularemia microbe has taken root in Kazakhstan as a result of natural conditions favourable for carriers and vectors, as well as the possibility of *Francisella tularensis* existence in their bodies. Mammals spread the infection widely by vectors. Cases of tularemia among people are registered in almost all regions of the Republic of Kazakhstan.

Focal tularemia territories occupy huge areas in Kazakhstan – more than 550 thousand km^2. In the 1930s–1950s, there was a high incidence of tularemia among humans. Specific preventive measures contributed to the improvement of the epizootic situation. Between 1999 and 2021, 86 cases of tularemia among humans were registered in Kazakhstan.

In Kazakhstan, the landscape complex of tularemia focality is represented by foothill brook, floodplain swamp, tugai and steppe types of foci. Floodplain swamp foci are located in Pavlodar, Kostanay Akmola, Aktobe, Almaty, Atyrau, East Kazakhstan, West Kazakhstan and Karaganda regions. Foothill-brook foci are located on the territory of Almaty and East Kazakhstan regions. Tugai foci were registered in Jambyl (or Zhambyl) and Kyzylorda regions. Steppe foci are present in the West Kazakhstan and Pavlodar regions.

Different routes of transmission have been recorded in floodplain swamp areas [2]. The highest percentage of cases is recorded with vector-borne infection.

During a focus inspection in the West Kazakhstan and North Kazakhstan regions we have found high infection rate among rodents and vectors. In Kostanay, Jambyl, Karaganda, East Kazakhstan, Akmola, Aktobe and Pavlodar regions, focal tularemia territories are currently inactive. There are no foci of tularemia on the territory of Mangystau and Turkistan regions [3].

THE AIM OF THE STUDY

To carry out retrospective analysis and to study modern spatiotemporal characteristics of tularemia in the West Kazakhstan and North Kazakhstan regions in order to improve the effectiveness of preventive measures.

MATERIALS AND METHODS

In our work, we used public records, the results of an epizootological survey of tularemia natural foci and the official data from the Departments of Sanitary and Epidemiological Control of two regions on the human cases of tularemia in 2000–2021. We used descriptive statistics methods, relative and absolute indicators of the tularemia incidence in the population for the analysis. Analyses of human cases of tularemia included the study of sources, factors, routes of transmission of the *F. tularensis* and clinical forms. The phenotypic and genetic properties of the strains isolated in 2000–2021 were studied according to the methodological recommendations "On approval of methodological recommendations on strengthening measures to prevent human diseases with tularemia in the Republic of Kazakhstan" (Order of the Ministry of Health of the Republic of Kazakhstan No. 88 dated 14.12.2005).

RESULTS AND DISCUSSION

The risk of human infection with tularemia varies in Kazakhstan. This article presents a retrospective analysis and modern characteristics of tularemia in the West Kazakhstan and North Kazakhstan regions.

In the West Kazakhstan region there are foci of floodplain swamp and steppe types, which are included in zone A – active natural foci of tularemia [4]. The floodplain swamp focus covers an area of more than 100,000 km^2. *F. tularensis* strains are detected annually in the foci, and sporadic infection cases among humans are registered [5]. The floodplain swamp focus occupies 37881.8 km^2 and is located in the floodplain of the Ural River with its tributaries (Kaztalovsky, Zhangalinsky, Akzhaiksky, Terektinsky, Burlinsky districts) [6]. The steppe focus occupies an area of 91081.1 km^2 and covers the territories of seven districts of the region (Akzhaiksky, Bokeyordinsky, Zhangalinsky, Kaztalovsky, Baitereksky (formerly Zelenovsky), Taskalinsky, Chingirlausky). In the steppe focus the causative agent circulates among hamsters, hares, field and house mice, water voles; its main vectors are ixodes ticks of the *Dermacentor* genus.

The main carrier of the tularemia causative agent in floodplain swamp foci is the water vole. In the West Kazakhstan region, 22 species of wild mammals are carriers of *F. tularensis* . The ecological and faunal list of mammals susceptible to *Francisella tularensis* was expanded by four species over the twenty years from 1994 to 2021 (stoat, grey dwarf hamster, isabelline wheatear and common shrew) and increased to 26 species. In the period 2015–2021, winter epizootics with involvement of all common rodent species were registered in eight districts of the West Kazakhstan region. The main vectors are ixodes ticks of the genera *Dermacentor, Rhipicephalus, Ixodes*. In 2020, the tick population was 47.8 specimens per 1 flag-km. The most active foci are located in the middle reaches of the Ural River, along the Bolshoy and Maly Uzen rivers, and in the Ural-Kushum interfluve (Fig. 1).

High level of epidemic activity before mass tularemia vaccination in the West Kazakhstan region was noted in the early twentieth century. In 1928, the first human cases of tularemia were registered in the Ural River floodplain, which were associated with mass harvesting of water vole skins [7]. Prior to 1960, large epidemic outbreaks associated with water vole hunting were recorded

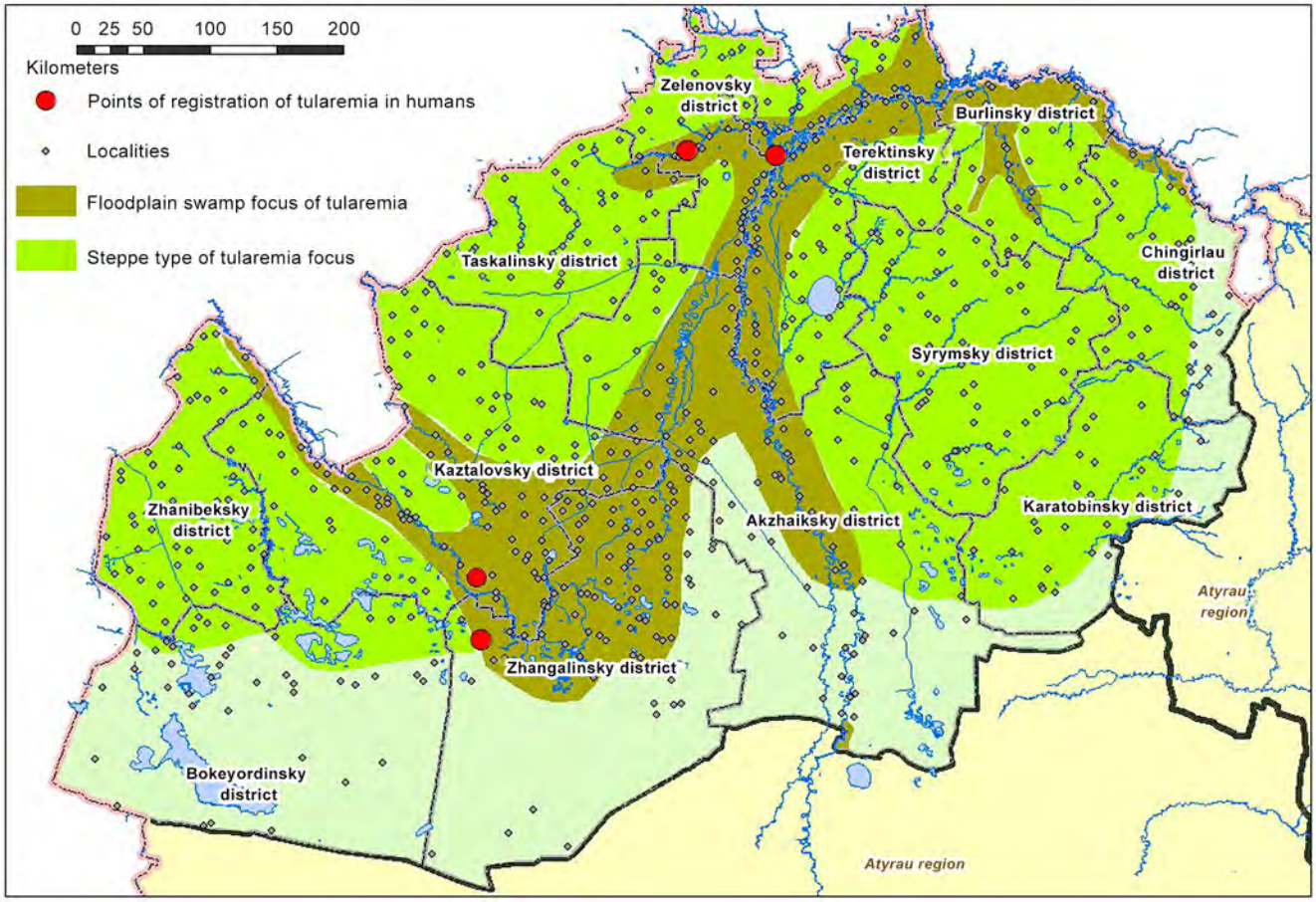

FIG. 1.
Tularemia foci in the territory of the West Kazakhstan region

in the West Kazakhstan region [8]. In 2002, a case of human tularemia in Kaztalovsky district of the West Kazakhstan region was registered. In 2007, a 26-year-old Uralsk resident fell ill with weakness, sweating, malaise, and a fever of up to 39 °C. A 4-fold increase of antibody titer in indirect hemagglutination reaction from 1:80 to 1:320, enlargement of lymph nodes was revealed, and on 18 May, 2007 the patient was admitted to the City Clinical Hospital of Infectious Diseases with the diagnosis: Tularemia, bubonic form, moderate form. An epidemiological investigation determined that there may have been contact with rodents that were in the house [9]. In 2018, 1 case of human tularemia was registered in the region, which resulted in recovery.

Analysis of human tularemia cases from 1928 to 1960 in the West Kazakhstan region showed that about 900 cases were registered in these years. 3 cases of tularemia were detected between 2000 and 2001. Since 1960, in the following 37 years the epidemiological situation in the region was favourable. The incidence of human disease in the West Kazakhstan region has decreased 200 times in the last 20 years compared to 1928–1960. At the same time, the epizootic potential is rather high: more than 300 strains of *F. tularensis* were isolated during the period. The comparative study of molecular biological properties of *F. tularensis* strains allowed to conclude both intraspecific variability and interspecific similarities. The range of genetic variability of tularemia microbe strains was determined using 25 *VNTR* markers; the strains were found to belong to the second cluster. Outbreak of foodplain swamp foci mostly assosiated by hunting of wild animals and by vector-borne transmission. The source of human infection was rodents, and the vectors were ticks of the *Dermacentor* genus. The patients had a cutaneous bubonic form of tularemia with moderate and mild severity of the infection course [10]. The incubation period averaged 3–7 days. Diagnosis was confirmed on the basis of epidemiological history, clinical manifestation and serological confirmation.

Thus, the study of the current spatial and temporal characterization of tularemia from 2000 to 2021 in the West Kazakhstan region showed that the epidemic potential of tularemia has significantly decreased.

On the territory of the North Kazakhstan region there are active natural foci of tularemia; endemic territories

are Mamlyutsky, Kyzylzhar, Magzhan Zhumabaev, Gabit Musrepov, Ayyrtau, Shal Akyn, Akkayynsky districts (Fig. 2). Tularemia epizootics were registered in the floodplain of the Ishim River, which crosses the region from south-west to north-east, and its tributaries [11]. The main carrier of the tularemia microbe in the floodplain swamp foci is the water vole (50 specimens per 1 km of coastline, 0.4–2.8 % infection rate). The epizootic process involves rodents and vectors – ticks of the *Dermacentor* genus. Periods of high water vole population were recorded in 1927–1929, 1937–1939, 1947–1949, 1952–1953, 1957–1958.

For the first time in 1958, a culture of the *F. tularensis* was isolated from a muskrat. In 1972, a spillover epizootic was registered in Akkayynsky, Zhambyl, and Mamlyutsky districts. In 1972, 12 strains of the tularemia agent were isolated from a field mouse and a water vole; in 1973 a strain was isolated from *D. marginatus ticks*. In the following years, serological confirmation of the epizootic process was obtained almost annually.

In 1945, 8 cases of human tularemia were registered in the North Kazakhstan region for the first time. In 1949, 200 human cases were reported. In 1972, there was a major outbreak of tularemia, affecting more than 40 people. The outbreak was transmissible and occurred in the vicinity of Petropavlovsk, Sokolovsky and Mamlyutsky districts.

As a result of retrospective analysis, it was determined that from 1945 to 1999 in the North Kazakhstan region 441 cases of tularemia among people were registered. Human infections were observed in summer, during the period of activity of blood-sucking two-winged insects, and clinically manifested in ulcerative and bubonic form [12].

Between 2000 and 2021, 11 human cases of tularemia have been reported. Infection of people occurred in the territory endemic for tularemia (Akkayynsky, Kyzylzhar, Magzhan Zhumabaev districts). The source was rodents, the transmission factors were arthropods, and the routes of transmission were contact and vector-borne.

A study of the contemporary spatial and temporal characterization of tularemia showed that the intensity of epizootics from 1945 to 1999 was high. *Fr. tularensis* strains were isolated during rodent and arthropod surveys. In recent years (2000–2021), single positive samples for specific tularemia antibodies and antigens have been detected in rodents, mammals and environ-

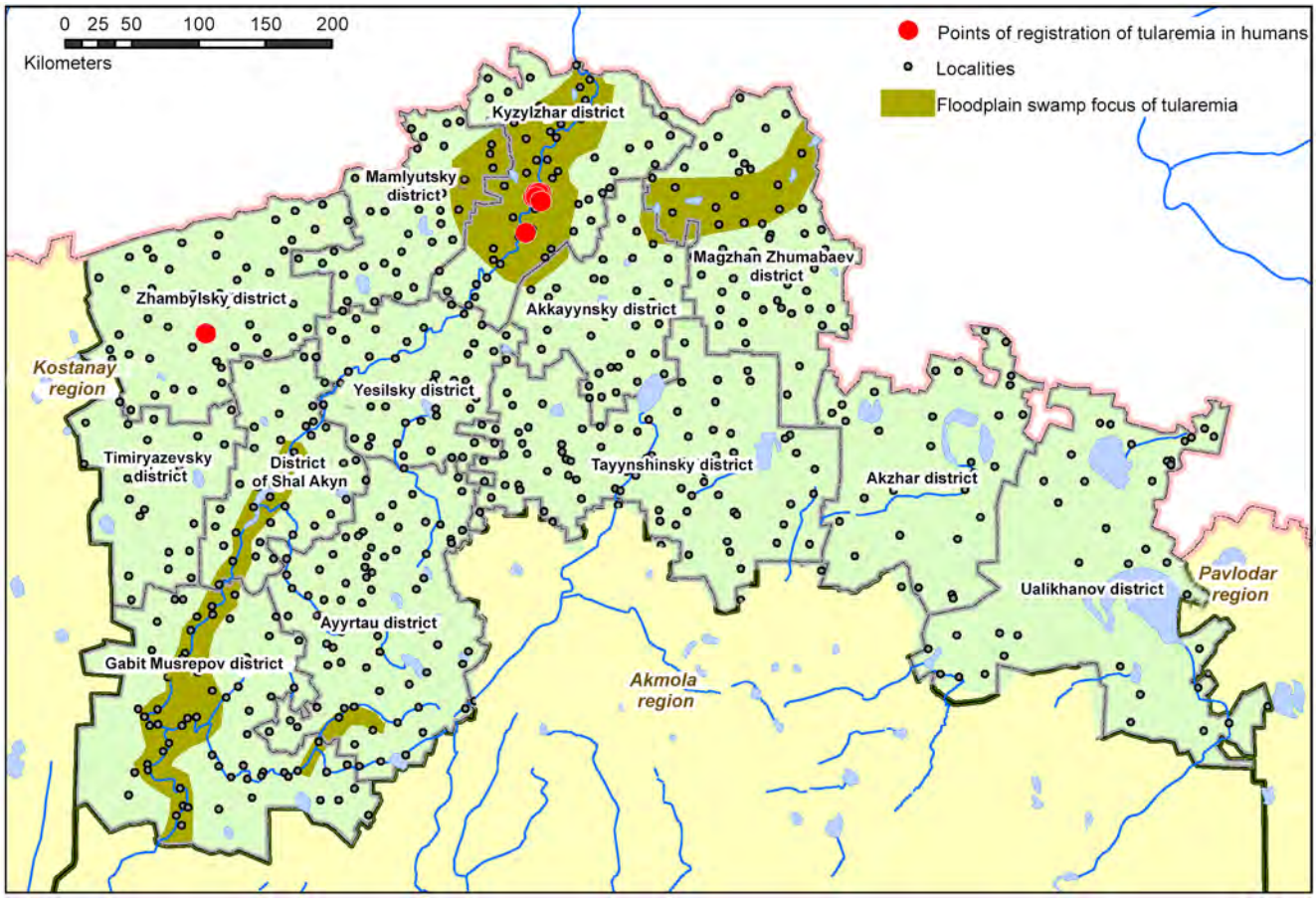

FIG. 2.
Tularemia foci in the North Kazakhstan region

TABLE 1

RELATIVE INCIDENCE RATE OF THE POPULATION FROM 2000 TO 2021 IN THE WEST KAZAKHSTAN AND NORTH KAZAKHSTAN REGIONS

Regions of Kazakhstan	Years of human cases of tularemia (relative indicator)						
	2002	2003	2004	2006	2007	2016	2018
West Kazakhstan Region	0.17	0	0	0	0.16	0.2	0.16
North Kazakhstan Region	0.15	0.59	0	0.3	0	0.2	0.18

mental objects; *Fr. tularensis* strains have not been isolated.

A study of the current spatial and temporal characterization of tularemia (2000–2021) in the North Kazakhstan region showed that the epidemic potential has significantly decreased. The incidence among humans between 2000 and 2021 decreased 39-fold compared to the period between 1945 and 1999. The relative incidence of human tularemia in the period 2000–2021 is shown in Table 1, which presents the years when human tularemia cases were registered.

Contact of the population with rodents infected with tularemia, consumption of water and food contaminated with *F. tularensis* causative agent and tick bites are the main causes of infection and disease among humans. Before 2000, the source of infection and factors of causative agent transmission were muskrat hunting, water and blood-sucking two-winged insects; after 2000, the main type of transmission was alimentary.

CONCLUSION

An analysis of long-term data on the epizootic and epidemic activity of tularemia natural foci, processed using descriptive statistics and GIS technology, made it possible to identify places of long-term persistence of the tularemia agent in the natural focus of the North Kazakhstan and West Kazakhstan regions and to create an electronic map of the territories endemic for tularemia to determine the scope of preventive measures.

A study of the current spatial and temporal characterization of tularemia (2000–2021) in the West Kazakhstan and North Kazakhstan regions showed that the epidemic potential has significantly decreased.

Characteristic epidemiological features of this period are single cases of the disease with infection in floodplain swamp natural foci; predominantly alimentary route of infection through consumption of food or water contaminated with tularemia.

In the West Kazakhstan and North Kazakhstan regions, no significant changes in the epizootic situation are currently expected, but there is a risk of sporadic cases of morbidity among the population. Under favorable conditions for increasing numbers of rodents, ticks and blood-sucking insects, the epizootic process may intensify to local and spillover epizootics.

It is necessary to continue monitoring studies and to carry out timely preventive measures, including vaccination of the population.

Conflict of interest

The authors declare the absence of any conflict of financial/non-financial interests related to the article.

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Authors' contributions

Uinkul A. Izbanova – study of archival and official documents, results of epizootological survey of natural foci of tularemia in two regions; literature search on epidemiological monitoring of tularemia in natural foci of Kazakhstan in PubMed, Thomson Reuters, Springer, eLibrary databases; analysis of data on the incidence of tularemia using descriptive statistics, relative and absolute indicators.

Larisa Yu. Lukhnova – literature review, assessment of the reliability of data in selected sources; analysis of tularemia incidence using descriptive statistics methods; conclusion to the article.

Tatyana V. Meka-Mechenko – literature review; search for archival documents in the scientific library of National Scientific Center of Especially Dangerous Infections named after Masgut Aikimbayev.

Nurbek S. Maykanov – study of archival and official documents, results of epizootological survey of natural foci of tularemia in the West Kazakhstan region.

Veronika P. Sadovskaya – formation of shapefiles and creation of GIS-map of tularemia foci in the North Kazakhstan and West Kazakhstan regions.

Vladimir G. Meka-Mechenko – search for archived data on the results of epizootological survey of the territory of two regions for tularemia.

Aisazhan A. Yusupov – study of archival and official documents, results of epizootological survey of natural foci of tularemia in the North Kazakhstan region; study of properties of the tularemia microbe strains isolated in 2000–2021.

Akbota B. Makulova – study of archival and official documents, results of epizootological survey of natural foci of tularemia in the West Kazakhstan region; study of properties of the tularemia microbe strains isolated in 2000–2021.

HISTORY OF MEDICINE AND ANNIVERSARIES

JEAN PECQUET (1622–1674). TO THE 400TH ANNIVERSARY OF THE BIRTH

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ABSTRACT

The article is dedicated to the 400th anniversary of the birth of the outstanding French anatomist, physician and philosopher Jean Pecquet (1622–1674). Pecquet's biography is connected with the city of Dieppe, where the future scientist was born and got his primary education, and with Paris, where he made his main discoveries in anatomy. Throughout his life, Pecquet collaborated with many prominent scientists of that time (Jacques Mentel, Louis Gayant, Jean Riolan (the Younger)), including not only physicians and anatomists, but also physicists such as Blaise Pascal, Edme Mariotte, Marin Mersenne and Evangelista Torricelli. Pecquet's most famous discovery is the chyle cictern, or cisterna chyli. The structure was named after of the scientist – "Pecquet's reservoir (cistern)". But more revolutionary discovery made by Pecquet is revealing and proving the fact that the lymphatic ducts flow into the superior vena cava indirectly through the venous angles and refuting the conventional opinion on the drainage of lymph into the liver. An important help in Pecquet's anatomical research and experiments was his passion for the physical and mathematical sciences. In collaboration with Edme Marriott, Pecquet studied the structure of the eyeball and turned out to be more foresighted, because, unlike Marriott, he correctly understood the role of the retina in the functioning of the eye as an organ of vision. Pecquet was one of William Harvey's supporters regarding his concept of blood circulation. He introduced cutting-edge at that moment technologies into the anatomy methodology, including animal experiments in vivo, and made a fateful contribution to the progress of anatomical science.

Key words: *history of medicine, history of anatomy, Jean Pecquet, lymphatic system, thoracic duct, blood circulation*

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ЖАН ПЕКЕ (1622–1674). К 400-ЛЕТИЮ СО ДНЯ ЕГО РОЖДЕНИЯ

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РЕЗЮМЕ

Статья посвящена 400-летию со дня рождения выдающегося французского анатома, врача и философа Жана Пеке (1622–1674). Биография Пеке связана с г. Дьепп, где будущий учёный родился и получил начальное образование, и с Парижем, где он совершил основные свои открытия в анатомии. На протяжении жизни Пеке сотрудничал со многими выдающимися учёными того времени (Жак Ментель, Луи Гайан, Жан Риолан – младший), в том числе не только с врачами и анатомами, но и с физиками, такими как Блез Паскаль, Эдм Мариотт, Марен Мерсенн и Эвангелиста Торричелли. Самое известное открытие Пеке – млечная цистерна, cisterna chyli, или цистерна грудного протока. В честь учёного данная структура носит его имя – «резервуар (цистерна) Пеке». Но более революционным открытием Пеке является то, что он, опровергая устоявшееся мнение о дренаже лимфы в печень, обнаружил и доказал факт впадения лимфатических протоков в верхнюю полую вену опосредованно через венозные углы. Важным подспорьем в анатомических исследованиях и экспериментах Пеке явилось его увлечение физико-математическими науками. В содружестве с Мариоттом Пеке занимался изучением строения глазного яблока и оказался более прозорливым, т. к., в отличие от Мариотта, правильно понял роль сетчатки в функционировании глаза как органа зрения. Пеке был одним из сторонников Уильяма Гарвея в отношении его концепции кровообращения, внедрял передовые на тот момент технологии в методологию анатомии, в том числе эксперименты на животных in vivo, и в целом внёс судьбоносный вклад в прогресс анатомической науки.

Ключевые слова: *история медицины, история анатомии, Жан Пеке, лимфатическая система, грудной проток, кровообращение*

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The year 2022 marks the 400th anniversary of the birth of Jean Pecquet (9 May 1622, Dieppe (Normandy) – 26 February 1674, Paris), a French scientist, anatomist, physician and educator who made indisputable contributions to anatomy and physiology (Fig. 1). As a child, Pecquet attended a Catholic school in Dieppe, then studied at the Jesuit College of Rouen. There he met Adrien Auzout and Blaise Pascal, with whom he shared interests in mathematics and natural philosophy. After graduation, the young man started looking for a job. His first employer was a noblewoman who employed Pecquet as a practicing physician in 1641. There is evidence that at that time the future scientist performed autopsies, the information about which he recorded (Memoirs of the Royal Academy of Surgery). When the woman moved to Paris, she took Pecquet with her. There, the elderly Marquise reportedly paid for anatomy lessons that Pecquet took from Louis Gayant, a renowned anatomist and president of the Paris Society of Surgeons. However, in 1646 the patient passed away and so the doctor was forced to find a new employer. According to history, the autopsy of his dead mistress was performed by young Pecquet himself [1, 2].

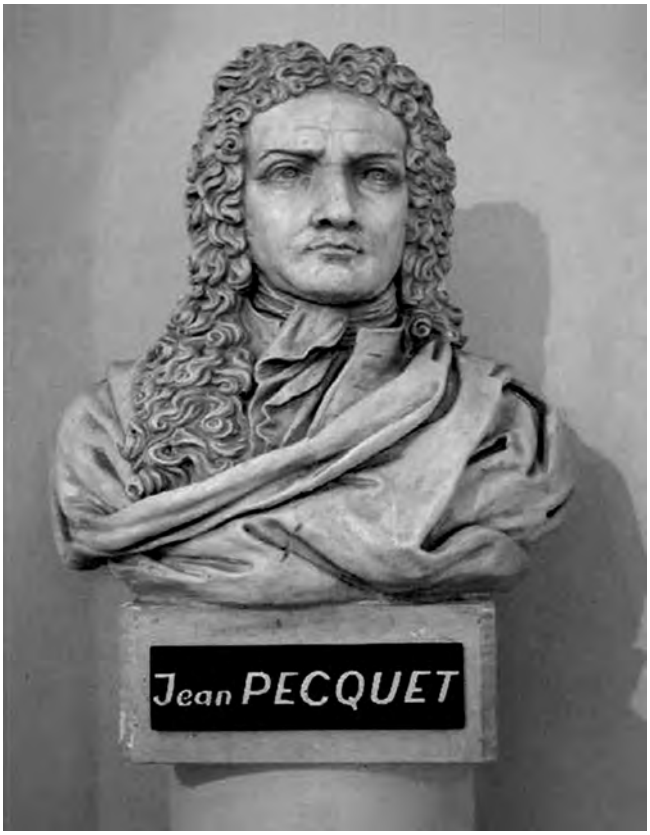

FIG. 1.
The bust of Jean Pecquet [3]

In 1645, the rector of the Jesuit college in Clermont (today, the Lycée Louis-le-Grand), which was part of the University of Paris, offered Pecquet a teaching (tutor) position, providing him with the financial means to further his studies. There the future scientist received a Master of Arts degree, necessary for admission to the Faculty of Paris [1]. Pecquet met many interesting people during that period, notably Marin Mersenne and Evangelista Torricelli, who were conducting research in acoustics, gravity and vacuum fields. By the mid-1640s Pecquet, probably through Pascal, had gained access to the circle of the famous physicist and mathematician Marin Mersenne, and, judging from the evidence of their correspondence, they became very close. Pecquet also met Mersenne's nephew Pierre, then a student at the University of Paris. Thanks to his acquaintance with Mersenne, Pecquet attended the Bourdelot Academy, later called the "true school" of medicine, and, according to some sources, by the mid-1640s he was conducting anatomical demonstrations (autopsies) there together with the surgeon of Saint-Côme Paul Emmerez [1]. In 1648, Mersenne introduced Pecquet to François Fouquet, physician to the faculty of Paris, Bishop of Agde, and his older brother Nicolas Fouquet (1615–1680), an official who was rapidly rising through the ranks of Cardinal Mazarini's administration at the time. The influential nobleman Nicolas Fouquet (superintendent of finance in France, 1653–1661) took Pecquet into his service and contributed to his further education. For this purpose, Pecquet travelled to Rome and returned to Paris in 1648. He was not only Fouquet's personal physician but also his confidant and friend; they discussed scientific, medical, and literary matters freely. In parallel with his practical medical work, Pecquet continued his medical research, which he had begun in Paris in 1646, and in 1651 he began work on his thesis, which he continued in Montpellier, where he received his doctorate in 1652 [2, 4].

Pecquet made extensive use of animal experimentation in his anatomical studies. When Jean Pecquet stepped into the Paris Faculty of Medicine in 1647, at the age of twenty-four, he already knew how to perform dissections. Most historians view the faculty by the 1640s as a dying institution steeped in the doctrines of Galen, which, under the leadership of Jean Riolan the Younger, refused to accept William Harvey's (1578–1657) theory of the blood circulation [1]. Between 1647 and 1650, Pecquet, with the support of his Parisian teachers Mentel and Mersenne, performed more than a hundred autopsies on various animals (bulls, horses, pigs, etc.). It is worth noting that Jacques Mentel (1599–1671) concluded in 1629, based on dissections of dogs, that mesenteric lymphatic vessels flow into the thoracic duct before entering the bloodstream [5].

Pecquet performed the majority of the autopsies at Fouquet's residence in Paris. Pecquet dedicated the book published as a result of these studies to François Fouquet as it was, in his words, "born in your house" [1, 6]. Thus, in 1647 (although A. Cunningham [7] believes that it happened in 1642), opening the chest of a living dog, he found a white fluid resembling milk, which he later interpreted as lymphatic fluid. He found that the structures conducting this "milky juice" end in the superior vena

cava and, on the other side, in a reservoir or lumbar cistern (called cisterna de Pecquet in French-speaking countries) behind the stomach. This discovery was made by accident: the white fluid that Pecquet initially mistook for pus after the removal of the dog's heart, but further investigation showed that the fluid appeared as a result of damage to a lymphatic vessel (thoracic duct) flowing into the venous angle [8, 9]. As a result, the scientist first described the thoracic duct he discovered and the differences between a vein and a lymphatic vessel [7, 10–12].

Thus, as a student, Pecquet challenged the prevailing notions of the time and took up not the "silent and frozen science" of cadaver anatomy, but the anatomy of animals – dogs, cattle, pigs and sheep. Using a living dog in the experiment, he showed the following:

1. If the heart is removed, pressure on the mesenteric root causes lymph to be released into the superior vena cava.

2. Lymph is channeled to the subclavian veins by two paravertebral channels, which swell when their distal ends are ligated.

3. The origin of the ascending lymphatic ducts is in the prevertebral and subdiaphragmatic ampullae – "this the sought-after sanctuary of chyle, this hard to palpate reservoir".

4. The posterior part of the "pancreas of Azelli" consists of lymph nodes.

5. The mesenteric lymphatic vessels do not go to the liver (a fact confirmed by Glisson in 1654), and the inferior vena cava, incised above the liver, shows no signs of lymph [5, 6].

By the way, the English anatomist Francis Glisson (1597–1677), who was engaged with his pupil George Joyliffe (1621–1658) in similar studies, claimed that he was the first to prove that lacteals are not connected to the liver. Glisson wrote that Joyliffe was "busy with other practice" an was unable to publish his findings, stating that the new knowledge of the course of the course of the mammary glands was obtained on behalf of his pupil [13, 14].

The discovery made by Pecquet as a result of experiments on animals was soon confirmed by other scientists, his contemporaries, but already on humans. Thus, Pecquet's colleague and associate, surgeon and anatomist Louis Gayant, soon repeated the study of the patterns of lymphatic drainage during the autopsy of a soldier killed in a fight with a comrade [14]. Nicholaes Tulp at Amsterdam described the thoracic duct and Pecquet's reservoir; Vesling (Padua) and Folli (Venice) made the same observation. There is an important testimony of the famous physicist Gassendi, who was present when Peyrac performed an autopsy on the corpse of a man who had just been hanged. To see the vessels of the criminal better, Peyrac fed him before the death sentence. Pecquet claims to have learnt this fact from Gassendi himself, who told him about it when they met in Paris. Thomas Bartholin, soon after the publication of Pecquet's work, demonstrated the human thoracic duct in Copenhagen; Jan van Horn did the same in Leiden in 1651. But while Bartholin gave Pecquet the praise he deserved, van Horn did the opposite. He officially appropriated the discovery and made no mention of Pecquet. Although there is a version that he was simply not familiar with Pecquet's work and thought he had made an independent discovery. Coincidentally, van Horn performed ligature experiments to prove that the chyle does not drain into the liver, as did Pecquet [15–17].

In the fundamental work "Experimenta nova anatomica, quibus incognitum hactenus chyli receptaculum, et abeo per thoracem in ramos usque subclavios vasa lactea deteguntur. Eiusdem dissertatio anatomica de circulatione sanguinis, et chyli motu" ("New anatomical experiments, in which a hitherto unknown reservoir of milky juice and milky vessels branching from it through the whole thoracic cavity up to the subclavian vein were discovered") (Fig. 2) Pecquet described the thoracic duct with its valves and reservoir, the so-called *cisterna chyli* (*receptaculum chyli*), which was later named Pecquet's cisterna in his honor.

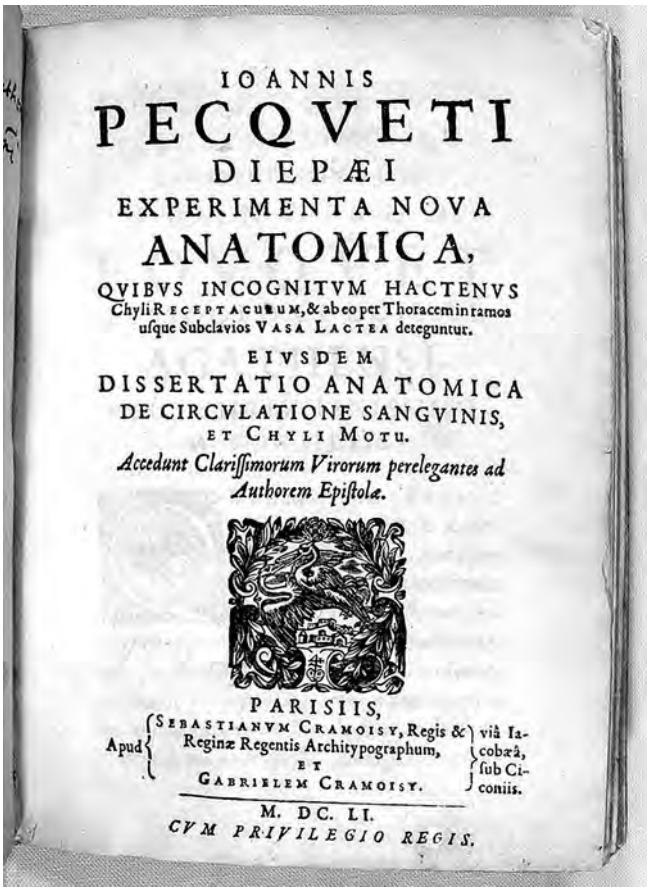
IOANNIS
PECQVETI
DIEPÆI
EXPERIMENTA NOVA
ANATOMICA,
QVIBVS INCOGNITVM HACTENVS
Chyli RECEPTACVLVM, & ab eo per Thoracem in ramos
usque Subclavios VASA LACTEA deteguntur.
EIVSDEM
DISSERTATIO ANATOMICA
DE CIRCVLATIONE SANGVINIS,
ET CHYLI MOTU.
Accedunt Clarissimorum Virorum perelegantes ad Authorem Epistolæ.
PARISIIS,
Apud SEBASTIANVM CRAMOISY, Regis & Reginæ Regentis Architypographum, ET GABRIELEM CRAMOISY. via Iacobæâ, sub Ciconiis.
M. DC. LI.
CVM PRIVILEGIO REGIS.

FIG. 2.
Frontpage of Jean Pecquet's "Experimenta nova anatomica" [21]

Importantly, he also definitively established that the intestinal duct containing milk-like fluid (lymph) flows into *cisterna chyli*, and then the lymph enters the thoracic duct, and not into the liver, as Azelli and other anatomists before him mistakenly thought [4, 18, 19]. Despite its relatively small volume and only one illustration, this work is considered the key point in research on the lymphatic system.

The scientist refutes Galen's ideas of hepatocentrism and refines Azelli's discovery. Numerous anatomists (Valleus, Harvey, Conring, Bartholin, and also Riolan the Younger) have argued on this point, stating that some of the lacteals scattered in the mesentery converge in the pancreas, some in the liver, some in the vena cava, and others in the portal vein. Riolan the Younger in particular supported Azelli's view that the "milky glands" drain into the liver, and rebuked Harvey for not thinking the same way. Pecquet demonstrated that chyle does not collect in any of these locations (Fig. 3) [2, 15, 20].

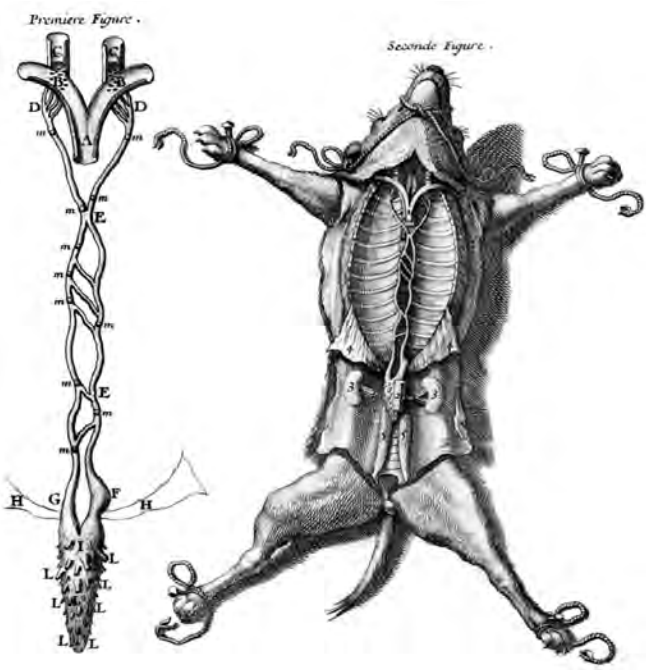

FIG. 3.
Dog's thoracic duct (from Jean Pecquet's «Experimenta nova anatomica», 1651) [21]

In the same work, in the "physico-mathematical" section, Pecquet describes experiments with vacuum conducted by means of a Torricelli tube. Evangelista Torricelli (1608–1648), student of Galileo, proposed an experiment in 1643 that investigated a phenomenon noted by Galileo in his Discorsi: a suction pump could only lift water to a certain height. Galileo argued that the internal force of a vacuum allows a column of water to rise until, stretched to its limit, it collapses under its own weight. Torricelli filled tubes of different lengths with mercury and inverted each over a bowl filled with the same liquid. In each case, the mercury fell into the tube to the same height, leaving an empty space at the top of the tube, which Torricelli claimed must be a vacuum, despite long-standing arguments that a vacuum could not exist in nature [1]. Pecquet witnessed a modification of the experiment belonging to the mathematician Gilles Personne de Roberval. The experiment consists in placing a deflated fish bladder in a vacuumized torricelli tube: as soon as it is in the empty space at the top of the tube, the bladder, working like a tiny balloon, inflates due to the elasticity of the air or "elatery" (the English translation of his work of 1653 is "elastrum"). As the upper part of the tube is almost empty, the tiny amount of air remaining inside the bladder expands and fills the bladder [22]. Pecquet introduced the concept of air elasticity ("elatery") as part of these experiments.

Pecquet had a number of reasons for resorting to such experiments: firstly, to explain the movement of chyle or digested food in a mechanical way within the body, without resorting to attraction solely as a result of elasticity and pressure, such as from respiration. Thus, elasticity is used here qualitatively rather than quantitatively in terms of pressure and volume. Another process associated with "elatery" is digestion, in which the fibres of the stomach and intestines expand and contract, "as in elatery". Another topical area concerns the "elatery" of blood vessels – both arteries and veins. Pecquet believed that immediately after cardiac systole, the arteries swell; the same happens to the veins when blood enters them. At one time, Harvey devoted attention to this issue, opposing Galen's views: Galen argued that arteries move because of the ability transmitted to them by the heart, and "ability" is called by the term *technicus* associated with his philosophical position. Harvey, on the other hand, argued that the arteries fill up because of the blood flow. Pecquet's analysis modified this dichotomy, as he attributed a more active role to the walls of arteries and veins: they were no longer purely passive vessels, but contributed to the movement of blood, facilitating by their dilation and contraction the activity of the heart [22].

These experiments had a profound influence on Pecquet and his ideas about the correlation between blood and lymph circulation. Pecquet later claimed that the knowledge he gained from dissecting human cadavers was "silent and cold" and that he gained "true knowledge" (*veram scientiam*) only by dissecting living animals. This technique allowed Pecquet to realize the mentioned discoveries in anatomy [1].

Pecquet may have been the first to introduce the concept of elasticity into anatomy, but he was not the last in the seventeenth century: other scientists followed his lead in various forms, some of which resonate with our current views and some of which do not. In those days, many researchers believed that physical and mathematical knowledge was necessary for a better understanding of anatomy. "New (or mechanistic) anatomy", of which Pecquet was also an adherent, refuted a number of statements existing at that time in anatomy. For example, the discovery of the thoracic lymphatic duct "deprived" the liver of its ability to produce blood and required a rethinking of its pathology [10, 11, 23]. Pecquet also formulated his reasoning on blood transfusion, capillary function and vascular permeability, by his own studies of the circulatory system confirming Harvey's theory of blood circulation. In his original work on blood circulation, William Harvey argued that the primary engine of blood circulation is the pulsation of the heart. Describing blood flow in the veins, Harvey argued that the movement of the ven-

tricles of the heart "is sufficient to distribute the blood throughout the body and to drain it from the vena cavae". Thus, for Harvey, a single heartbeat was enough to "draw" blood from the vena cava, despite the fact that the vena cava is the largest vein of the body, into which almost all other veins drain blood, Harvey was virtually silent about the patterns of blood circulation within it. One of the first anatomists to specifically state venous blood flow was the young scientist Jean Pecquet in his book "Experimenta nova anatomica..." (Paris, 1651). In this book, Pecquet included a thesis on the problem of blood circulation in veins. There he claimed that the initial impulse of cardiac contraction was insufficient to explain the return of blood to the heart through the veins (what today is called venous return). More importantly, in some parts of the venous outflow, Pecquet noticed that the blood moved in a direction opposite to the direction of its own weight or, to put it in modern terms, against the law of gravity. This opposite movement of blood is particularly problematic in upright humans and animals because more than half of the blood flows upward to the heart through the inferior vena cava. How can such a large amount of blood move upward without the initial impulse of the heart? [23].

In his work, Pecquet shows the "circular movement of blood throughout the animal's body" by means of ligatures applied to arteries and veins. Similar experiments have been carried out before him, but Pecquet shows great originality in methodology and in the directness of his conclusions. He starts with arteries and veins in general. If a ligature is applied to the femoral, brachial, or carotid artery of a living animal, the vessel empties beyond the ligature, but becomes swollen on the side towards the heart; and if it is opened beyond the ligature there is no hemorrhage, whereas a puncture on the side towards the heart causes profuse bleeding. But a similar experience on the femoral or brachial vein gives the opposite result: the vein shrinks towards the heart and swells towards the periphery; a puncture below the ligature is accompanied by hemorrhage, and above it has no effect. To make sure that the blood flowing from the wounded vein came from the arteries, he applies a ligature to the appropriate artery for the duration of the hemorrhage. When this ligature is tightened, the bleeding from the vein first diminishes, and then ceases; but when it is relaxed, the bleeding begins again with the same intensity. As part of these experiments, Pecquet was able to disprove the idea of Riolan the Younger that "portal vein blood does not pass through the liver into the vena cava". By studying blood flow in the portal vein using ligatures, Pecquet proved that blood entering the liver through the *v. porta* leaves the organ through the hepatic veins flowing into the inferior vena cava [24].

With the publication of "Experimenta nova anatomica..." in 1651, Pecquet's position on the Faculty of Paris was no longer secure. Apparently, the author did not receive permission from the faculty to publish because he was a student, which constituted a breach of etiquette [1]. The publication created a great sensation in scientific circles. Even Harvey questioned the importance of Pecquet's work. In writings dated 1652, Harvey stated that he had observed these "milky glands" (perhaps even before Aselli), but doubted their importance in the circulation process. Ironically, he believed that the network of mammary glands was "too extensive" to move all the nutrients from the digestive tract into the bloodstream. Harvey believed that while an embryo can receive nourishment from the umbilical veins, an adult can receive nourishment to the liver via the mesenteric veins [17, 20]. Pecquet's work also received harsh criticism from the influential Riolan the Younger, causing Pecquet to retire to Montpellier, where he completed his medical research and submitted his finished thesis on 23 March 1652. Pecquet was famous in Montpellier, where he gave public anatomical demonstrations, and remained there, coming occasionally to Paris, until 1654. After receiving his doctorate, Pecquet practiced successfully in Paris and even became a physician at the court of Louis XIV. In particular, he served as personal doctor to the Marquise de Sévigné and Jean de la Fontaine [1, 2, 12].

While the discoveries of Harvey and Azelli in the anatomy of the lymphatic system caused a burst of activity in the scientific world of that time, the work of Jean Pecquet, first published in Paris in 1651, served as a stimulus for subsequent studies by Thomas Bartholin and Olof Rudbeck [9]. It is important to note that by the mid-seventeenth century, comparative anatomy had become not only a descriptive but also an experimental discipline. Pecquet's work combined dissection and mechanical philosophy and paved the way for the mechanical theories of bodily functions that dominated the second half of the century [1].

In 1661, Nicolas Fouquet was arrested for abuse of official position. Pecquet, as his personal physician, voluntarily followed his master to the Bastille until February 1665. Fouquet was then transferred to the prison of Pignerol (where he died in 1680), and Pecquet was ordered to go to his sister in Dieppe. He was to remain there until further notice. This stay lasted a year before King Louis XIV and Secretary of State Jean-Baptiste Colbert concluded that Pecquet was not to blame for his master's misdemeanours. Colbert went even further by nominating Pecquet to the French Academy of Sciences as an anatomist in 1666, enabling him to participate in the blood transfusion experiments conducted at the Academy between 1666 and 1667. In the academy, the scientist had a difficult time: despite the fact that by that time Pecquet was already famous, he was a native of a provincial university and he had to fight with representatives of the Faculty of Paris, who believed that only doctors who came from this faculty could practice their art. Pecquet was one of the doctors who founded the Royal Chamber of Physicians of Provincial Universities. A few years later, between 1666 and 1670, Jean Pecquet was appointed personal physician to the king, which provided him with a solid career [2, 6].

Already a member of the Academy, Pecquet carried out research on the eyeball with Edme Marriott, the dis-

coverer of the blind spot, and published with him in 1668 the work "Nouvelle découverte touchant la veüe". Unlike Marriott, Pecquet believed that the retina, not the vasculature of the eye, was the main formation responsible for vision. He also experimented with mercury tubes because he suspected that atmospheric pressure affected blood circulation [2].

Summarizing the above, it can be stated that the work of Jean Pecquet has had a decisive influence on the formation of the modern concept of body structure. With his demonstration of the thoracic duct, Pecquet launched one of the strongest attacks on Galenism: "since blood does not flow into the liver, the liver cannot carry out the cooking process of converting chyle into blood". As a consequence, the liver has lost its privileged role in the body. In addition, Pecquet proved that the flow of chyle (lymph) is circulatory. In 1653, the Danish anatomist and physician Thomas Bartholin, in his work "Vasa lymphatica", supported Pecquet's conclusions and showed that the vessels described belonged to a new vascular system called the "lymphatic system". Finally, Pecquet's three-year experiments on living animals raised the problem of assessing the impact of dissection on the life sciences. Moreover, the attempt to adapt physical research to medicine shows the importance of co-operation between physicians and mathematicians in the foundation of "mechanistic anatomy" and, more generally, in the development of late seventeenth-century medicine [10].

Conflict of interest

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

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