

НАУЧНО-ПРАКТИЧЕСКИЙ ЖУРНАЛ

БСМ

ISSN 1682-0363 (print)
ISSN 1819-3684 (online)

БЮЛЛЕТЕНЬ СИБИРСКОЙ МЕДИЦИНЫ

BULLETIN OF SIBERIAN MEDICINE

BSM



Том 20

№ 3. 2021



7 сентября 2021 г.



Кафедра патофизиологии. 2021 г.



23 августа
2021 г.



Открытие горельефа В.В. Новицкого. 7 сентября 2021 г.

РЕКТОР НАВСЕГДА

BULLETIN OF SIBERIAN MEDICINE

Peer-reviewed scientific-practical journal
Issued quarterly

Volume 20, No. 3, 2021

ISSN 1682-0363 (print)
ISSN 1819-3684 (online)

FOUNDER AND PUBLISHER:

Siberian State Medical University, the Ministry
for Healthcare of the Russian Federation

Registered by the Ministry of the Russian Federation for
Affairs of the Press, Television and Radio Broadcasting and
Mass Communication Media

Certificate of registration PI No. 77-7366
dated to 26.03.2001

Bulletin of Siberian Medicine is included:
in the List of peer-reviewed scientific journals
and publications issued in the Russian Federation,
which should publish main scientific results
of doctor's and candidate's theses

Bulletin of Siberian Medicine is indexed:

Scopus
Web of Science (WoS (ESCI))
Science Index
RSCI

Ulrich's International Periodicals Directory
Cyberleninka
DOAS

Editorial Board Office:
107, Lenin Av., Tomsk, 634050, Russian Federation
Telephone: +7-(382-2)-51-41-53.
<http://bulletin.tomsk.ru>
E-mail: bulletin@bulletin.tomsk.ru

Publisher: the Siberian State Medical University.
2, Moscow Trakt, Tomsk, 634050,
Russian Federation.
Editors: E.E. Stepanova, Yu.P. Gotfrid
Translate: M.E. Orlova, D. Palazza
Electronic makeup, cover design
L.D. Krivtsova

Printed in Litburo LLC,
4, Korolev Str., Tomsk, 634055, Russian Federation

Signed to print 28.09.2021
Format 60 × 84/8. Offset print.
Coated paper. Times font.
P.l. 29,5. C.p.l. 29,0.
500 copies. Odder no 621.

The price – free.
Date of publication 30.09.2021.

Pass-through copyright.
Advertising providers are liable for the truthfulness
of information in advertising materials.

© Siberian State Medical University, 2021

EDITOR-IN-CHIEF

O.I. Urazova, *Corresponding Member of RAS (Tomsk)*

DEPUTY-EDITORS-IN-CHIEF:

L.M. Ogorodova, *Corresponding Member of RAS (Tomsk)*

SCIENCE EDITOR

V.V. Kalyuzhin, *Professor (Tomsk)*

EDITORIAL BOARD:

V.M. Alifirova, *Professor (Tomsk)*
L.I. Aftanas, *Academician of RAS (Novosibirsk)*
A.A. Baranov, *Academician of RAS (Moscow)*
A.I. Vengerovsky, *Professor (Tomsk)*
Ye.G. Grigoriyev, *Corresponding Member of RAS (Irkutsk)*
A.M. Dygai, *Academician of RAS (Tomsk)*
M.V. Zav'yalova, *Professor (Tomsk)*
L.V. Kapilevich, *Professor (Tomsk)*
S.I. Karas, *Professor (Tomsk)*
R.S. Karpov, *Academician of RAS (Tomsk)*
V.I. Kiselyov, *Corresponding Member of RAS (Barnaul)*
S.V. Logvinov, *Professor (Tomsk)*
A.D. Makatsaria, *Corresponding Member of RAS (Moscow)*
L.S. Namazova-Baranova, *Academician of RAS (Moscow)*
S.A. Nekrylov, *Professor (Tomsk)*
V.P. Puzyryov, *Academician of RAS (Tomsk)*
V.I. Starodubov, *Academician of RAS (Moscow)*
Ye.A. Stepovaya, *Professor (Tomsk)*
A.T. Teplyakov, *Professor (Tomsk)*
V.A. Tkachuk, *Academician of RAS (Moscow)*
V.A. Trufakin, *Academician of RAS (Novosibirsk)*
O.S. Fedorova, *Professor (Tomsk)*
I.A. Khlusov, *Professor (Tomsk)*
Ye.L. Choinzonov, *Academician of RAS (Tomsk)*
A.G. Chuchalin, *Academician of RAS (Moscow)*
A.V. Shabrov, *Academician of RAS (St.-Petersburg)*
V.A. Shkurupiy, *Academician of RAS (Novosibirsk)*
M.S. Yusubov, *Professor (Tomsk)*
A. Antsaklis, *Professor (Greece)*
F. Chervenak, *Professor (USA)*
C. Dadak, *Professor (Austria)*
Y. Dekhtyar, *Professor (Latvia)*
M. Epple, *Professor (Germany)*
D. Gailani, *Professor (USA)*
P. Odermatt, *(Switzerland)*
J. Odland, *(Norway)*
M. Poyurovsky, *Professor (Israel)*
V. Zhdankin, *Professor (USA)*

БЮЛЛЕТЕНЬ СИБИРСКОЙ МЕДИЦИНЫ

Научно-практический журнал
Выходит 4 раза в год

Том 20, № 3, 2021

ISSN 1682-0363 (print)
ISSN 1819-3684 (online)

УЧРЕДИТЕЛЬ И ИЗДАТЕЛЬ:

ФГБОУ ВО «Сибирский государственный
медицинский университет» Минздрава России

Журнал основан в 2001 году
Зарегистрирован в Министерстве РФ
по делам печати, телерадиовещания
и средств массовых коммуникаций
Свидетельство регистрации ПИ
№ 77-7366 от 26.03.2001 г.

Журнал входит в Перечень ведущих
рецензируемых научных журналов и изданий,
выпускаемых в РФ, в которых должны быть
опубликованы основные научные результаты
диссертаций на соискание ученой степени
доктора и кандидата наук

Индексация:

Scopus
Web of Science (WoS (ESCI))
РИНЦ (Science Index)
RSCI
Ulrich's International Periodicals Directory
Cyberleninka
DOAS

Редакция:

634050, г. Томск, пр. Ленина, 107.
Тел.: (382-2)-51-41-53.
<http://bulletin.tomsk.ru>
E-mail: bulletin@bulletin.tomsk.ru

Оригинал-макет:

Издательство СибГМУ.
634050, г. Томск, Московский тракт, 2.
Редакторы: Е.Е. Степанова, Ю.П. Готфрид
Перевод: М.Е. Орлова, Дж. Палацца
Электронная верстка, дизайн обложки
Л.Д. Кривцова

Отпечатано в ООО «Литбюро»,
634055, г. Томск, ул. Королёва, 4.

Подписано в печать 28.09.2021 г.
Формат 60 × 84/8. Печать офсетная.
Бумага мелованная. Гарнитура «Times».
Печ. л. 29,5. Усл. печ. л. 29,0.
Тираж 500 экз. Заказ 621.

Цена – свободная.
Дата выхода в свет 30.09.2021.

При перепечатке ссылка на
«Бюллетень сибирской медицины» обязательна.

Ответственность за достоверность информации,
содержащейся в рекламных материалах, несут рекламодатели.

ГЛАВНЫЙ РЕДАКТОР

О.И. Уразова, *член-корреспондент РАН (Томск)*

ЗАМЕСТИТЕЛЬ ГЛАВНОГО РЕДАКТОРА:

Л.М. Огородова, *член-корреспондент РАН (Томск)*

НАУЧНЫЙ РЕДАКТОР

В.В. Калюжин, *профессор, д-р мед. наук (Томск)*

РЕДКОЛЛЕГИЯ:

В.М. Алифирова, *профессор, д-р мед. наук (Томск)*
Л.И. Афтанас, *академик РАН (Новосибирск)*
А.А. Баранов, *академик РАН (Москва)*
А.И. Венгеровский, *профессор, д-р мед. наук (Томск)*
Е.Г. Григорьев, *член-корреспондент РАН (Иркутск)*
А.М. Дыгай, *академик РАН (Томск)*
М.В. Завьялова, *профессор, д-р мед. наук (Томск)*
Л.В. Капильевич, *профессор, д-р мед. наук (Томск)*
С.И. Карась, *профессор, д-р мед. наук (Томск)*
Р.С. Карпов, *академик РАН (Томск)*
В.И. Киселев, *член-корреспондент РАН (Барнаул)*
С.В. Логвинов, *профессор, д-р мед. наук (Томск)*
А.Д. Макацария, *член-корреспондент РАН (Москва)*
Л.С. Намазова-Баранова, *академик РАН (Москва)*
С.А. Некрылов, *профессор, д-р ист. наук (Томск)*
В.П. Пузырев, *академик РАН (Томск)*
В.И. Стародубов, *академик РАН (Москва)*
Е.А. Степанова, *профессор, д-р мед. наук (Томск)*
А.Т. Тепляков, *профессор, д-р мед. наук (Томск)*
В.А. Ткачук, *академик РАН (Москва)*
В.А. Труфакин, *академик РАН (Новосибирск)*
О.С. Федорова, *профессор, д-р мед. наук (Томск)*
И.А. Хлусов, *профессор, д-р мед. наук (Томск)*
Е.Л. Чойнзонов, *академик РАН (Томск)*
А.Г. Чучалин, *академик РАН (Москва)*
А.В. Шабров, *академик РАН (Санкт-Петербург)*
В.А. Шкурупий, *академик РАН (Новосибирск)*
М.С. Юсубов, *профессор, д-р хим. наук (Томск)*
A. Antsaklis, *профессор (Греция)*
F. Chervenak, *профессор (США)*
C. Dadak, *профессор (Австрия)*
Y. Dekhtyar, *профессор (Латвия)*
M. Epple, *профессор (Германия)*
D. Gailani, *профессор (США)*
P. Odermatt, *(Швейцария)*
J. Odland, *(Норвегия)*
M. Poyurovsky, *профессор (Израиль)*
V. Zhdankin, *профессор (США)*

ОРИГИНАЛЬНЫЕ СТАТЬИ	ORIGINAL ARTICLES
Бирulina Ю.Г., Иванов В.В., Буйко Е.Е., Трубаева О.А., Петрова И.В., Гречишникова А.Ю., Носарев А.В., Гусакова С.В. Влияние высокожировой и высокоуглеводной диеты на клетки крови крыс	Birulina J.G., Ivanov V.V., Buyko E.E., Trubacheva O.A., Petrova I.V., Grechishnikova A.Yu., Nosarev A.V., Gusakova S.V. Effects of a high-fat, high-carbohydrate diet on blood cells of rats
Ванеева Е.В., Росин В.А., Дьяконов Д.А., Лучинин А.С., Самарина С.В., Кочетов Н.Л. Значение экспрессии pAKT1 при диффузной В-крупноклеточной лимфоме	Vaneeva E.V., Rosin V.A., Diakonov D.A., Luchinin A.S., Samarina S.V., Kochetov N.L. The role of pAKT1 expression in diffuse large B-cell lymphoma
Ветлугина Т.П., Епимахова Е.В., Савочкина Д.Н., Плотников Е.В., Бойко А.С., Иванова С.А., Бохан Н.А. Оценка влияния солей лития на продукцию цитокинов клетками крови в опытах <i>in vitro</i>	Vetlugina T.P., Epimakhova E.V., Savochkina D.N., Plotnikov E.V., Boiko A.S., Ivanova S.A., Bokhan N.A. Estimation of the effect of lithium salts on cytokine production by blood cells in <i>in vitro</i> experiments
Гельцер Б.И., Сергеев Е.А., Котельников В.Н., Силаев А.А., Карпов Р.С. Гемодинамический статус пациентов с различными вариантами коморбидности ишемической болезни сердца до и после коронарного шунтирования	Geltser B.I., Sergeev E.A., Kotelnikov V.N., Silaev A.A., Karpov R.S. Hemodynamic status of patients with different comorbidities of ischemic heart disease before and after coronary artery bypass grafting
Казаков С.Д., Королева Е.С., Бразовская Н.Г., Зайцев А.А., Иванова С.А., Алифиров В.М. Оценка уровня сывороточного BDNF при комплексной реабилитации пациентов с ишемическим инсультом с использованием традиционных подходов к восстановлению моторных функций	Kazakov S.D., Koroleva E.S., Brazovskaya N.G., Zaytsev A.A., Ivanova S.A., Alifirova V.M. Assessment of serum BDNF levels in complex rehabilitation of patients with ischemic stroke using traditional approaches to the restoration of motor functions
Кит О.И., Франциянц Е.М., Нескубина И.В., Черярина Н.Д., Шихлярова А.И., Сурикова Е.И., Каплиева И.В., Немашкалова Л.А. Влияние варианта развития меланомы B16/F10 на содержание Bcl-2 в митохондриях клеток различных органов самок мышей	Kit O.I., Frantsiyants E.M., Neskubina I.V., Cheryarina N.D., Shikhlyarova A.I., Surikova E.I., Kaplieva I.V., Nemashkalova L.A. Influence of a B16/F10 melanoma variant on the Bcl-2 levels in mitochondria in various organs of female mice
Клюев Е.А., Шейко Г.Е., Дунаев М.Г., Лобанова Е.В., Растеряева М.В., Шарабрин Е.Г. Нейровизуализационные особенности строения головного мозга у детей с детским церебральным параличом, полученные методом магнитно-резонансной трактографии	Klyuev E.A., Sheiko G.E., Dunaev M.G., Lobanova E.V., Rasteryaeva M.V., Sharabrin E.G. Neurovisualization features of brain anatomy in children with spastic cerebral palsy revealed by magnetic resonance tractography
Комар А.А., Скуратовская Д.А., Вульф М.А., Ву Х.К., Даринская А., Газатова Н.Д., Тодосенко Н.М., Затолокин П.А., Кириенкова Е.В., Литвинова Л.С. Влияние sTNFSF14 на митохондриальную динамику в печени у пациентов с ожирением	Komar A.A., Skuratovskaia D.A., Vulf M.A., Vu H.Q., Darinskaya A., Gazatova N.D., Todosenko N.M., Zatolokin P.A., Kirienkova E.V., Litvinova L.S. The role of sTNFSF14 in the liver mitochondrial dynamics in obese patients

Лебедев Д.А., Лаевская М.Ю., Бабенко А.Ю. Маркеры фиброза и эхокардиографические параметры у пациентов с сахарным диабетом 2-го типа в зависимости от уровня альбуминурии	72	Lebedev D.A., Laevskaya M.Yu., Babenko A.Yu. Fibrosis biomarkers and echocardiographic parameters in patients with type 2 diabetes depending on the degree of albuminuria
Осипова Н.Н., Бардеништейн Л.М., Алешкина Г.А., Дмитриева Е.В. Структура аффективных колебаний в неклинической выборке	79	Osipova N.N., Bardenstein L.M., Aleshkina G.A., Dmitrieva E.V. The structure of affective fluctuations in a non-clinical sample
Прокудина Е.С., Маслов Л.Н., Нарыжная Н.В., Курбатов Б.К., Мухомедзянов А.В., Шипулин В.В., Крылатов А.В., Цибульников С.Ю., Воронков Н.С. Роль вегетативной нервной системы в стресс-индуцированном повреждении сердца	88	Prokudina E.S., Maslov L.N., Naryzhnaya N.V., Kurbatov B.K., Mukhomedyanov A.V., Shipulin V.V., Krylatov A.V., Tsibulnikov S.Yu., Voronkov N.S. The role of the autonomic nervous system in stress cardiomyopathy
Рюмишина Н.И., Кошельская О.А., Кологривова И.В., Харитонова О.А., Насекина К.А., Усов В.Ю. Магнитно-резонансная томографическая оценка абдоминальной жировой ткани и состояние брюшной аорты у пациентов с ишемической болезнью сердца: связь с нарушениями метаболизма	95	Ryumshina N.I., Koshelskaya O.A., Kologrivova I.V., Kharitonova O.A., Nasekina K.A., Ussov V.Yu. MRI assessment of the abdominal adipose tissue and the state of the abdominal aorta in patients with coronary artery disease: association with metabolic disorders
Смирнова О.В., Елманова Н.Г. Роль цитокинов в патогенезе инфекционных осложнений при хирургическом лечении механической желтухи желчнокаменного генеза	105	Smirnova O.V., Elmanova N.G. The role of cytokines in the pathogenesis of infectious complications in surgical treatment of obstructive jaundice of gallstone origin
Украинцев И.И., Счастный Е.Д., Бохан Н.А. Частота встречаемости и клинические характеристики сезонного аффективного расстройства у студентов-медиков старшего курса обучения	112	Ukrainets I.I., Schastnyy E.D., Bokhan N.A. Incidence rate and clinical characteristics of seasonal affective disorders in senior medical students
ОБЗОРЫ И ЛЕКЦИИ		REVIEWS AND LECTURES
Андреев К.А., Горбенко А.В., Скирденко Ю.П., Николаев Н.А., Ливзан М.А., Бикбавова Г.Р., Федорин М.М. Приверженность лечению больных воспалительными заболеваниями кишечника	120	Andreev K.A., Gorbenko A.V., Skirdenko Yu.P., Nikolaev N.A., Livzan M.A., Bikbavova G.R., Fedorin M.M. Adherence to treatment in patients with inflammatory bowel disease
Гареев И.Ф., Бейлерли О.А., Измайлов А.А. Потенциал использования микроРНК в судебно-медицинской экспертизе	129	Gareev I.F., Beylerli O.A., Izmailov A.A. The potential use of miRNAs in forensic science
Деева Н.С., Шабалдин А.В., Антонова Л.В. Роль нарушений обмена кальция в индукции иммунной гиперчувствительности при сердечно-сосудистых заболеваниях	141	Deeva N.S., Shabaldin A.V., Antonova L.V. The role of calcium metabolism disorders in induction of hypersensitivity in cardiovascular diseases
Долгушин И.И., Зарипова З.З., Карпова М.И. Роль нейтрофилов в патогенезе ишемического инсульта	152	Dolgushin I.I., Zaripova Z.Z., Karpova M.I. The role of neutrophils in the pathogenesis of ischemic stroke

<i>Климов А.В., Климов В.В., Коваль С.А., Свиридова В.С., Кошкарлова Н.С.</i> Диагностические маркеры локального аллергического ринита	161	<i>Klimov A.V., Klimov V.V., Koval S.A., Sviridova V.S., Koshkarova N.S.</i> Diagnostic markers of local allergic rhinitis
<i>Лопин К.Н., Рыжков И.А., Мальцева В.А., Удут Е.В.</i> Катетеризация сосудов мелких лабораторных животных при проведении биомедицинских исследований: технологические аспекты метода (обзор)	168	<i>Lapin K.N., Ryzhkov I.A., Maltseva V.A., Udut E.V.</i> Vascular catheterization in small laboratory animals in biomedical research: technological aspects of the method (review article)
<i>Омельчук Е.П., Кутилин Д.С., Димитриади С.Н., Гусарева М.А., Тимошкина Н.Н.</i> Молекулярно-генетические аспекты радиорезистентности рака предстательной железы	182	<i>Omelchuk E.P., Kutilin D.S., Dimitriadi S.N., Gusareva M.A., Timoshkina N.N.</i> Molecular genetic aspects of prostate cancer radioresistance
<i>Савинова А.В., Шнайдер Н.А., Насырова Р.Ф.</i> Генетика семейных форм бокового амиотрофического склероза	193	<i>Savinova A.V., Shnayder N.A., Nasyrova R.F.</i> Genetics of familial amyotrophic lateral sclerosis
<i>Чаулин А.М., Дупляков Д.В.</i> МикроРНК: роль в патофизиологии фибрилляции предсердий и возможности использования в качестве биомаркера	203	<i>Chaulin A.M., Duplyakov D.V.</i> MicroRNA: the role in the pathophysiology of atrial fibrillation and potential use as a biomarker
СЛУЧАЙ ИЗ КЛИНИЧЕСКОЙ ПРАКТИКИ		CLINICAL CASES
<i>Артюхина Е.А., Дедух Е.В., Яшков М.В., Ревивили А.Ш.</i> Первый опыт использования неинвазивного картирования в режиме реального времени в условиях электрофизиологической лаборатории для лечения желудочковой аритмии	213	<i>Artyukhina E.A., Dedukh E.V., Yashkov M.V., Revishvili A.Sh.</i> The first experience of using non-invasive real-time mapping in an electrophysiology laboratory for treatment of ventricular arrhythmia
<i>Крапошина А.Ю., Демко И.В., Собко Е.А., Гордеева Н.В., Соловьева И.А., Матвеева И.В., Казмерчук О.В., Кацер А.Б., Абрамов Ю.И., Мальчик Н.В.</i> Клинический случай: поражение легких при гранулематозе с полиангиитом	219	<i>Kraposhina A.Yu., Demko I.V., Sobko E.A., Gordeeva N.V., Soloveva I.A., Matveeva I.V., Kazmerchuk O.V., Katser A.B., Abramov Yu.I., Malchik N.V.</i> A clinical case of lung damage in granulomatosis with polyangiitis
<i>Тетенева А.В., Калюжнин В.В., Чернявская Г.М., Беспалова И.Д., Черногорюк Г.Э., Завадовская В.Д., Жогина Т.В., Устюжанина Е.А., Кузин Е.В., Варфоломеева И.А., Седляр О.В., Медикова Е.А., Кошчавцева Ю.И., Потапов К.В., Карзилов А.И., Поровский Я.В., Соловьев М.М.</i> Клинический случай идиопатического легочного фиброза на фоне коморбидной патологии	225	<i>Teteneva A.V., Kalyuzhin V.V., Chernyavskaya G.M., Bepalova I.D., Chernogoryuk G.E., Zavadovskaya V.D., Zhogina T.V., Ustyuzhanina E.A., Kuzin E.V., Varfolomeeva I.A., Sedlyar O.V., Medikova E.A., Koshchavtseva Yu.I., Potapov K.V., Karzilor A.I., Porovsky Ya.V., Solovev M.M.</i> A clinical case of idiopathic pulmonary fibrosis against the background of comorbid pathology
ПАМЯТЬ		MEMORY
К 75-летию со дня рождения Вячеслава Викторовича Новицкого	232	75 th anniversary of the birth of Vyacheslav Novitsky

УДК 612.111.33:591.11:599.323.45
<https://doi.org/10.20538/1682-0363-2021-3-6-12>

Effects of a high-fat, high-carbohydrate diet on blood cells of rats

Birulina J.G.¹, Ivanov V.V.¹, Buyko E.E.^{1,2}, Trubacheva O.A.^{1,3}, Petrova I.V.¹, Grechishnikova A.Yu.¹, Nosarev A.V.¹, Gusakova S.V.¹

¹ Siberian State Medical University (SSMU)

2, Moscow Trakt, Tomsk, 634050, Russian Federation

² National Research Tomsk Polytechnic University (NR TPU)

30, Lenina Av., Tomsk, 634050, Russian Federation

³ Cardiology Research Institute, Tomsk National Research Medical Center (TNRMC), Russian Academy of Sciences (RAS)

111a, Kievskaya Str., Tomsk, 634012, Russian Federation

ABSTRACT

Aim. To study the effects of a high-fat, high-carbohydrate diet on erythrocytes and platelets of rats.

Materials and methods. Male Wistar rats ($n = 23$) were used for the study. The rats were divided into a control group and an experimental group. The rats from the control group were fed with standard rat chow. The rats from the experimental group had received a high-fat and high-carbohydrate diet for 12 weeks. In the rats, body weight and blood pressure (BP) were measured, an oral glucose tolerance test was carried out, and hematological and lipid metabolism parameters were analyzed. The conductance of erythrocyte K_{Ca} -channels was measured by the potentiometric method, and platelet aggregation was determined by the turbidimetric method.

Results. Feeding the rats with a high-fat, high-carbohydrate diet for 12 weeks resulted in obesity, BP elevation, hyperglycemia, impaired glucose tolerance, and dyslipidemia with pronounced triglyceridemia. In the experimental group, a rise in the number of leukocytes, mainly due to granulocytes, and an increase in the number of platelets and their collagen-induced aggregation were observed. The red blood cell count in the rats of the experimental group did not significantly differ from that of the control group. In the experimental group, multidirectional changes in the membrane potential were observed in response to the stimulation of the K_{Ca} -channels in the erythrocyte membrane with the Ca^{2+} ionophore A23187 or artificial redox systems.

Conclusion. The obtained data indicate that a high-fat, high-carbohydrate diet leads to metabolic and hemorheological disorders that are typical of metabolic syndrome.

Key words: high-fat, high-carbohydrate diet, metabolic syndrome, obesity, dyslipidemia, blood cells, K_{Ca} -channels, aggregation.

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

Source of financing. The study was supported by the Council for Grants of the President of the Russian Federation (MK-143.2020.4).

Conformity with the principles of ethics. The study was approved by the local Ethics Committee at SSMU (Protocol No. 8201 of 27.03.2020).

For citation: Birulina J.G., Ivanov V.V., Buyko E.E., Trubacheva O.A., Petrova I.V., Grechishnikova A.Yu., Nosarev A.V., Gusakova S.V. Effects of a high-fat, high-carbohydrate diet on blood cells of rats. *Bulletin of Siberian Medicine*. 2021; 20 (3): 6–12. <https://doi.org/10.20538/1682-0363-2021-3-6-12>.

Влияние высокожировой и высокоуглеводной диеты на клетки крови крыс

Бирулина Ю.Г.¹, Иванов В.В.¹, Буйко Е.Е.^{1,2}, Трубачева О.А.^{1,3}, Петрова И.В.¹,
Гречишникова А.Ю.¹, Носарев А.В.¹, Гусакова С.В.¹

¹ Сибирский государственный медицинский университет (СибГМУ)
Россия, 634050, г. Томск, Московский тракт, 2

² Национальный исследовательский Томский политехнический университет (НИ ТПУ)
634050, г. Томск, пр. Ленина, 30

³ Научно-исследовательский институт (НИИ) кардиологии, Томский национальный исследовательский медицинский центр (НИМЦ) Российской академии наук
Россия, 634012, г. Томск, ул. Киевская, 111а

РЕЗЮМЕ

Цель — изучить воздействие высокожировой и высокоуглеводной диеты на эритроциты и тромбоциты крови крыс.

Материалы и методы. Исследование выполнено на 23 самцах крыс линии Вистар, которые были разделены на контрольную и опытную группу. Крысы контрольной группы находились на стандартной диете. Крысы опытной группы в течение 12 нед получали высокожировую и высокоуглеводную диету. Животным измеряли массу тела, артериальное давление (АД), выполняли глюкозотолерантный тест, определяли гематологические показатели и параметры липидного обмена. Потенциометрическим методом изучали проводимость K_{Ca} -каналов мембраны эритроцитов, турбидиметрическим — агрегационную способность тромбоцитов.

Результаты. Содержание животных на высокожировой и высокоуглеводной диете приводило к ожирению, повышению АД, гипергликемии, снижению толерантности к глюкозе, дислипидемии с выраженной триглицеридемией. У животных опытной группы происходило увеличение количества лейкоцитов, главным образом, за счет гранулоцитов, повышение числа тромбоцитов и их коллаген-индуцированной агрегации. Количественные показатели клеток красной крови крыс опытной группы не отличались от контрольной группы. В ответ на стимуляцию K_{Ca} -каналов мембраны эритроцитов животных экспериментальной группы с помощью Ca^{2+} -ионофора A23187 или редокс-системы наблюдались разнонаправленные изменения мембранного потенциала.

Заключение. Полученные результаты свидетельствуют, что высокожировая и высокоуглеводная диета приводит к метаболическим и гемореологическим нарушениям, характерным для метаболического синдрома.

Ключевые слова: высокожировая и высокоуглеводная диета, метаболический синдром, ожирение, дислипидемия, клетки крови, K_{Ca} -каналы, агрегация.

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

Источник финансирования. Исследование выполнено при финансовой поддержке Совета по грантам Президента Российской Федерации (МК-143.2020.4).

Соответствие принципам этики. Исследование одобрено локальным этическим комитетом СибГМУ (заключение № 8201 от 27.03.2020).

Для цитирования: Бирулина Ю.Г., Иванов В.В., Буйко Е.Е., Трубачева О.А., Петрова И.В., Гречишникова А.Ю., Носарев А.В., Гусакова С.В. Влияние высокожировой и высокоуглеводной диеты на клетки крови крыс. *Бюллетень сибирской медицины*. 2021; 20 (3): 6–12. <https://doi.org/10.20538/1682-0363-2021-3-6-12>.

INTRODUCTION

Metabolic syndrome (MS) is one of the most topical problems in modern medicine. This is a combination of metabolic, hormonal, and hemodynamic disorders that increase the risk of cardiovascular diseases [1, 2]. The most significant factors in the MS development are abdominal obesity and insulin resistance [3, 4]. In recent years, the number of obese people has increased significantly, that has resulted in the increased prevalence of MS [5, 6].

Currently, it has been documented that MS is accompanied by various pathological processes of the blood system, which play an important role in the pathogenesis of many complications of cardiovascular diseases [7, 8]. Structural and functional disorders of erythrocytes and platelets contribute to the development of the latter. Disorganization and modulation of physicochemical properties in the erythrocyte membrane in metabolic disorders lead to dysregulation of its cation-transporting function, in which an important role belongs to Ca^{2+} -activated potassium channels – Gardos channels (K_{Ca} -channels), the activity of which determines red blood cell eryptosis [9] and deformability [10]. Dyslipidemia and systemic oxidative stress in MS [11] cause an increase in platelet aggregation and elevate the risk of thrombosis [12].

The need to clarify the fundamental basis of hemorheological disorders and their role in the progression of cardiovascular pathology during MS requires an adequate experimental model of MS. One of the most common methods for modeling MS in animals is a special diet. In this respect, a diet with a high content of fats and carbohydrates proved to be the most effective, reproducing the typical characteristics of MS in animals [13, 14].

Therefore, the aim of the study was to investigate the effect of experimental MS, induced by a high-fat and high-carbohydrate diet, on erythrocytes and platelets of rats.

MATERIALS AND METHODS

An experimental MS was modeled on male Wistar rats (23 rats aged 6 weeks, average weight 226.5 ± 20.9 g). The studies were carried out in compliance with the principles of humanity set out in the directives of the European Community (86/609/EEC) and the Declaration of Helsinki. The animals were divided into a control group and an experimental group (11 and 12 animals, respectively). The rats of the control group were fed with a standard diet (Delta Feeds, Biopro, Russia, total calories 3,000 kcal / kg). The rats of the experimental group had received a diet containing standard food (66%) with the addition of animal fat (17%), fructose (17%), and cholesterol (0.25%) for 12 weeks. Drinking water was replaced with a 20% fructose solution (total calories 4,400 kcal/

kg).

Before and at the end of the study, body weight and blood pressure (BP) (Systola, Neurobotics, Russia) were measured in the animals. At the 12th week of the experiment, a glucose tolerance test (GTT) was performed [15]. Fasted rats (12 h of food deprivation) were injected intragastrically with a glucose solution at a dose of 2 g / kg (D-glucose, Sigma-Aldrich, USA). The concentration of glucose was determined in the blood drawn from the rat tail vein spectrophotometrically at 0, 15, 30, 60, 90, and 120 min of the experiment using an enzymatic kit (Chronolab, Spain).

The animals were euthanized with CO_2 after 12 weeks from the start of the experiment. Blood from the heart was collected in BD Vacutainer® vacuum tubes with anticoagulants: K_2 EDTA (to assess hematological and biochemical parameters) or sodium citrate (to study platelet aggregation). The liver and visceral adipose tissue were isolated and weighed using the analytical balance, and their specific gravity was calculated. Hematological parameters were assessed using an automatic analyzer (BC-2800 Vet, Mindray, China). Blood plasma was obtained by centrifugation of whole blood (4 °C, 8,000 g, 6 min). The blood plasma lipid spectrum of the animals was determined using an automatic biochemical analyzer (Architect c4000, Abbott, USA).

To study the activity of K_{Ca} -channels, the erythrocyte sediment obtained after centrifugation was resuspended in an incubation medium containing 150 mM NaCl, 10 mM glucose, 1 mM KCl, and 1 mM MgCl_2 . The amplitude of an erythrocyte hyperpolarization response (HR) was determined after addition of 0.5 μM Ca^{2+} ionophore A23187 or the artificial ascorbate (10 mM) – phenazine methosulfate (PMS, 0.1 mM) redox system. Rat platelet aggregation was studied by the turbidimetric method using a laser analyzer (Biola, Russia). Collagen (2 μg / ml) was used as a platelet aggregation inducer. The degree and rate of platelet aggregation were determined according to the average aggregate size curve.

The data were analyzed using the SPSS Statistics 23 software. The data are presented as the mean and standard deviation ($M \pm SD$) for normally distributed variables and as the median (Me) and the interquartile range (Q_1 ; Q_3) for non-normally distributed variables. To analyze the differences between the samples, Student's *t*-test or the Mann – Whitney U-test were used. The result was considered statistically significant at $p < 0.05$. Correlation was assessed using the Pearson's correlation coefficient.

RESULTS

Feeding the rats with a high-fat, high-carbohydrate diet for 12 weeks resulted in an increase in blood pressure and specific gravity of the adipose tissue and liver

of the studied animals (Table 1). It was shown that the specific gravity of abdominal fat, including mesenteric, epididymal, and retroperitoneal fatty tissue, increased by 2.5 times in the experimental rats.

The rats of the experimental group showed an increase in the level of triacylglycerols (TAG) in blood plasma by 2.5 times compared with the control group. The total cholesterol (Ch) level in the blood of the animals fed with a high-fat, high-carbohydrates diet increased by 1.5 times. Such an increase in the level of total Ch is determined by a rise in the level of low-density lipoprotein cholesterol (LDL-Ch), while the level of high-density lipoprotein cholesterol (HDL-Ch) did not change (Table 1). A direct relationship was found between the concentration of TAG and an increase in the specific gravity of the adipose tissue ($r = 0.689$, $p = 0.001$), weight of the liver ($r = 0.434$, $p = 0.03$), the content of total Ch, and the specific gravity of the liver ($r = 0.418$, $p = 0.04$). Furthermore, in the animals receiving a special diet, an elevated level of glucose in the blood plasma after fasting was observed (Table 1). A positive correlation was found between the concentration of glucose in the blood and the specific gravity of the adipose tissue ($r = 0.823$, $p = 0.001$).

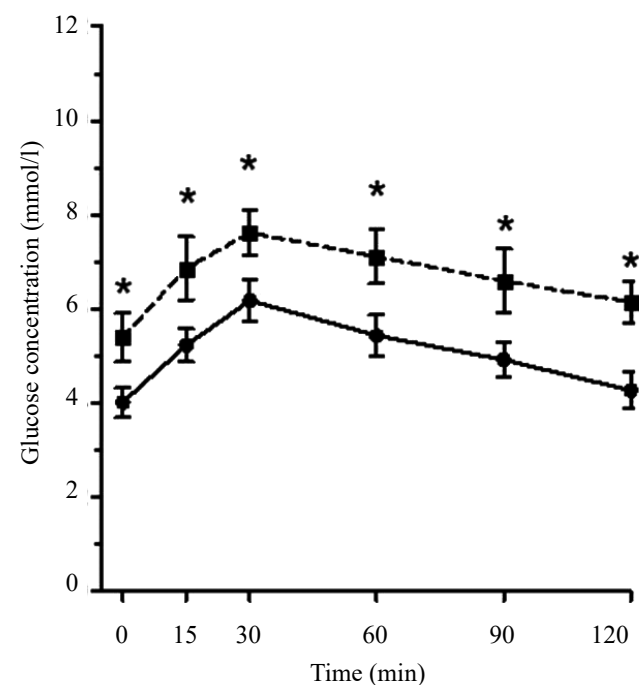
Table 1

Effects of a high-fat, high-carbohydrate diet on the physiological and biochemical parameters of rats, $M \pm SD$		
Parameter	Group	
	Controls ($n = 11$)	Metabolic syndrome ($n = 12$)
Body weight, g	376.6 ± 28.9	388.2 ± 30.7 ($p = 0.241$)
SBP, mm Hg.	123.2 ± 8.8	139.2 ± 9.1 ($p = 0.01$)
DBP, mm Hg.	86.5 ± 9.3	101.4 ± 12.2 ($p = 0.028$)
Fasting blood glucose, mmol/l	4.0 ± 0.3	5.4 ± 0.5 ($p = 0.001$)
Total Ch, mmol/l	1.7 ± 0.2	2.5 ± 0.3 ($p = 0.009$)
HDL-Ch, mmol/l	± 0.2	8 ± 0.1 ($p = 0.09$)
LDL-Ch, mmol/l	0.7 ± 0.3	1.6 ± 0.4 ($p = 0.03$)
TAG, mmol/l	0.9 ± 0.4	2.3 ± 0.8 ($p = 0.001$)
Specific gravity of the adipose tissue, g	1.1 ± 0.3	2.8 ± 0.4 ($p = 0.001$)
Specific gravity of the liver, g	3.2 ± 0.5	4.3 ± 0.6 ($p = 0.001$)

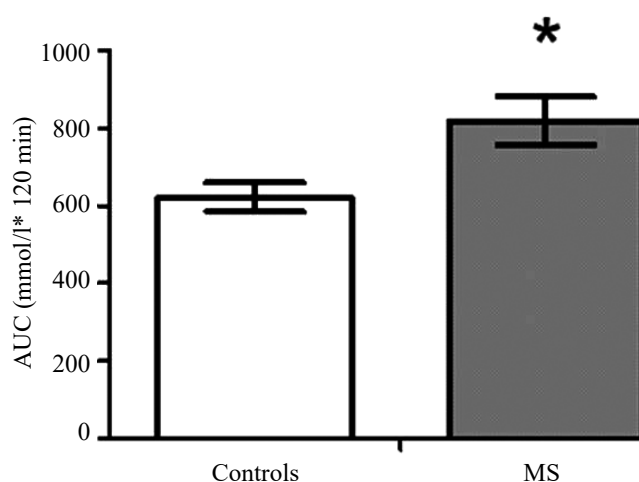
Note: SBP – systolic blood pressure, DBP – diastolic blood pressure. Here and in Table 2, 3: p – the level of statistical significance in comparison with the controls.

Following GTT, it was found that 30 min after glucose load in the rats of the experimental group, the blood glucose level exceeded the level in the control group by 23.3% ($p = 0.005$); after 60 and 120 min – by 30.9% ($p = 0.001$) and 28.6% ($p = 0.003$), respectively (Figure, a). The area under the glucose concentration – time

curve (AUC_{0-120}) in the experimental group of animals was 818.2 ± 61.4 mmol / $l \times 120$ min and exceeded the control group value (622.1 ± 36.8 mmol / $l \times 120$ min, $p = 0.001$) (Figure, b).



a



b

Figure. Changes in the blood glucose concentration in rats (a) and the area under the glucose concentration – time curve (b) in the oral glucose tolerance test: the control group (solid line) and the experimental group (dotted line). * $p < 0.05$ in comparison with the control group

Evaluation of hematological parameters revealed that in the animals fed with a high-fat and high-carbohydrate diet the leukocyte count increased by 1.5 times in comparison with the control group, mainly due to granulocytes (Table 2). A relationship between the leukocyte count ($r = 0.589$, $p = 0.015$) and the specific gravity of

the adipose tissue was established in the experimental rats. There were no statistically significant changes in the count of red blood cells in the animals of the experimental group. However, the platelet count in the blood was 1.4 times higher than in the rats receiving a standard diet (Table 2).

In the rats fed with a high-fat, high-carbohydrate diet, the amplitude of the erythrocyte HR stimulated by the Ca^{2+} ionophore A23187 decreased compared with the control group (Table 3). In contrast, the amplitude of HR generated by adding the artificial electron-donor system (ascorbate – PMS) to the erythrocyte suspension was elevated in the rats of the experimental group. In the animals of the experimental group, an increase in the degree and rate of collagen-induced platelet aggregation was observed (Table 3).

Table 2

Effects of a high-fat, high-carbohydrate diet on the hematological parameters of rats, $M \pm SD$		
Parameter	Group	
	Controls (n = 11)	Metabolic syndrome (n = 12)
White blood cells, $10^9/\text{l}$	14.1 ± 3.0	20.7 ± 5.5 ($p = 0.022$)
Lymphocytes, %	69.7 ± 6.4	63.4 ± 8.9 ($p = 0.048$)
Monocytes, %	3.7 ± 0.3	3.9 ± 0.7 ($p = 0.078$)
Granulocytes, %	26.6 ± 5.8	35.9 ± 7.6 ($p = 0.001$)
Red blood cells, $10^{12}/\text{l}$	9.4 ± 0.5	9.2 ± 0.8 ($p = 0.28$)
Hemoglobin, g/l	208 ± 10.9	198 ± 11.5 ($p = 0.088$)
Hematocrit, %	47.1 ± 2.4	45.6 ± 6.8 ($p = 0.072$)
Mean cell volume, fl	50.3 ± 1.8	49.3 ± 1.1 ($p = 0.118$)
Platelets, $10^9/\text{l}$	$1,010.8 \pm 70.5$	$1,450.5 \pm 84.6$ ($p = 0.02$)

Table 3

Effects of a high-fat, high-carbohydrate diet on the hyperpolarization response of erythrocytes and collagen-induced platelet aggregation of rats, $Me (Q_1; Q_3)$				
Group	Parameter			
	A23187-induced HR, mV	Redox-induced HR, mV	Degree of aggregation, rel. units	Rate of aggregation, rel. units/min
Controls (n = 11)	$-38.4 (-40.2; -35.3)$	$-40.2 (-45.6; -35.5)$	$1.6 (1.4; 1.8)$	$1.9 (1.7; 2.6)$
Metabolic syndrome (n = 12)	$-30.1 (-34.6; -27.6)$ $p = 0.022$	$-49.1 (-51.3; -44.3)$ $p = 0.015$	$2.9 (2.4; 4.2)$ $p = 0.008$	$2.9 (2.7; 5.2)$ $p = 0.011$

DISCUSSION

Experimental animal models are used to study the mechanisms of development and progression of metabolic and hemorheological disorders in MS [16–18]. They are aimed at reproducing the characteristic signs of MS, such as overweightness, obesity, insulin resistance, impaired glucose tolerance, dyslipidemia, and arterial hypertension [1, 3]. One of the possible ways to induce MS in animals is a high-fat, high-carbohydrate diet.

In our study, it was found that feeding rats with a diet containing animal fat, fructose, and Ch for 12 weeks resulted in obesity, increased BP, hyperglycemia, decreased glucose tolerance, and dyslipidemia with hypertriglyceridemia. In the meantime, a positive correlation between a significant increase in the specific gravity of the liver, total Ch, and TAG level may indicate emergence of metabolic disorders in the liver of the experimental animals. The results are consistent with the literature data on diet-induced MS models [14, 18] and indicate that the chosen diet effectively reproduces the clinical signs of MS.

Experimental and clinical studies carried out in recent years suggest that the pathogenetic mechanisms of MS are inextricably linked with the functioning of the blood system. Thus, it was shown that MS is accompa-

nied by an increase in blood viscosity, aggregation of erythrocytes, a change in the number of red [7, 17] and white blood [19] cells, and an increase in the content and functional activity of platelets [20]. In this work, it was demonstrated that the leukocyte count increased in the animals of the experimental group mainly due to granulocytes. A correlation was also established between the white blood cell count and the specific gravity of the adipose tissue. It is known that obesity, as one of the important components of MS, is accompanied by a chronic inflammatory process in the adipose tissue, infiltrated by leukocytes, with high production of proinflammatory cytokines [15, 19].

Feeding animals with a high-fat, high-carbohydrate diet led to a rise in the content of platelets in the blood and an increase in their collagen-induced aggregation. According to existing data, an increase in the sensitivity of platelets to collagen in experimental rats can be caused by an increase in glycoprotein VI (GPVI) on the cell surface [20, 21], as well as by a change in the content of lipids in platelet membranes due to dyslipidemia [12]. This is inevitably accompanied by activation of phospholipases C and A_2 and protein kinase C followed by phosphorylation of platelet contractile proteins. It was shown that prothrombotic conditions associated with metabolic disorders increase blood clotting, reduce fi-

brinolysis and endothelial thromboresistance, and cause platelet hyperactivity [22].

The quantitative indicators of red blood cells of the experimental animals did not differ from those of the control group. However, after the stimulation of K_{Ca} -channels in the erythrocyte membrane with the Ca^{2+} ionophore A23187 or the artificial electron-donor system (ascorbate – PMS), multidirectional changes in the HR amplitude were observed. This effect can be explained by the increased level of calcium ions in erythrocytes of the rats with MS [9]. Thus, equal addition of Ca^{2+} to a suspension of normal erythrocytes and a suspension with an increased level of calcium ions will cause a decrease in the HR amplitude in the latter. The change in the HR amplitude caused by the Ca^{2+} ionophore and the artificial ascorbate – PMS redox system may be determined by different mechanisms of K_{Ca} -channel opening [23].

In turn, a rise in the amplitude of the ascorbate – PMS-induced HR in the erythrocyte membrane in the animals of the experimental group indicates increased conductance of K_{Ca} -channels in the erythrocyte membrane. At the same time, structural rearrangement of the membrane lipid composition caused by increased cholesterol incorporation and phosphatidylserine externalization significantly affects the activity of Gardos channels, which leads to an increase in microviscosity of the lipid bilayer [10] and a decrease in cell deformability in the vascular bed.

CONCLUSION

It was found that the proposed high-fat, high-carbohydrate diet effectively reproduces the typical symptoms of MS. Analysis of hematological parameters revealed significant changes in white blood cells and platelets of the animals with MS. The erythrocyte membrane of the rats fed with a special diet was characterized by increased permeability to potassium ions, and platelets were characterized by high sensitivity to collagen. The obtained results are valuable for studying the causes of development and progression of cardiovascular diseases associated with MS.

REFERENCES

- Potenza M.V., Mechanick J.I. The metabolic syndrome: definition, global impact, and pathophysiology. *Nutr. Clin. Pract.* 2009; 24 (5): 560–577. DOI: 10.1177/0884533609342436.
- Saklayen M.G. The global epidemic of the metabolic syndrome. *Curr. Hypertens. Rep.* 2018; 20 (2): 12. DOI: 10.1007/s11906-018-0812-z.
- Aydin S., Aksoy A., Aydin S., Kalayci M., Yilmaz M., Kuloğlu T., Cıtil C., Catak Z. Today's and yesterday's of pathophysiology: biochemistry of metabolic syndrome and animal models. *Nutrition.* 2014; 30 (1): 1–9. DOI: 10.1016/j.nut.2013.05.013.
- Catoi A.F., Parvu A.E., Andreicut A.D., Mironiuc A., Craciun A., Catoi C., Pop I.D. Metabolically healthy versus unhealthy morbidly obese: chronic inflammation, nitro-oxidative stress, and insulin resistance. *Nutrients.* 2018; 10 (9): 1199. DOI: 10.3390/nu10091199.
- Chen Y.Y., Fang W.H., Wang C.C., Kao T.W., Chang Y.W., Yang H.F., Wu C.J., Sun Y.S., Chen W.L. Association of percentage body fat and metabolic health in offspring of patients with cardiovascular diseases. *Sci. Rep.* 2018; 8 (1): 13831. DOI: 10.1038/s41598-018-32230-7.
- Lee M.K., Han K., Kim M.K., Koh E.S., Kim E.S., Nam G.E., Kwon H.S. Changes in metabolic syndrome and its components and the risk of type 2 diabetes: a nationwide cohort study. *Sci. Rep.* 2020; 10 (1): 2313. DOI: 10.1038/s41598-020-59203-z.
- Zeng N.F., Mancuso J.E., Zivkovic A.M., Smilowitz J.T., Ristenpart W.D. Red blood cells from individuals with abdominal obesity or metabolic abnormalities exhibit less deformability upon entering a constriction. *PLoS One.* 2016; 11 (6): e0156070. DOI: 10.1371/journal.pone.0156070.
- Li P.F., Chen J.S., Chang J.B., Chang H.W., Wu C.Z., Chuang T.J., Huang C.L., Pei D., Hsieh C.H., Chen Y.L. Association of complete blood cell counts with metabolic syndrome in an elderly population. *BMC Geriatr.* 2016; 16: 10. DOI: 10.1186/s12877-016-0182-9.
- Lang F., Lang E., Föller M. Physiology and pathophysiology of eryptosis. *Transfus. Med. Hemother.* 2012; 39 (5): 308–314. DOI: 10.1159/000342534.
- Gyawali P., Richards R.S., Uba Nwose E. Erythrocyte morphology in metabolic syndrome. *Expert. Rev. Hematol.* 2012; 5 (5): 523–531. DOI: 10.1586/ehm.12.47.
- Marseglia L., Manti S., D'Angelo G., Nicotera A., Parisi E., Di Rosa G., Gitto E., Arrigo T. Oxidative stress in obesity: a critical component in human diseases. *Int. J. Mol. Sci.* 2014; 16 (1): 378–400. DOI: 10.3390/ijms16010378.
- Zavalishina S.Yu., Kutafina N.V., Vatinikov Yu.A., Makurina O.N., Kulikov E.V., Rystsova E.O., Gurina R.R., Sotnikova E.D. Platelet-activity dependence on the age of rats with experimental dyslipidemia. *Biol. Med. (Aligarh).* 2016; 8: 326. DOI: 10.4172/0974-8369.1000326.
- Dupas J., Feray A., Goanvec C., Guernec A., Samson N., Bougaran P., Guerrero F., Mansourati J. Metabolic syndrome and hypertension resulting from fructose enriched diet in Wistar rats. *Biomed. Res. Int.* 2017; 2017: 2494067. DOI: 10.1155/2017/2494067.
- Kwitek A.E. Rat models of metabolic syndrome. *Methods Mol. Biol.* 2019; 2018: 269–285. DOI: 10.1007/978-1-4939-9581-3_13.
- Francisqueti F.V., Nascimento A.F., Minatel I.O., Dias M.C., Luvizotto R., Berchieri-Ronchi C., Ferreira A., Correa C.R. Metabolic syndrome and inflammation in adipose tissue occur at different times in animals submitted to a high-sugar/fat diet. *J. Nutr. Sci.* 2017; 6: e41. DOI: 10.1017/jns.2017.42.
- Suman R.K., Mohanty I.R., Borde M.K., Maheshwari U., Deshmukh Y.A. Development of an experimental model of diabetes co-existing with metabolic syndrome in rats. *Adv. Pharmacol. Sci.* 2016; 2016: 9463476. DOI: 10.1155/2016/9463476.

17. Monteomo G.F., Kamagate A., Yapo A.P. Effects of metabolic syndrome on blood cells to Wistar rats. *J. Diabetes Metab. Disord. Control.* 2018; 5 (6): 222–225. DOI: 10.15406/jdm-dc.2018.05.00170.
18. Marques C., Meireles M., Norberto S., Leite J., Freitas J., Pestana D., Faria A., Calhau C. High-fat diet-induced obesity rat model: a comparison between Wistar and Sprague Dawley rat. *Adipocyte.* 2015; 5 (1): 11–21. DOI: 10.1080/21623945.2015.1061723.
19. Sait S., Alqassab M., Othman S., Shihon S.B., Alqalayta L., Alqusair S., Qari M. Obesity correlates with neutrophilia. *Hematol. Transfus. Int. J.* 2016; 3 (2): 159–162. DOI: 10.15406/htij.2016.03.00062.
20. Barrachina M.N., Moran L.A., Izquierdo I., Casanueva F.F., Pardo M., Garcia A. Analysis of platelets from a diet-induced obesity rat model: elucidating platelet dysfunction in obesity. *Sci. Rep.* 2020; 10 (1): 13104. DOI: 10.1038/s41598-020-70162-3.
21. Barrachina M.N., Sueiro A.M., Izquierdo I., Hermida-Nogueira L., Guitian E., Casanueva F.F., Farndale R.W., Moroi M., Jung S.M., Pardo M., Garcia A. GPVI surface expression and signalling pathway activation are increased in platelets from obese patients: elucidating potential anti-atherothrombotic targets in obesity. *Atherosclerosis.* 2019; 281: 62–70. DOI: 10.1016/j.atherosclerosis.2018.12.023.
22. Ishida K., Taguchi K., Matsumoto T., Kobayashi T., Activated platelets from diabetic rats cause endothelial dysfunction by decreasing akt/endothelial NO synthase signaling pathway. *PLoS One.* 2014; 9 (7): e102310. DOI: 10.1371/journal.pone.0102310.
23. Birulina Y.G., Petrova I.V., Rozenbaum Y.A., Shefer E.A., Smaglyi L.V., Nosarev A.V., Gusakova S.V. H₂S-Mediated changes in erythrocyte volume: role of gardos channels, Na⁺,K⁺,2Cl⁻ cotransport and anion exchanger. *Bull. Exp. Biol. Med.* 2019; 167 (4): 508–511. DOI: 10.1007/s10517-019-04561-6.

Authors contribution

Birulina J.G., Ivanov V.V. – conception and design, drafting of the manuscript. Petrova I.V., Grechishnikova A. Yu. – analysis and interpretation of data. Nosarev A.V., Gusakova S.V. – substantiation of the manuscript, final approval of the manuscript for publication. Buyko E.E., Trubacheva O.A. – carrying out of the experiment.

Authors information

Birulina Julia G., Cand.Sci. (Biology), Associate Professor, Biophysics and Functional Diagnostics Division, SSMU, Tomsk, Russian Federation. ORCID 0000-0003-1237-9786.

Ivanov Vladimir V., Cand. Sci. (Biology), Head of the Center for Preclinical Research, Central Research Laboratory, SSMU, Tomsk, Russian Federation. ORCID 0000-0001-9348-4945.

Buyko Evgeniy E., Laboratory Assistant, Center for Preclinical Research, Central Research Laboratory, SSMU; Post-Graduate Student, Research School of Chemistry & Applied Biomedical Sciences, TPU, Tomsk, Russian Federation. ORCID 0000-0002-6714-1938.

Trubacheva Oksana A., Cand. Sci. (Med.), Physical Education and Health Division, SSMU; Researcher, Department of Functional and Laboratory Diagnostics, Cardiology Research Institute, TNRM RAS, Tomsk, Russian Federation. ORCID 0000-0002-1253-3352.

Petrova Irina V., Dr. Sci. (Biology), Professor, Biophysics and Functional Diagnostics Division, SSMU, Tomsk, Russian Federation. ORCID 0000-0001-9034-4226.

Grechishnikova Aleksandra Yu., Assistant, Medical and Biological Cybernetics Division, SSMU, Tomsk, Russian Federation. ORCID 0000-0001-9891-2910.

Nosarev Alexey V., Dr. Sci. (Med.), Professor, Biophysics and Functional Diagnostics Division, SSMU, Tomsk, Russian Federation. ORCID 0000-0002-0119-9707.

Gusakova Svetlana V., Dr. Sci. (Med.), Head of the Biophysics and Functional Diagnostics Division, SSMU, Tomsk, Russian Federation. ORCID 0000-0001-5047-8668.

(✉) **Birulina Julia G.**, e-mail: birulina20@yandex.ru

Received 04.11.2020

Accepted 28.12.2020

The role of pAKT1 expression in diffuse large B-cell lymphoma

Vaneeva E.V., Rosin V.A., Diakonov D.A., Luchinin A.S., Samarina S.V., Kochetov N.L.

*Kirov Research Institute of Hematology and Blood Transfusion (KRIHBT)
72, Krasnoarmeyskaya Str., Kirov, 610027, Russian Federation*

ABSTRACT

Aim. To evaluate the prognostic value of pAKT1 expression by tumor cells in patients with diffuse large B-cell lymphoma.

Materials and methods. The study included 90 patients with newly diagnosed diffuse large B-cell lymphoma (DLBCL), who were treated at the clinic of Kirov Research Institute of Hematology and Blood Transfusion from 2014 to 2017 and received standard first-line polychemotherapy according to the R-CHOP regimen. Using immunohistochemical and morphometric methods, the relative number of tumor cells expressing pAKT1 was determined. Using the two-sided Fisher's exact test, the relationship of different levels of marker expression with clinical and laboratory parameters of patients and long-term treatment results was analyzed. The impact of pAKT1 on the risk of an adverse event was assessed using the Cox regression analysis.

Results. Overexpression of pAKT1 is associated with unfavorable clinical characteristics of patients with DLBCL, excessive expression of the BCL2 and c-Myc oncoproteins, as well as with low rates of overall and progressive survival. Overexpression of pAKT1 is an independent prognostic factor and statistically significantly affects the risk of an adverse outcome in DLBCL.

Conclusion. The degree of pAKT1 expression is an informative criterion that allows to predict the course of diffuse large B-cell lymphoma. It is advisable to use the indicated marker when stratifying patients into risk groups.

Key words: diffuse large B-cell lymphoma, pAKT1, overexpression, survival, biomarker.

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

Source of financing. The study was supported by Kirov Research Institute of Hematology and Blood Transfusion.

Conformity with the principles of ethics. All patients signed an informed consent to participate in the study. The study was approved by the local Ethics Committee at Kirov Research Institute of Hematology and Blood Transfusion (Protocol No. 48 of 12.10.2019).

For citation: Vaneeva E.V., Rosin V.A., Diakonov D.A., Luchinin A.S., Samarina S.V., Kochetov N.L. The role of pAKT1 expression in diffuse large B-cell lymphoma. *Bulletin of Siberian Medicine*. 2021; 20 (3): 13–20. <https://doi.org/10.20538/1682-0363-2021-3-13-20>.

✉ Vaneeva Elena V., e-mail: vaneeva.elena.vic@mail.ru

Значение экспрессии pAKT1 при диффузной В-крупноклеточной лимфоме

Ванеева Е.В., Росин В.А., Дьяконов Д.А., Лучинин А.С., Самарина С.В., Кочетов Н.Л.

Кировский научно-исследовательский институт гематологии и переливания крови (КНИИГиПК)
Россия, 610027, г. Киров, ул. Красноармейская, 72

РЕЗЮМЕ

Цель – оценить прогностическое значение экспрессии pAKT1 опухолевыми клетками у больных диффузной В-крупноклеточной лимфомой.

Материалы и методы. В исследование включены 90 пациентов с впервые диагностированной диффузной В-крупноклеточной лимфомой (ДВККЛ), наблюдавшиеся в клинике института с 2014 по 2017 г. и получавшие стандартную полихимиотерапию первой линии по схеме R-CHOP. С помощью иммуногистохимического и морфометрического методов определено относительное количество экспрессирующих pAKT1 опухолевых клеток. С помощью точного двухстороннего критерия Фишера проанализирована взаимосвязь различных уровней экспрессии маркера с клинико-лабораторными показателями пациентов и отдаленными результатами лечения. Оценку влияния pAKT1 на риск наступления неблагоприятного события проводили с помощью регрессионного анализа Кокса.

Результаты. Гиперэкспрессия pAKT1 ассоциирована с неблагоприятными клиническими характеристиками больных диффузной В-крупноклеточной лимфомой, избыточной экспрессией онкобелков BCL2, cMyc, а также низкими показателями общей и беспрогрессивной выживаемости. Гиперэкспрессия pAKT1 является независимым фактором прогноза и статистически значимо влияет на риск возникновения неблагоприятного исхода при ДВККЛ.

Заключение. Степень экспрессии pAKT1 является информативным критерием, позволяющим прогнозировать течение диффузной В-крупноклеточной лимфомы. Указанный маркер целесообразно использовать при стратификации пациентов на группы риска.

Ключевые слова: диффузная В-крупноклеточная лимфома, pAKT1, гиперэкспрессия, выживаемость, биомаркер.

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

Источник финансирования. Работа выполнена при финансовой поддержке КНИИГиПК ФМБА России.

Соответствие принципам этики. Все пациенты подписали информированное согласие на участие в исследовании. Исследование одобрено локальным этическим комитетом КНИИГиПК ФМБА России (протокол № 48 от 10.12.2019).

Для цитирования: Ванеева Е.В., Росин В.А., Дьяконов Д.А., Лучинин А.С., Самарина С.В., Кочетов Н.Л. Значение экспрессии pAKT1 при диффузной В-крупноклеточной лимфоме. *Бюллетень сибирской медицины*. 2021; 20 (3): 13–20. <https://doi.org/10.20538/1682-0363-2021-3-13-20>.

INTRODUCTION

Diffuse large B-cell lymphoma (DLBCL) is known as the most common type of lymphoid neoplasia in adults. On average, it accounts for 40% of all non-Hodgkin lymphomas, varying slightly across geographic regions. The disease is characterized by high biological and clinical heterogeneity, rapid tumor growth, and early extranodal lesions [1–3].

Standard treatment for common variants of DLBCL is first-line polychemotherapy according to the

R-CHOP regimen (rituximab, cyclophosphamide, doxorubicin, vincristine, prednisolone). It provides good overall survival (OS) and progression-free survival (PFS). However, the disease is refractory in more than one-third of patients [4, 5].

The International Prognostic Index (IPI) and age-adjusted International Prognostic Index (aaIPI) are used as the main tools for assessing individual risk of early disease progression. These indices are quite successfully used in clinical practice, but in some cases, they do not allow to accurately determine the

prognosis of the disease, since they mainly rely on the clinical characteristics of patients and do not take into account aspects related to biology of neoplastic cells [4, 6, 7].

It was found that molecular and biological characteristics of the tumor can influence the course of DLBCL and play an important role in the onset of unfavorable outcomes. Thus, based on the gene expression profiling (GEP), the main molecular subgroups of DLBCL (ABC and GCB) were identified. These subgroups to a large extent correlate with the disease prognosis when using standard chemotherapy regimens [2, 8]. Unfortunately, integration of GEP in clinical practice is limited due to its technological complexity and high cost. Surrogate markers in immunohistochemical analysis determined using various algorithms do not fully correlate with GEP, which reduces the accuracy of obtained prognostic information [9]. Therefore, search for other informative molecular and biological predictors of the disease course remains relevant [4].

In recent years, much attention has been paid to studying various signaling pathways involved in the pathogenesis of DLBCL. One of them is the PI3K / AKT / mTOR pathway, which has crosstalk with many other oncogenic signaling pathways and mediates a wide range of cellular functions, including proliferation, apoptosis, metabolism, and angiogenesis [10]. The main components of this signaling pathway are phosphoinositide 3-kinase (PI3K), AKT1 serine / threonine kinase, and the mTOR protein. The most important of them is the AKT1 enzyme, a key regulatory protein of this pathway.

Intact AKT1 is located in the cytoplasm of the cell. The pathway is activated by binding of a ligand to a cellular receptor and subsequent initiation of an enzymatic cascade of phosphorylation reactions. As a result, a complex of phospholipids is formed on the cell membrane, the most important of which being PIP₃ (phosphatidylinositol-3-4-5-triphosphate). It binds to the N-terminal domain of AKT1 and recruits it to the cytoplasmic membrane. Here, the protein kinase is activated (pAKT1) in the catalytic and hydrophobic domains through sequential interaction with PDK1 / 2 (3-phosphoinositide-dependent protein kinase-1, 2) and mTOR. Then it leaves the membrane and moves to the cytoplasm and the cell nucleus. In normal conditions, protein phosphorylation is a temporary and strictly controlled process [11].

According to foreign studies, the PI3K / AKT / mTOR signaling pathway is constitutively activated

in 25–50% of DLBCL cases [12]. This contributes to a rise in the level of intracellular signaling and an increase in the survival rate and proliferation of tumor cells and is accompanied by tumor resistance to standard chemotherapy and high incidence of relapses. It was shown that this pathway can activate the nuclear factor-kappa B (NF-κB), triggering the oncogenic JAK / STAT signaling pathway. This enhances expression of apoptosis inhibitors (survivin and TIMP1) and promotes the development of immortality of tumor cells [8, 10].

There is evidence that an increased level of pAKT1 indirectly affects tumor resistance to chemotherapy through negative regulation of the activity of transport proteins that control the entry and exit of substances from the cell [13]. At the same time, the prognostic value of pAKT1 expression in DLBCL has been studied insufficiently. The results of the published works are contradictory. There is no information on this topic in the domestic scientific literature.

The aim of the study was to evaluate the prognostic value of pAKT1 expression in tumor cells in DLBCL.

MATERIALS AND METHODS

The retrospective study included 90 patients with a newly diagnosed DLBCL who were treated at the clinic of Kirov Scientific Research Institute of Hematology and Blood Transfusion from 2014 to 2017. All patients received standard first-line therapy according to the R-CHOP regimen. The average age of patients was 58 years (from 24 to 83 years). All patients signed an informed consent to participate in the study. The study was approved by the local Ethics Committee. Clinical and laboratory characteristics of patients with DLBCL are presented in Table 1.

Formalin-fixed, paraffin-embedded (FFPE) lymph node and other organ and tissue samples were used, from which 3–5 μm-thick histological sections were prepared according to the generally accepted method. Verification of DLBCL diagnosis and visualization of pAKT1-positive tumor cells in the studied samples were carried out by the immunohistochemical method using antibodies to CD3, CD20, CD10, BCL6, MUM1, Ki67, BCL2, cMyc, and pAKT1 (phosphor Ser 473). The tumor subtype (GCB or non-GCB) was determined according to the classification proposed by C.P. Hans [14].

Immunoreactivity of primary antibodies was detected using secondary antibodies conjugated with peroxidase included in the reagent kit. Immunohistochemical reactions were set up according to the stan-

standard technique following the protocol for ENVISION imaging systems (DAB+, Dako, Denmark). The relative number of tumor cells with nuclear expression of pAKT1 was calculated on the AxioScope.A1 microscope (Carl Zeiss Microscopy GmbH, Germany) with a built-in photo / video camera and image analysis software. The study was carried out in five fields of view for each sample using the x10 eyepiece lens and the x100 objective lens.

The cut-off threshold for assessing the level of pAKT1 expression was calculated using the ROC analysis. For the oncoproteins BCL2 and cMyc, the expression thresholds used in international studies and showing the most reproducible results (50 and 40% of positive tumor cells, respectively) were used [15–17]. Statistical assessment of the presence / absence of an association between the degree of pAKT1 expression and the clinical and laboratory characteristics of patients was carried out using the two-sided Fisher's exact test. OS and PFS were calculated using the Kaplan – Meier method with graphical plotting of the corresponding curves.

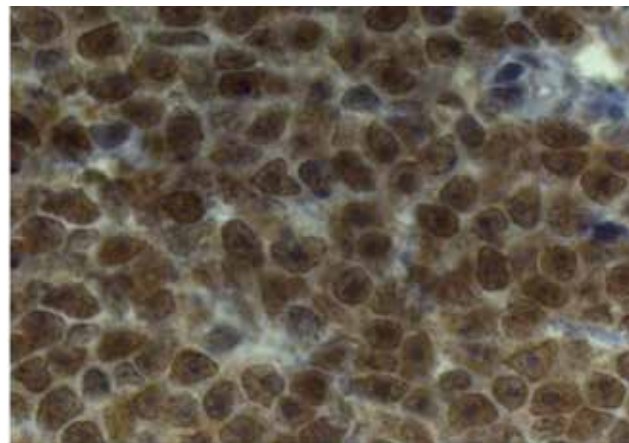
A comparative analysis of survival rates was performed using the log-rank test. To identify prognostic factors influencing the risk of developing an adverse event for OS and PFS, we used univariate and multivariate Cox regression analysis with the determination of a 95% confidence interval (CI) and hazard ratio (HR). The study was carried out using the IBM SPSS version 19.0 software. Differences between indicators were considered statistically significant at $p < 0.05$.

RESULTS

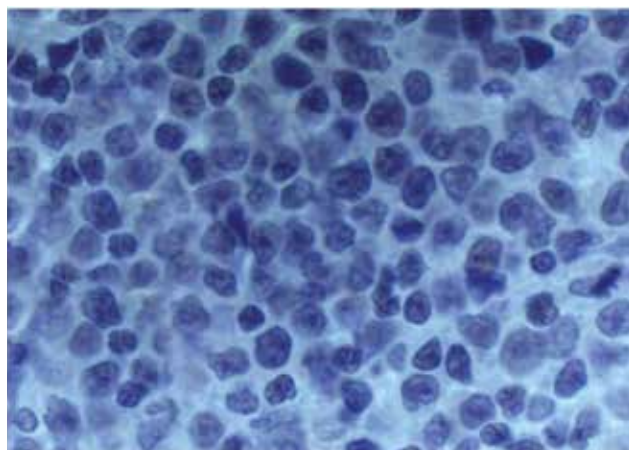
During the research, all patients were divided into two groups depending on the number of pAKT1-positive tumor elements. According to the results of the ROC analysis, the optimal cut-off threshold for protein expression was set at 70% with sensitivity of 84% and specificity of 24%; the area under the curve was 0.78 ± 0.04 (95% CI 0.66–0.91). Group 1 included 36 patients (40%) with protein overexpression ($\geq 70\%$ of cells, Fig. 2a), group 2 contained 54 (60%) patients with a low degree of marker expression ($< 70\%$ of cells, Fig. 1b).

Following a comparative assessment of the degree of pAKT1 expression by DLBCL tumor cells with the clinical and laboratory characteristics of patients, statistically significant intergroup differences were revealed (Table 1). It was noted that the values of IPI > 2 were more often detected in the patients of group

1 compared with its values in the patients of group 2 (58% vs. 33%, $p = 0.029$). The frequency of occurrence of stage III–IV of the pathological process was significantly higher in the patients with pAKT1 overexpression than in the patients with low expression of this marker (72% vs. 48%, $p = 0.030$).



a



b

Fig. 1. Immunohistochemical staining of tumor cells with an antibody to pAKT1: high (a) and low (b) expression, x 1,000

The patients of group 1 were 1.5 times more likely to have B symptoms of the disease than those of group 2 (69% vs. 46%, $p = 0.034$). When analyzing the results of R-CHOP chemotherapy, it was found that the patients with low marker expression were characterized by higher frequency of achieving complete remissions than patients with overexpression of the marker (44% vs. 70%, respectively, $p = 0.017$). In addition, the supra-threshold values for the number of pAKT1-positive tumor cells were associated with high expression of the oncoproteins BCL2 ($p = 0.036$) and c-Myc ($p = 0.015$). No significant intergroup differences were found for other clinical and laboratory parameters.

Table 1

Patient characteristics and results of analysis of clinical and laboratory parameters in the groups with different pAKT1 expression in patients with DLBCL				
Parameters	Number of patients, <i>n</i> = 90, abs. (%)	pAKT1 expression		Fisher's exact test, <i>p</i>
		high, abs., <i>n</i> = 36	low, abs., <i>n</i> = 54	
Gender:				
males	47 (52)	18	25	0.830
females	43 (48)	18	29	
Age, years:				
≥ 60	40 (44)	17	23	0.672
< 60	50 (56)	19	31	
B symptoms:				
present	50 (56)	25	25	0.034*
absent	40 (44)	11	29	
Stage (according to Ann Arbor staging):				
I–II	38 (42)	10	28	0.030*
III–IV	52 (58)	26	26	
Extranodal lesions:				
present	41 (46)	18	23	0.523
absent	49 (54)	18	31	
Level of lactate dehydrogenase (LDH):				
within the normal values	30 (33)	25	35	0.820
exceeds the normal values	60 (66)	11	19	
Immunohistochemical subtype:				
GCB	34 (38)	10	24	0.126
non-GCB	56 (62)	26	30	
IPI, risk groups:				
IPI ≤ 2	49 (54)	15	36	0.029*
IPI > 2	41 (45)	21	18	
Response to first-line therapy:				
partial response	54 (60)	20	16	0.017*
response / relapse / refractory disease	36 (40)	16	38	
complete response				
BCL2 expression:				
≥ 50%	28 (31)	16	12	0.036*
< 50%	62 (69)	20	42	
c-Myc expression:				
≥ 40%	18 (20)	12	6	0.015*
< 40%	72 (80)	24	48	

Note: Fisher's exact test – *p*.

* statistically significant differences between the groups (here and in Table 2).

Three-year OS in group 1 was 44% (the median survival was 23 months) as opposed to 87% in group 2 (the median survival was not reached), $p < 0.001$, (Fig. 2a). Significant differences were found in the assessment of PFS: 39% – in the patients with high expression of pAKT1, 71% – in the patients with low expression of the marker, $p = 0.005$. The median sur-

vival in group 1 was 14 months, and it was not reached in group 2 (Fig. 2, b).

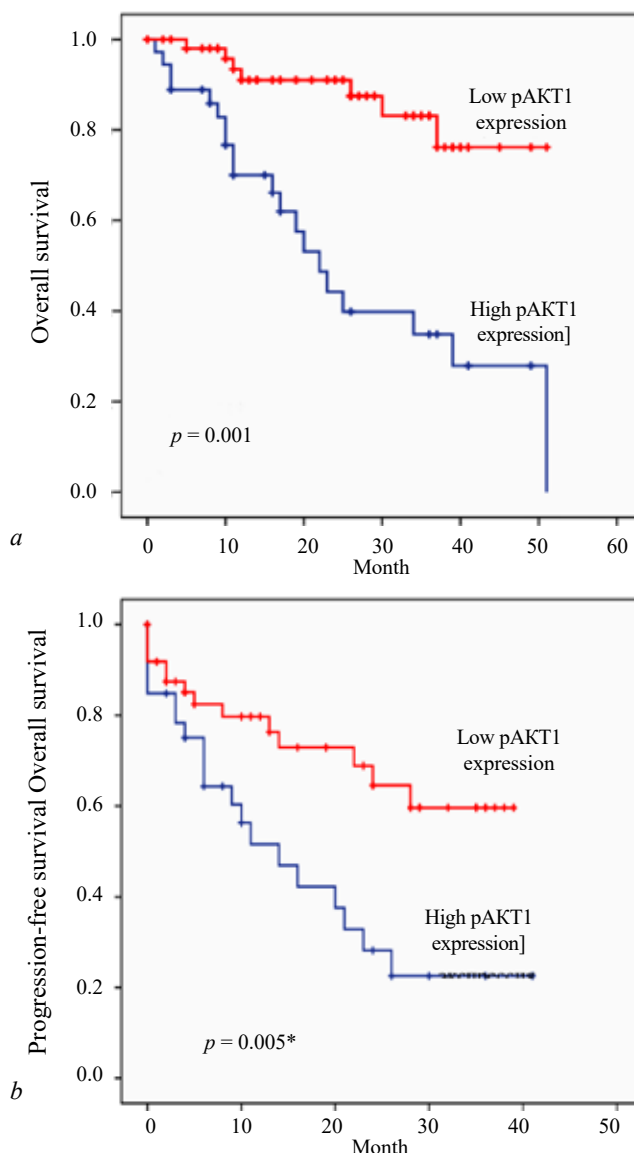


Fig. 2. Overall (a) and progression-free (b) survival of patients with diffuse large B-cell lymphoma depending on the degree of pAKT1 expression by tumor cells

Clinical and laboratory parameters of patients were initially included in the univariate Cox analysis, following which the factors adversely affecting OS in patients with DLBCL were: IPI > 2, non-GCB subtype, age ≥ 60 years, and pAKT1 expression ≥ 70% (Table 2). In the course of the multivariate regression analysis, it was found that the most significant independent predictive factors were only IPI and overexpression of pAKT1 ($p = 0.039$ and $p = 0.001$, respectively). At the same time, the content of pAKT1-positive tumor cells ≥ 70% determined a two times higher risk of an unfavorable outcome than the IPI index.

Table 2

Univariate and multivariate Cox analysis of predictors of overall and progression-free survival in patients with diffuse B-large cell lymphoma						
Parameter	Univariate analysis					
	OS			PFS		
	HR	95% CI	<i>p</i>	HR	95% CI	<i>p</i>
Gender	1.893	0.865–4.140	0.112	1.954	0.965–3.955	0.063
Age ≥ 60 years	2.254	1.013–5.015	0.046*	1.511	0.763–2.993	0.236
IPI > 2	6.319	2.377–16.800	< 0.001*	3.727	1.810–7.675	< 0.001*
non-GCB subtype	0.351	0.132–0.932	0.035*	0.367	0.160–0.844	0.018*
Expression of pAKT1 ≥ 70%	9.954	3.432–28.870	< 0.001*	3.527	1.170–7.275	0.001*
Parameter	Multivariate analysis					
	OS			PFS		
	HR	95% CI	<i>p</i>	HR	95% CI	<i>p</i>
Age ≥ 60 years	1.933	0.813–4.595	0.135	–	–	–
IPI > 2	3.061	1.056–8.868	0.039*	2.577	1.191–5.574	0.016*
non-GCB subtype	0.623	0.219–1.772	0.375	0.607	0.252–1.467	0.268
Expression of pAKT1 ≥ 70%	6.171	2.069–18.402	0.001*	2.456	1.141–5.287	0.022*

For PFS, the same factors showed statistical significance in the univariate Cox analysis, except for patient age. When assessing the effect of several predictors on disease progression, IPI > 2 and pAKT1 expression ≥ 70% were also significant independent prognostic factors with a comparable risk of an adverse event.

DISCUSSION

DLBCL is the most common non-Hodgkin's lymphoma, which encompasses a large group of tumors that differ in their clinical, morphological, and molecular genetic characteristics. Standard therapeutic approaches provide good treatment results in only 50–60% of cases. At the stage of early diagnosis, the existing systems of stratification (IPI) do not always provide an opportunity to accurately predict the course of the disease in each individual patient, since they do not take into account the molecular and biological aspects of the pathology. This is especially relevant for low- and intermediate-risk patients. Therefore, the search for additional prognostic criteria is justified in this disease.

The results obtained in this study indicate a relationship between overexpression of pAKT1 and unfavorable prognostic clinical and laboratory characteristics: common stages of the disease, IPI values > 2, the presence of B symptoms, and high expression of the BCL2 and c-Myc oncoproteins. This association may be due to uncontrolled activation of the Mdm2 protein and impaired functioning of the p53 and p21 tumor suppressors. This leads to inhibition of apoptosis and excessive proliferation of neoplastic cells [10, 16]. In patients with supra-threshold values of pAKT1 expression, lower frequency of achieving complete re-

missions was noted. However, for some parameters, there is significant variation in the research results.

In a number of studies, in addition to the indicated clinical characteristics, a relationship was found between the pAKT1-positive variant of the disease and gender, age, and LDH level [8, 18, 19]. We believe that these disagreements may be associated with a lack of unified criteria for determining the threshold values for pAKT1 expression, comorbidity, and different sample sizes. In some foreign studies, an association of pAKT1 overexpression with low survival in DLBCL patients was shown [17, 18]. The results of our study confirm this.

At the same time, based on the multivariate analysis data, it was revealed that overexpression of pAKT1 is an independent predictor of low OS, which is more significant in prognostic terms than the IPI index. For PFS, the studied marker was also a significant independent prognostic factor. There is no consensus among the authors regarding this fact, the research results are contradictory. There are foreign studies in which the role of pAKT1 expression as an independent prognostic factor was not proven. It is assumed that adverse clinical manifestations associated with excessive activation of the PI3K / AKT / mTOR pathway are indirect and depend on other effectors [17]. At the same time, there are publications confirming the independent prognostic role of this marker in relation to the course of the disease [8, 18, 19].

CONCLUSION

Overexpression of pAKT1 is associated with unfavorable clinical and laboratory parameters in patients

with DLBCL and treatment failures according to the R-CHOP regimen. This biomarker could be used as an independent predictor of the course of the disease.

REFERENCES

1. Cabattini E., Pileri S.A., Dirnhofer S., Went P., Ascani S., Marafioti T., Tzankov A., Leoncini L., Falini B., Zinzani P.L. Diffuse large B-cell lymphoma: one or more entities? Present controversies and possible tools for its sub classification. *Histopathology*. 2002; 41 (6): 482–509. DOI: 10.1046/j.1365-2559.2002.01538.x.
2. Sehn L.H., Gascoyne R.D. Diffuse large B-cell lymphoma: optimizing outcome in the context of clinical and biologic heterogeneity. *Blood*. 2015; 125 (1): 22–32. DOI: 10.1182/blood-2014-05-577189.
3. Polyatskin I.L., Artemyeva A.S., Krivolapov Yu.A. Revised WHO classification of tumors of hematopoietic and lymphoid tissues. *Archives of Pathology*. 2019; 81 (3): 59–65 (in Russ.). DOI: 10.17116/patol.20188006143.
4. Rastorguev S.M., Koroleva D.A., Bulygina E.S., Tsygankova S.V., Goncharov N.G., Naraykin O.S., Gabeeva N.G., Zvonkov E.E., Nedoluzhko A.V. Clinical and prognostic value of molecular markers of diffuse large B-cell lymphoma. *Clinical Oncohematology*. 2019; 12 (1): 95–100 (in Russ.). DOI: 10.21320/2500-2139-2019-12-1-95-100.
5. Samarina S.V., Nazarova E.L., Minaeva N.V., Zotina E.N., Paramonov I.V., Gritsaev S.V. Clinical and hematological prognostic parameters of a response to first-line therapy in patients with diffuse large B-cell lymphoma. *Clinical Oncohematology*. 2019; 12 (1): 68–72 (in Russ.). DOI: 10.21320/2500-2139-2019-12-1-68-72.
6. Kaplanov K.D., Volkov N.P., Klitochenko T.Yu., Matveeva I.V., Shipaeva A.L., Shirokova M.N., Davydova N.V., Gemdjyan E.G., Abramov D.S., Kononov D.M., Snigur G.L., Redbkina N.A. Results of the analysis of the national register of patients with diffuse large B-cell lymphoma: risk factors and problems of immunochemotherapy. *Clinical Oncohematology*. 2019; 12 (2): 154–164 (in Russ.). DOI: 10.21320/2500-2139-2019-12-2-154-164.
7. Vaneeva E.V., Posin V.A., Diakonov D.A., Samarina S.V., Rylov A.V. Assessment of the prognostic value of pSTAT3 expression in diffuse large B-cell lymphoma in a Russian sample of patients. *Siberian Scientific Medical Journal*. 2019; 39(5): 125–133 (in Russ.). DOI: 0.15372/SSMJ20190515.
8. Zhang H., Wang X., Dong L., Lv H., Li W., Song Z., Li L., Zhou S., Qiu L., Qian Z., Liu X., Feng L., Meng B., Fu K., Wang X., Pan-Hammarstrom Q., Wang P. Co-expression of PD-L1 and p-AKT is associated with poor prognosis in diffuse large B-cell lymphoma via PD-1/PD-L1 axis activating intracellular AKT/mTOR pathway in tumor cells. *Oncotarget*. 2016; 7 (22): 33350–33362. DOI: 10.18632/oncotarget.9061.
9. Zeynalova P.A., Sholokhova E.N., Tupitsyn N.N. Prognosis in diffuse large B-cell lymphoma: some aspects. *Bulletin of N.N.Blokhin Russian Cancer Research Center*. 2015; 26 (1): 43–49 (in Russ.).
10. Courtney K.D., Corcoran R.B., Engelman J.A. The PI3K pathway as drug target in human cancer. *Journal of Clinical Oncology*. 2010; 28 (6): 1075–1083. DOI: 10.1200/JCO.2009.25.3641.
11. Kumar A., Rajendran V., Sethumadhavan R., Purohit R. AKT kinase pathway: a leading target in cancer research. *Scientific World Journal*. 2013; 2013: 756134. DOI: 10.1155/2013/756134.
12. Wang X., Cao X., Sun R., Tang C., Tzankov A., Zhang J., Manyam G.C., Xiao M., Miao Y., Jabbar K., Tan X., Pang Y., Visco C., Xie Y., Dybkaer K., Chiu A., Orazi F., Young K.H. Clinical significance of *PTEN* deletion, mutation, and loss of pten expression in *de novo* diffuse large B-cell lymphoma. *Neoplasia*. 2018; 20 (6): 574–593 DOI: 10.1016/j.neo.2018.03.002.
13. Stavrovskaya A.A., Gens G.P. New trends in studying multi-drug resistance of breast cancer cells. *Advances in Molecular Oncology*. 2015; 2 (1): 39–51 (in Russ.). DOI: 10.17650/2313-805X.2015.2.1.039–051.
14. Hans C.P., Weisenburger D.D., Timothy C. et al. Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. *Blood*. 2004; 103 (1): 275–282. DOI: 10.1182/blood-2003-05-1545.
15. Nasr M.R., Perry A.M., Skrabek P. Lymph node pathology for clinicians; translation from English edited by Yu.A.Krivolapov. M.: Practical Medicine, 2020: 224 (in Russ.).
16. Altomare D.A., Testa J.R. Perturbations of the AKT signaling pathway in human cancer. *Oncogene*. 2005; 24 (50): 7455–7464. DOI: 10.1038/sj.onc.1209085.
17. Wang J., Xu-Monette Z.Y., Jabbar K.J., Shen Q., Manyam G.C., Tzankov A., Visco C., Wang J., Montes-Moreno S., Dybkaer K., Tam W., Bhagat G., His E.D., van Krieken J.H., Ponzoni M., Ferreri A.J.M., Wang S., Møller M.B., Piris M.A., Medeiros L.J., Li Y., Pham L.V., Young K.H. AKT hyperactivation and the potential of akt-targeted therapy in diffuse large B-cell lymphoma. *American Journal Pathology*. 2017; 187 (8): 1700–1716. DOI: 10.1016/j.ajpath.2017.04.009.
18. Hasselblom S., Hansson U., Olsson M., Tore'n L., Bergström A., Nilsson-Ehle H., Andersson P.-O. High immunohistochemical expression of p-AKT predicts inferior survival in patients with diffuse large B-cell lymphoma treated with immunochemotherapy. *British Journal of Haematology*. 2010; 149 (4): 560–568. DOI: 10.1111/j.1365-2141.2010.08123.x.
19. Hong J.Y., Hong M.E., Choi M.K., Kim Y.S., Chang W., Maeng C.H., Park S., Lee S.J., Do I.-G., Jo J.-S., Jung S.H., Kim S.J., Ko Y.H., Kim W.S. The impact of activated p-AKT expression on clinical outcomes in diffuse large B-cell lymphoma: A clinicopathological study of 262 cases. *Annals of Oncology*. 2014; 25 (1): 182–188. DOI: 10.1093/annonc/mdt530.

Authors contribution

Vaneeva E.V., Rosin V.A. – design of the study, collection of data, carrying out of the practical part of the study, analysis and interpretation of data, drafting of the manuscript. Diakonov D.A., Rosin V.A. – development of the research direction, critical revision of the article for important intellectual content. Luchinin A.S. – analysis and interpretation of statistical data. Samarina S.V. – collection of clinical data of DLBCL patients. Kochetov N.L. – selection of biopsy samples.

Authors information

Vaneeva Elena V., Post-Graduate Student, Junior Researcher, Laboratory of Pathomorphology, KRIHBT, Kirov, Russian Federation.
Rosin Vitaliy A., Cand. Sci. (Med.), Senior Researcher, Laboratory of Pathomorphology, KRIHBT, Kirov, Russian Federation. ORCID 0000-0003-2054-2870.

Diakonov Dmitriy A., Cand. Sci. (Med.), Head of the Laboratory of Pathomorphology, KRIHBT, Kirov, Russian Federation. ORCID 000-0001-8688-1344.

Luchinin Aleksander S., Cand. Sci. (Med.), Hematologist, KRIHBT, Kirov, Russian Federation. ORCID 0000-0002-5016-210X.

Samarina Svetlana V., Head of the Clinical and Diagnostic Department, KRIHBT, Kirov, Russian Federation. ORCID 0000-0001-8639-719X.

Kochetov Nikolai L., Forensic Pathologist, KRIHBT, Kirov, Russian Federation.

(✉) **Vaneeva Elena V.**, e-mail: vaneeva.elena.vic@mail.ru

Received 14.01.2020

Accepted 25.05.2021

Estimation of the effect of lithium salts on cytokine production by blood cells in *in vitro* experiments

Vetlugina T.P.¹, Epimakhova E.V.¹, Savochkina D.N.¹, Plotnikov E.V.^{1,2}, Boiko A.S.¹,
Ivanova S.A.¹, Bokhan N.A.¹

¹ Mental Health Research Institute, Tomsk National Research Medical Center (NRMC), Russian Academy of Sciences
4, Aleutskaya Str., Tomsk, 634014, Russian Federation

² National Research Tomsk Polytechnic University
30, Lenina Av., Tomsk, 634050, Russian Federation

ABSTRACT

Aim. To study the effects of lithium salts on production of cytokines by immunocompetent cells in the whole-blood culture of patients with alcohol dependence and affective disorders.

Materials and methods. The study materials were blood samples from 25 patients with alcohol dependence (AD) and 12 patients with bipolar disorder (BD). Blood diluted 1:1 with complete RPMI-1640 medium (Gibco, UK) was added to the wells of the culture plate, then new lithium salts (succinate, fumarate, pyruvate, ascorbate) and a reference salt – lithium carbonate at a final concentration of 1.2 mmol / l per lithium ion – were added. In parallel, control samples without lithium salts were tested; the samples were incubated for a day. The concentration of cytokines (interferon (IFN) γ , interleukin (IL)-1 β , IL-2, IL-4, IL-6, IL-8, IL-10, IL-17A, tumor necrosis factor (TNF) α) was determined in the culture supernatants on the MAGPIX multiplex analyzer (Luminex, USA) (Center for Collective Use “Medical Genomics”, Tomsk NRMC) using the Human Cytokine / Chemokine Magnetic Bead Panel (Merck, Germany).

Results. All lithium salts had a unidirectional effect on the production of cytokines by immunocompetent cells (ICC), except for lithium ascorbate and IL-8. The concentrations of cytokines in the supernatants of loaded and control samples (spontaneous production) were comparable, which indicates an absence of stimulating or suppressing effects of salts on the functional activity of ICC under the experimental conditions. The effect of lithium ascorbate as an IL-8 inducer was detected: the production of IL-8 induced by lithium ascorbate was 2.3–2.5 times higher than its spontaneous production.

Conclusion. The obtained results, as well as the previously revealed antioxidant and cytoprotective properties of new lithium salts, confirmed that they are promising for development of pharmacological agents with combined action.

Key words: lithium salts, cytokines, blood cells, alcoholism, bipolar disorder.

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

Source of financing. The study was carried out with the financial support of the Russian Science Foundation (project No. 17-75-20045), partly (recruitment of patients) – within the financing of the research project No. 0550-2019-0007.

Conformity with the principles of ethics. All patients signed an informed consent to participate in the study. The study was approved by the local Ethics Committee at Mental Health Research Institute of Tomsk NRMC (Protocol No. 361 of 23.10.2017).

✉ Vetlugina Tamara P., e-mail: vetlug@mail.tomsknet.ru

For citation: Vetlugina T.P., Epimakhova E.V., Savochkina D.N., Plotnikov E.V., Boiko A.S., Ivanova S.A., Bokhan N.A. Estimation of the effect of lithium salts on cytokine production by blood cells in *in vitro* experiments. *Bulletin of Siberian Medicine*. 2021; 20 (3): 21–28. <https://doi.org/10.20538/1682-0363-2021-3-21-28>.

Оценка влияния солей лития на продукцию цитокинов клетками крови в опытах *in vitro*

Ветлугина Т.П.¹, Епимахова Е.В.¹, Савочкина Д.Н.¹, Плотников Е.В.^{1,2}, Бойко А.С.¹, Иванова С.А.¹, Бохан Н.А.¹

¹ Научно-исследовательский институт (НИИ) психического здоровья, Томский национальный исследовательский медицинский центр (НИМЦ) Российской академии наук
Россия, 634014, г. Томск, ул. Алеутская, 4

² Национальный исследовательский Томский политехнический университет (НИ ТПУ)
Россия, 634050, г. Томск, пр. Ленина, 30

РЕЗЮМЕ

Цель – изучить влияние солей лития на продукцию цитокинов иммунокомпетентными клетками в культуре цельной крови больных с алкогольной зависимостью и аффективными расстройствами.

Материалы и методы. Материалом для исследования послужили образцы крови 25 больных с алкогольной зависимостью и 12 пациентов с биполярным аффективным расстройством. В лунки культурального планшета вносили кровь, разведенную 1 : 1 полной средой RPMI-1640 (Gibco, Великобритания), добавляли новые соли лития (сукцинат, фумарат, пируват, аскорбат) и соль сравнения – карбонат лития в конечной концентрации 1,2 ммоль/л в расчете на ион лития, параллельно ставили контрольные пробы без солей лития; пробы инкубировали в течение суток. В супернатантах суточной культуры на мультиплексном анализаторе MAGPIX (Luminex, США) (ЦКП «Медицинская геномика», Томский НИМЦ) определяли концентрацию цитокинов (интерферона γ , интерлейкина (ИЛ) 1 β , ИЛ-2, ИЛ-4, ИЛ-6, ИЛ-8, ИЛ-10, ИЛ-17A, фактора некроза опухоли α) с использованием наборов реагентов Human Cytokine/Chemokine Magnetic Bead Panel (Merck, Германия).

Результаты. Все соли лития оказывали однонаправленное действие на продукцию спектра цитокинов иммунокомпетентными клетками (ИКК) за исключением аскорбата лития и ИЛ-8. Концентрации цитокинов в супернатантах нагрузочных и контрольных проб (спонтанная продукция) были сопоставимы, что свидетельствует об отсутствии стимулирующего или супрессирующего действия солей на функциональную активность ИКК в условиях эксперимента. Обнаружен эффект аскорбата лития как индуктора ИЛ-8: индуцированная аскорбатом лития продукция ИЛ-8 в 2,3–2,5 раза превышала его спонтанную продукцию.

Заключение. Полученные результаты, а также выявленные ранее антиоксидантные, цитопротекторные свойства новых солей лития подтверждают их перспективность для разработки фармакологических средств комбинированного действия.

Ключевые слова: соли лития, цитокины, клетки крови, алкоголизм, биполярное аффективное расстройство.

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

Источник финансирования. Исследование выполнено при финансовой поддержке Российского научного фонда (проект № 17-75-20045), частично (набор пациентов) – в рамках финансирования темы НИР № 0550-2019-0007.

Соответствие принципам этики. Все пациенты подписали информированное согласие на участие в исследовании. Исследование одобрено локальным этическим комитетом НИИ психического здоровья Томского НИМЦ (протокол № 361 от 23.10.2017).

Для цитирования: Ветлугина Т.П., Епимахова Е.В., Савочкина Д.Н., Плотников Е.В., Бойко А.С., Иванова С.А., Бохан Н.А. Оценка влияния солей лития на продукцию цитокинов клетками крови в опытах *in vitro*. *Бюллетень сибирской медицины*. 2021; 20 (3): 21–28. <https://doi.org/10.20538/1682-0363-2021-3-21-28>.

INTRODUCTION

Lithium-containing drugs are often used as mood stabilizers in the complex therapy of patients with mood disorders and alcohol dependence complicated by affective disorders; lithium carbonate is commonly used for such treatment [1–3]. At the same time, the data of biological studies indicate a significant role of inflammation and oxidative stress factors in these diseases [4–8]. This determines the relevance of developing combined action drugs targeted to the main links in the pathogenesis of affective spectrum disorders.

Mental Health Research Institute of Tomsk NRMC is engaged in search for lithium salts with combined normothymic, neuroprotective, cytoprotective, and antioxidant effect. To select the most promising compounds as the basis for combined action drugs, studies are being carried out on the biological effects of new lithium salts synthesized on the basis of ascorbic acid and citric acid cycle substrates (succinate, fumarate, pyruvate).

The aim of this work was to study the effect of lithium salts on the production of cytokines by immunocompetent cells in the whole-blood culture of patients with alcohol dependence and affective disorders.

MATERIALS AND METHODS

The material for the study was blood samples from 25 men with alcohol dependence (AD) according to the International Classification of Diseases, Tenth Revision (ICD-10) (“Mental and behavioral disorders due to alcohol use: dependence syndrome – F10.21 and withdrawal syndrome – F10.30”), aged 29 to 60 (46.82 ± 9.48) years and 12 patients (2 men, 10 women) diagnosed with ICD-10 “Bipolar disorder – F31” (BD), aged 20 to 59 (35.61 ± 13.63) years; the level of statistical significance of age differences between the groups $p = 0.0152$.

The patients were admitted for treatment to the Addictive States and the Affective States Departments of the Mental Health Research Institute clinic, Tomsk NRMC. Blood sampling from the patients was carried out from the cubital vein in the morning on an empty stomach before the start of standard therapy; Vacutainer systems with EDTA anticoagulant were used. All patients signed an informed consent to participate in the study. The study was carried out in compliance with ethical standards developed in accordance with the Declaration of Helsinki of the World Medical Association and approved by the local Ethics Committee at Mental Health Research Institute of Tomsk NRMC (protocol No. 361 of 23.10.2017).

Lithium salts (lithium succinate – $\text{Li}_2 \text{C}_4\text{H}_4\text{O}_4$; lithium fumarate – $\text{Li}_2 \text{C}_4\text{H}_2\text{O}_4$; lithium pyruvate – $\text{LiC}_3\text{H}_3\text{O}_3$; lithium ascorbate – $\text{LiC}_6\text{H}_7\text{O}_6$) were synthesized at Research School of Chemical and Biomedical Technologies of National Research Tomsk Polytechnic University. Lithium carbonate – Li_2CO_3 (Sigma-Aldrich, USA) was used as a reference salt, since this salt is the basis of most lithium preparations used in medical practice. Lithium salts were dissolved in physiological saline, and stock solutions were obtained with a convenient concentration for further addition to experimental samples. The final standard concentration was 1.2 mmol / l per lithium ion (Li^+), which correlated with the therapeutic dose of lithium in treatment of affective spectrum disorders.

Peripheral venous blood was sampled in BD Vacutainer tubes with EDTA anticoagulant. Blood was diluted 1: 1 with the RPMI-1640 medium (Gibco, UK) with addition of inactivated fetal bovine serum (Gibco, UK), HEPES (Sigma-Aldrich, USA), and gentamicin (Dalkhimpharm, Russian Federation). A certain volume of diluted blood was added to the wells of the Cell Culture Plate (24-Well, Eppendorf). The samples were added with stock solutions of lithium salts to a final Li^+ concentration of 1.2 mmol / l (loaded samples). To evaluate the spontaneous production of cytokines by immunocompetent cells (ICC), in parallel with loaded samples, control samples without addition of lithium salts were used.

The samples were incubated for 24 hours at 37° C in 5% carbon dioxide in a CO_2 -incubator. After incubation, the supernatants were carefully taken, poured into aliquots, and stored in a low-temperature chamber at –80° C until cytokines were determined.

The level of cytokine production was assessed by their concentration in the supernatants of loaded and control samples using the LuminexMAP technology on a MAGPIX multiplex analyzer (Luminex, USA) (Center for Collective Use “Medical Genomics”, Tomsk NRMC). The concentration of cytokines (interferon γ ($\text{IFN}\gamma$), interleukin (IL)-1 β , IL-2, IL-4, IL-6, IL-8, IL-10, IL-17A, and tumor necrosis factor (TNF) α) was determined using the MILLIPLEX MAP Human Cytokine / Chemokine Magnetic Bead Panel (Merck, Germany) The analysis was performed in a standard 96-well plate according to the instructions for the kits. The results were presented in pg / ml.

Statistical analysis was performed using the STATISTICA software package for Windows, version 12.0. Descriptive statistics were presented by the me-

dian and the interquartile range Me ($LQ-UQ$). For intergroup comparison, the Mann – Whitney test was used. The differences were considered statistically significant at $p < 0.05$.

RESULTS

During the cultivation of blood samples from patients with AD, most lithium salts did not have either a stimulating or inhibitory effect on the production of cytokines, except for lithium ascorbate and IL-8 (Table 1). There were no statistically significant differences in the level of cytokines in the loaded and control samples, which indicates the presence of cytokines spontaneously produced by ICC in the samples with lithium salts, including lithium carbonate. The highest concentration in blood cell culture supernatants was found for the anti-inflammatory cytokine IL-4, the

median values in all the samples ranged from 19.52 to 19.85 pg / ml. The lowest concentrations were found for proinflammatory cytokines IL-2 (1.43–1.50 pg / ml) and IL-6 (1.54–1.69 pg / ml).

At the same time, the addition of lithium ascorbate to the whole-blood culture resulted in a pronounced increase in the production of IL-8 (Table 1), and its content in the supernatants of the loaded samples exceeded the corresponding value in the samples with other lithium salts (the level of statistical significance of the differences between the samples ranged from 0.0060 to 0.0003). In addition, the concentration of IL-8 in the samples with lithium ascorbate was 2.5 times higher than the corresponding value of its spontaneous production in the control samples (25.37 (17.19–36.04) and 10.07 (8.41–15.99) pg / ml, respectively, $p_c = 0.0010$).

Table 1

Cytokine concentration in the supernatants of diurnal blood cell culture of patients with alcohol dependence, pg / ml, Me ($LQ-UQ$)							
Cytokines, pg / ml	Loaded samples, Li^+ dose – 1.2 mmol / l					Control 6 (n = 25)	p
	1 (n = 25)	2 (n = 23)	3 (n = 25)	4 (n = 24)	5 (n = 24)		
IFN γ	6.62 (5.72–7.59)	6.93 (6.00–7.41)	6.69 (6.02–6.96)	6.92 (6.24–7.41)	6.58 (6.02–7.46)	6.46 (5.94–7.18)	$p > 0.05$
IL-1 β	4.41 (2.83–6.56)	4.06 (3.24–5.54)	3.78 (2.95–5.28)	3.44 (2.74–4.81)	4.00 (3.10–6.21)	4.10 (3.03–5.08)	$p > 0.05$
IL-2	1.47 (1.30–1.69)	1.43 (1.27–1.68)	1.45 (1.26–1.60)	1.50 (1.38–1.67)	1.49 (1.29–1.57)	1.46 (1.27–1.60)	$p > 0.05$
IL-4	19.85 (18.25–21.30)	19.65 (18.25–21.20)	19.65 (18.21–21.50)	19.68 (18.24–21.15)	19.62 (18.23–20.77)	19.52 (18.25–20.28)	$p > 0.05$
IL-6	1.69 (1.31–2.25)	1.62 (1.36–2.07)	1.54 (1.39–2.13)	1.65 (1.36–2.07)	1.66 (1.34–2.55)	1.64 (1.36–2.33)	$p > 0.05$
IL-8	12.66 (8.57–20.26)	11.05 (8.66–18.04)	11.01 (8.52–12.64)	11.57 (9.02–17.88)	25.37 (17.19–36.04) $p_c = 0.001$	10.07 (8.41–15.99)	0.006 (between samples 1 and 5); 0.002 (between samples 2 and 5); 0.0003 (between samples 3 and 5); 0.004 (between samples 4 and 5)
IL-10	7.29 (6.88–8.9)	7.29 (6.59–7.98)	7.27 (6.59–8.35)	7.30 (6.92–8.66)	7.23 (6.80–8.33)	7.29 (6.49–7.94)	$p > 0.05$
IL-17	5.56 (5.01–6.07)	5.37 (5.16–5.91)	5.56 (5.20–5.88)	5.59 (5.19–6.06)	5.74 (5.21–6.18)	5.64 (5.00–5.95)	$p > 0.05$
TNF α	5.81 (4.7–7.31)	5.52 (4.88–7.33)	5.43 (4.96–7.00)	5.71 (4.91–6.40)	5.81 (4.42–6.54)	5.62 (4.64–7.30)	$p > 0.05$

Note: 1 – lithium succinate; 2 – lithium fumarate; 3 – lithium pyruvate; 4 – lithium carbonate; 5 – lithium ascorbate; 6 – control samples.

p_c – the level of statistical significance of differences in relation to sample 6 (control);

p – the level of statistical significance of differences between the samples 1 and 5; 2 and 5; 3 and 5; 4 and 5.

Similar results were obtained in a series of experiments with blood samples from patients with bipolar disorder (Table 2), although this group of patients differed from the group of patients with alcoholism in terms of age and sex characteristics.

In the diurnal cell culture supernatants of patients with BD, as well as in the first series of experiments with blood samples from patients with AD, no statis-

tically significant differences were found between the samples loaded with lithium salts (except for ascorbate and IL-8) and the control samples. In all the samples, IL-4 (18.38–20.09 pg / ml) and IL-17A (14.15–15.12 pg / ml) had the highest average values of the cytokine content (Me), whereas IL-2 (1.70–1.85 pg / ml) and IL-6 (1.47–1.69 pg / ml) were characterized by the lowest values.

Table 2

Cytokine concentration in the supernatants of diurnal blood cell culture of patients with bipolar disorder, pg / ml, Me (LQ–UQ)							
Cytokines, pg / ml	Loaded samples, Li ⁺ dose – 1.2 mmol / l					Control 6 (n = 12)	p
	1 (n = 12)	2 (n = 12)	3 (n = 11)	4 (n = 12)	5 (n = 11)		
IFN γ	5.88 (4.50–6.99)	5.16 (4.71–6.41)	5.29 (4.41–6.58)	5.35 (4.75–7.37)	5.77 (4.56–6.93)	5.35 (4.45–8.51)	$p > 0.05$
IL-1 β	2.8 (2.37–4.44)	3.12 (2.56–3.49)	2.68 (2.09–2.98)	2.88 (2.32–3.22)	3.22 (2.43–3.44)	2.83 (1.98–3.27)	$p > 0.05$
IL-2	1.76 (1.68–1.90)	1.74 (1.64–1.92)	1.74 (1.56–1.81)	1.70 (1.60–1.87)	1.81 (1.60–1.96)	1.85 (1.60–2.02)	$p > 0.05$
IL-4	19.89 (18.25–20.10)	18.38 (18.29–20.06)	19.08 (16.51–20.44)	19.14 (18.21–20.03)	20.09 (18.20–21.84)	19.85 (18.25–20.13)	$p > 0.05$
IL-6	1.67 (1.47–2.04)	1.61 (1.54–1.96)	1.47 (1.35–1.74)	1.58 (1.47–1.84)	1.67 (1.39–2.24)	1.69 (1.54–1.99)	$p > 0.05$
IL-8	10.00 (6.63–13.00)	10.52 (8.33–16.45)	8.83 (6.72–13.30)	10.66 (7.39–20.33)	19.80 (18.84–31.74) $p_c = 0.002$	8.48 (6.66–10.30)	0.002 (between samples 1 and 5); 0.01 (between samples 2 and 5); 0.005 (between samples 3 and 5)
IL-10	5.63 (5.32–6.05)	5.42 (5.11–5.85)	5.28 (4.87–5.72)	5.75 (5.21–7.17)	5.58 (5.15–6.43)	5.31 (5.07–5.75)	$p > 0.05$
IL-17	14.62 (13.28–15.34)	14.25 (13.35–15.02)	14.15 (12.84–15.24)	14.69 (13.17–15.32)	15.12 (14.04–15.77)	14.63 (13.60–15.19)	$p > 0.05$
TNF α	2.96 (2.74–3.17)	3.09 (2.81–3.23)	2.86 (2.61–3.01)	3.16 (2.76–3.19)	2.96 (2.76–3.26)	3.06 (2.50–3.36)	$p > 0.05$

Note: p_c – the level of statistical significance of differences in relation to sample 6 (control);

p – the level of statistical significance of differences between the samples 1 and 5; 2 and 5; and 3 and 5.

In this series of experiments, the ability of lithium ascorbate to enhance the production of IL-8 by ICC was confirmed. The concentration of IL-8 in the supernatants of the samples loaded with lithium ascorbate significantly exceeded the corresponding value both in the control samples ($p_c = 0.002$) and in the samples with other lithium salts (the levels of statistical significance of differences were from 0.010 to 0.002). The mean (Me) lithium ascorbate-induced IL-8 production / the mean spontaneous IL-8 production ratio was 2.33.

DISCUSSION

Despite the fact that lithium has been used as a mood stabilizer for more than half a century, it remains the gold standard for treatment of affective spectrum disorders, as evidenced by a number of reviews over the past decade [9–11]. Along with a high therapeutic effect of lithium therapy, it poses a risk of toxicity, to decrease which various ways are proposed, in particular, to reduce the dose of the drug and monitor constantly the level of lithium in patients' blood.

To reduce toxicity and increase the therapeutic effect, one of the approaches is to expand the spectrum

of lithium salts synthesized on the basis of various anionic components. We previously identified antioxidant and cytoprotective effects in certain lithium compounds synthesized from substrates of the citric acid cycle (succinate, fumarate, pyruvate) and ascorbic acid in plasma and blood cell models [12, 13].

Considering the role of inflammation in the pathogenesis of affective spectrum disorders, this study continued to evaluate the biological effects of new lithium salts, as well as the reference salt of lithium carbonate, on the production of cytokines by ICC in the diurnal whole-blood culture of patients with AD and BD in the complete RPMI-1640 medium. Analysis of the data showed that almost all the salts had a unidirectional effect on the cytokine production in blood samples from patients with different pathologies, except for the lithium ascorbate effect on the production of IL-8.

At the same time, there were no statistically significant differences in the level of cytokines in the supernatants of the loaded and control samples. Thus, the analyzed salts did not have negative effects on blood cells, which, under the experimental conditions,

retained the ability to spontaneously produce cytokines. A new property of lithium ascorbate was discovered – to enhance the production of IL-8 by ICC. It was found that the level of IL-8 in the samples loaded with lithium ascorbate was 2.3–2.5 times higher than the level of its spontaneous production, regardless of the pathology and gender and age characteristics of patients.

IL-8 (CXCL8) belongs to the group of CXC chemokines involved in the regulation of the biological activity of almost all ICC; the main role of IL-8 is enhancing the chemotaxis of leukocytes to the focus of infection, its localization and removal. The role of chemokines in the development of an immune response to the pathogen is widely covered in the literature [14–16]. The effect of lithium ascorbate on IL-8 synthesis, revealed in our experiments, may be due to the unique properties of ascorbate in regulating redox processes [17, 18].

In the blood culture medium with lithium ascorbate in the presence of iron ions, a prooxidant system can be created. This leads to stimulation of leukocytes and expression of CXCR1 and CXCR2 receptors, which initiate the early phase of a nonspecific immune response and are able to bind to CXCL1, CXCL2, CXCL5, CXCL6, CXCL7, and CXCL8 [16, 19]. To some extent, the obtained results may depend on the experimental conditions (drug dose, incubation time, etc.). It should be noted that the revealed effect of lithium ascorbate was manifested at a Li^+ dose of 1.2 mmol / l, corresponding to the therapeutic dose in treatment of affective disorders.

High levels of IL-8 are typical of a wide range of chronic inflammatory diseases [20–22]. At the same time, production of IL-8 associated with innate immunity activation does not weaken subsequent adaptive mechanisms of antigen-specific immune defense [23]. On the contrary, suppression of IL-8 secretion in the initial phases of inflammation can result in a decrease in the influx of neutrophils into the foci of inflammation, impaired elimination of the pathogen, and transition of the infectious process to a chronic course due to immunodeficiency [24]. The revealed property of lithium ascorbate makes it possible to expand the arsenal of IL-8 / CXCL8 inducers – a regulator of natural immunity, which is especially necessary in the initial phases of inflammation.

CONCLUSION

Lithium succinate, lithium fumarate, lithium pyruvate, lithium ascorbate, and lithium carbonate (the reference salt) have a unidirectional effect on the

production of cytokines by immunocompetent cells during 24-hour incubation in the RPMI medium of blood samples from patients with alcohol dependence and bipolar disorder. Cytokine concentrations in the supernatants of all loaded and control samples (spontaneous production) were comparable. This indicates the absence of both stimulating and suppressive effects of these compounds on the functional activity of ICC and can be considered as their positive characteristic. This characteristic also applies to lithium ascorbate. In addition, the property of lithium ascorbate as an IL-8 inducer was discovered.

The data obtained in this work and the previously revealed antioxidant and cytoprotective properties of some new lithium salts confirm the relevance of further study of their biological effects in order to select the most promising compounds for the development of import-substituting combined action drugs both for treatment of affective spectrum disorders and for possible wider use in other diseases, the pathogenesis of which involves inflammation and oxidative stress.

REFERENCES

1. Mosolov S.N., Kostyukova H.G., Fedotov D.D. Comparative efficacy and tolerability of lithium carbonate and valproate sodium during the prophylactic therapy in patients with bipolar affective disorder after reduction of manic episode. *Mental health*. 2009; 11 (42): 32–39 (in Russ.).
2. Prisciandaro J.J., Brown D.G., Brady K.T., Tolliver B.K. Comorbid anxiety disorders and baseline medication regimens predict clinical outcomes in individuals with co-occurring bipolar disorder and alcohol dependence: Results of a randomized controlled trial. *Psychiatry Res*. 2011; 188 (3): 361–365. DOI: 10.1016/j.psychres.2011.04.030.
3. Mashkovskiy M.D. Medicines. 16th ed. M.: Novaya Volna. 2017: 114–116 (in Russ.).
4. Prokopyeva V.D., Yarygina E.G., Mandel A.I. Dynamics of oxidative modification of proteins and lipids of blood plasma in alcoholic patients in the process of the therapy. *Siberian Herald of Psychiatry and Addiction Psychiatry*. 2017; 3 (96): 11–15 (in Russ.).
5. Bokhan N.A., Prokopyeva V.D., Ivanova S.A., Vetlugina T.P., Epimakhova E.V., Plotnikov E.V., Yarygina E.G., Boyko A.S. Oxidative stress and its correction in patients with alcohol dependence: results from research at the Mental Health Research Institute of the Tomsk National Research Medical Center. *Journal of Addiction Problems*. 2018; 3(163): 27–59 (in Russ.).
6. Martinez P., Lien L., Zemore S., Bramness J.G., Neupane S.P. Circulating cytokine levels are associated with symptoms of depression and anxiety among people with alcohol and drug use disorders. *J. Neuroimmunol*. 2018; 318: 80–86. DOI: 10.1016/j.jneuroim.2018.02.011.
7. Zhao G., Liu X. Neuroimmune advance in depressive disorder.

- Adv. Exp. Med. Biol.* 2019; 1180: 85–98. DOI: 10.1007/978-981-32-9271-0_4.
8. Rossetti A.C., Paladini M.S., Riva M.A., Molteni R. Oxidation-reduction mechanisms in psychiatric disorders: A novel target for pharmacological intervention. *Pharmacol. Ther.* 2020; 2010: 107520. DOI: 10.1016/j.pharmthera.2020.107520.
 9. Licht R.W. Lithium: still a major option in the management of bipolar disorder. *CNS Neurosci. Ther.* 2012; 18 (3): 219–226. DOI: 10.1111/j.1755-5949.2011.00260.x.
 10. Dell’Osso L., De Grande C., Gesi C., Carmassi C., Musetti L. A. New look at an old drug: neuroprotective effects and therapeutic potentials of lithium salts. *Neuropsychiatr. Dis. Treat.* 2016; 12: 1687–1703. DOI: 10.2147/NDT.S106479.
 11. Won E., Kim Y.K. An oldie but goodie: lithium in the treatment of bipolar disorder through neuroprotective and neurotrophic mechanisms. *Int. J. Mol. Sci.* 2017; 18 (12): 2679. DOI: 10.3390/ijms18122679.
 12. Epimakhova E.V., Losenkov I.S., Roshchina O.V., Plotnikov E.V. Evaluation of cytoprotective and antioxidant activities of lithium pyruvate on the peripheral blood mononuclear cells of patients with alcohol addiction. *Journal of Addiction Problems.* 2018; 12 (171): 36–47 (in Russ.).
 13. Prokopyeva V.D., Vetlugina T.P., Yarygina E.G., Plotnikov E.V., Bokhan N.A. Role of the anionic component in manifestation of effects of organic lithium salts on oxidative modification of blood proteins and lipids in patients with depressive disorders. *Pathological physiology and experimental therapy.* 2020; 64 (2): 13–20 (in Russ.). DOI: 10.25557/0031-2991.2020.02.13-20.
 14. Sokol C.L., Luster A.D. The chemokine system in innate immunity. *Cold Spring Harb Perspect Biol.* 2015; 7 (5): a016303. DOI: 10.1101/cshperspect.a016303.
 15. Moskalev A.V., Rudoy A.S., Apchel V.Ya. Chemokines, their receptors and features of the development of the immune response answer. *Bulletin of Russian Military Medical Academy.* 2017; 2 (58): 182–187 (in Russ.).
 16. Menyaylo M.E., Malashchenko V.V., Shmarov V.A., Gazatova N.D., Melashchenko O.B., Goncharov A.G., Seledtsova G.V., Seledtsov V.I. Interleukin-8 is able to support the pro-inflammatory activity of human monocytes (macrophages). *Genes & Cells.* 2018; 13 (1): 65–69 (in Russ.). DOI: 10.23868/201805007.
 17. Halliwell B., Frei B., England L., Ames B. N. Vitamin C: anti-oxidant or pro-oxidant *in vivo*? *Free Radic. Res.* 1996; 25 (5): 439–454. DOI: 10.3109/10715769609149066.
 18. Plotnikov E., Korotkova E., Voronova O., Dorozhko E., Bohan N., Plotnikov S. Lithium-based antioxidants: electrochemical properties and influence on immune cells. *Physiology and Pharmacology.* 2015; 19 (2): 107–113.
 19. De Filippo K., Dudeck A., Hasenberg M., Nye E., van Rooijen N., Hartmann K., Gunzer M., Roers A., Hogg N. Mast cell and macrophage chemokines CXCL1/CXCL2 control the early stage of neutrophil recruitment during tissue inflammation. *Blood.* 2013; 121 (24): 4930–4937. DOI: 10.1182/blood-2013-02-486217.
 20. Chumakova S.P., Urazova O.I., Shipulin V.M., Novitsky V.V., Khardikova S.A. Cytokines as inducers of postperfusion systemic inflammatory response in cardiosurgical patients with different duration of coronary pathology. *Bulletin of Siberian Medicine.* 2017; 16 (4): 260–268 (in Russ.). DOI: 10.20538/1682-0363-2017-4-260–268.
 21. Shetelig C., Limalanathan S., Hoffmann P., Seljeflot I., Gran J.M., Eritsland J., Andersen G.Ø. Association of IL-8 with infarct size and clinical outcomes in patients with STEMI. *J. Am. Coll. Cardiol.* 2018; 72 (2): 187–198. DOI: 10.1016/j.jacc.2018.04.053.
 22. Chernykh V.V., Konenkov V.I., Ermakova O.V., Orlov N.B., Obukhova O.O., Eremina A.V., Trunov A.N. Content of cytokines and growth factors in the intraocular fluid of patients with primary open-angle glaucoma. *Bulletin of Siberian Medicine.* 2019; 18 (1): 257–265 (in Russ.). DOI: 10.20538/1682-0363-2019-1-257-265.
 23. Menyaylo M.E., Malashchenko V.V., Shmarov V.A., Gazatova N.D., Todosenko N.M., Melashchenko O.B., Goncharov A.G., Seledtsov V.I. Direct effect of interleukin-8 on T-cell activation. *Russian Journal of Immunology.* 2016; 10 (2): 174–178 (in Russ.).
 24. Marks D.J., Segal A.W. Innate immunity in inflammatory bowel disease: a disease hypothesis. *J. Pathol.* 2008; 214 (2): 260–266. DOI: 10.1002/path.2291.

Authors contribution

Vetlugina T.P. – development of experimental technology and research design, analysis and interpretation of data, critical revision of the manuscript for important intellectual content, drafting of the manuscript. Epimakhova E.V. – carrying out of the experimental part of the study, statistical analysis of data. Savochkina D.N. – carrying out of the experimental part of the study. E.V. Plotnikov – synthesis of lithium preparations, editing of the manuscript. Boiko A.S. – determination of the cytokine concentration using the Luminex xMAP technology. Bokhan N.A. – critical revision of the manuscript for important intellectual content, editing of the manuscript, final approval of the manuscript for publication.

Authors information

Vetlugina Tamara P., Dr. Sci. (Biology), Professor, Honored Scientist of the Russian Federation, Leading Researcher, Laboratory of Clinical Psychoneuroimmunology and Neurobiology, Mental Health Research Institute, Tomsk NRMC, Tomsk, Russian Federation. ORCID 0000-0003-2068-0931.

Epimakhova Elena V., Cand. Sci. (Biology), Researcher, Addictive States Department, Mental Health Research Institute, Tomsk NRMC, Tomsk, Russian Federation. ORCID 0000-0002-9304-4496.

Savochkina Dariya N., Cand. Sci. (Med.), Researcher, Laboratory of Clinical Psychoneuroimmunology and Neurobiology, Mental Health Research Institute, Tomsk NRMC, Tomsk, Russian Federation. ORCID 0000-0003-1263-5516.

Plotnikov Evgeny V., Cand. Sci. (Chemistry), Senior Researcher, Department of Endogenous Disorders, Mental Health Research Institute, Tomsk NRMC; Associate Professor, Research School of Chemistry and Applied Biomedical Sciences, Tomsk Polytechnic University, Tomsk, Russian Federation. ORCID 0000-0002-4374-6422.

Boiko Anastasiia S., Cand. Sci. (Med.), Researcher, Laboratory of Molecular Genetics and Biochemistry, Mental Health Research Institute, Tomsk NRMC, Tomsk, Russian Federation. ORCID 0000-0002-2955-9057.

Bokhan Nikolay A., Dr. Sci. (Med.), Professor, Academician of the Russian Academy of Sciences, Honored Scientist of the Russian Federation, Head of the Addictive States Department, Mental Health Research Institute, Tomsk NRMC, Tomsk, Russian Federation. ORCID 0000-0002-1052-855X.

(✉) **Vetlugina Tamara P.**, e-mail: vetlug@mail.tomsknet.ru

Received 01.08.2020

Accepted 28.12.2020

Hemodynamic status of patients with different comorbidities of ischemic heart disease before and after coronary artery bypass grafting

Geltser B.I.¹, Sergeev E.A.¹, Kotelnikov V.N.¹, Silaev A.A.¹, Karpov R.S.²

¹ Far Eastern Federal University (FEFU)
6, Sukhanova Str., Vladivostok, 690091, Russian Federation

² Cardiology Research Institute, Tomsk National Research Medical Center (NRMCC), Russian Academy of Sciences
111a, Kievskaya Str., Tomsk, 634012, Russian Federation

ABSTRACT

Aim. To assess the hemodynamic status by transpulmonary thermodilution (TPTD) in patients with ischemic heart disease (IHD) with different comorbidities before and after coronary artery bypass grafting (CABG).

Materials and methods. 66 patients with IHD (40 men and 26 women) aged 53 to 77 years who were admitted for planned CABG were examined. The patients were divided into three groups according to the comorbidity: cardiovascular, respiratory, and metabolic. The first comorbidity was represented by a combination of IHD and multifocal atherosclerosis, the second – by IHD and chronic obstructive pulmonary disease (COPD), and the third – by IHD and metabolic syndrome (MS). All patients underwent CABG with the use of cardiopulmonary bypass. Hemodynamic parameters were recorded by the TPTD method using the Pulsion Picco Plus module (Germany) at 3 stages: after the start of mechanical ventilation (stage I), after the completion of cardiopulmonary bypass (stage II), and 24 hours after CABG (stage III).

Results. The patients with IHD with different comorbidities differed in characteristic signs of hemodynamic changes. In IHD with comorbid COPD, after withdrawal from the cardiopulmonary bypass and 24 hours after CABG, the highest index of systemic vascular resistance, the minimum values of the global ejection fraction, and a decrease in the global end-diastolic volume and pulmonary blood volume less noticeable compared with other groups of patients were noted. With comorbid respiratory and metabolic disorders, the maximum values for the indices of extravascular lung water and pulmonary vascular permeability were recorded. In the patients with a comorbid cardiovascular disease, hemodynamic and volume status violations in the dynamic follow-up were less pronounced.

Conclusion. The use of the TPTD method in patients with IHD before and after CABG makes it possible to specify the functional state of the circulatory system in different comorbidities, which increases the effectiveness of risk stratification and the accuracy of predicting possible complications.

Key words: ischemic heart disease, coronary artery bypass grafting, comorbidity, transpulmonary thermodilution.

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

Source of financing. This study was carried out within the framework and with financial support of the RFBR grant No. 19-29-01077.

Conformity with the principles of ethics. All patients signed an informed consent to participate in the study. The study was approved by the local Ethics Committee at Far Eastern Federal University (Protocol No. 12 of 15.11.2017).

For citation: Geltser B.I., Sergeev E.A., Kotelnikov V.N., Silaev A.A., Karpov R.S. Hemodynamic status of patients with different comorbidities of ischemic heart disease before and after coronary artery bypass grafting. *Bulletin of Siberian Medicine*. 2021; 20 (3): 29–37. <https://doi.org/10.20538/1682-0363-2021-3-29-37>.

✉ Kotelnikov Vladimir N., e-mail: 671235@mail.ru

Гемодинамический статус пациентов с различными вариантами коморбидности ишемической болезни сердца до и после коронарного шунтирования

Гельцер Б.И.¹, Сергеев Е.А.¹, Котельников В.Н.¹, Силаев А.А.¹, Карпов Р.С.²

¹ Дальневосточный федеральный университет (ДВФУ)

Россия, 690091, г. Владивосток, ул. Суханова, 8

² Научно-исследовательский институт (НИИ) кардиологии, Томский национальный исследовательский медицинский центр (НИМЦ) Российской академии наук

Россия, 634012, г. Томск, ул. Киевская, 111а

РЕЗЮМЕ

Цель – оценить гемодинамический статус методом транспульмональной термодилуции (ТПТД) у больных ишемической болезнью сердца (ИБС) с различными вариантами коморбидности до и после коронарного шунтирования (КШ).

Материалы и методы. Обследованы 66 больных ИБС (40 мужчин и 26 женщин) в возрасте 53–77 лет, поступивших на плановое КШ, которые были разделены на три группы коморбидности: кардиоваскулярную, респираторную и метаболическую. Первая из них представлена сочетанием ИБС и мультифокального атеросклероза, вторая – ИБС и хронической обструктивной болезни легких (ХОБЛ), третья – ИБС и метаболического синдрома (МС). Всем больным проведено КШ в условиях искусственного кровообращения (ИК). Показатели гемодинамики регистрировали методом ТПТД с использованием модуля Pulsion Picco Plus (Германия) на трех этапах: после начала искусственной вентиляции легких (I этап), после завершения ИК (II этап) и через 24 ч после КШ (III этап).

Результаты. Больные ИБС с различными вариантами коморбидности отличались характерными признаками гемодинамических изменений. При сочетании ХОБЛ и ИБС после отхода от ИК и через 24 ч после КШ отмечен наиболее высокий индекс системного сосудистого сопротивления, минимальные значения глобальной фракции изгнания, менее заметное по отношению к другим группам больных снижение глобального конечного диастолического объема и легочного объема крови. При респираторной и метаболической коморбидности фиксировались максимальные значения индексов внесосудистой жидкости в легких и проницаемости легочных сосудов. У больных с кардиоваскулярной коморбидностью нарушения гемодинамического и волемического статуса в динамике наблюдения были менее выраженными.

Заключение. Использование метода ТПТД у больных ИБС до и после КШ позволяет детализировать функциональный статус системы кровообращения при различных вариантах коморбидности, что повышает эффективность риск-стратификации и точность прогнозирования возможных осложнений.

Ключевые слова: ишемическая болезнь сердца, коронарное шунтирование, коморбидность, транспульмональная термодилуция.

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

Источник финансирования. Работа выполнена при финансовой поддержке гранта РФФИ (№ 19-29-01077).

Соответствие принципам этики. Все пациенты подписали информированное согласие на участие в исследовании. Исследование одобрено локальным этическим комитетом Дальневосточного федерального университета (протокол № 12 от 15.11.2017).

Для цитирования: Гельцер Б.И., Сергеев Е.А., Котельников В.Н., Силаев А.А., Карпов Р.С. Гемодинамический статус пациентов с различными вариантами коморбидности ишемической болезни сердца до и после коронарного шунтирования. *Бюллетень сибирской медицины*. 2021; 20 (3): 29–37. <https://doi.org/10.20538/1682-0363-2021-3-29-37>.

INTRODUCTION

Ischemic heart disease (IHD) remains one of the leading causes of death worldwide. Previous research showed that almost 70% of IHD patients have different comorbidities, which often modify clinical presentation of the underlying disease and reduce the effectiveness of pharmacotherapy [1]. The most common types of IHD comorbidity include its combination with other cardiovascular diseases, metabolic syndrome (MS), and chronic obstructive pulmonary disease (COPD). Thus, the combination of IHD with an atherosclerotic occlusion of the carotid and coronary arteries (CA) occurs in 75% of patients, and the combination of IHD with chronic lower limb ischemia accounts for 37–78% of cases [2]. MS is verified in 24% of patients sent for coronary artery bypass grafting (CABG), and COPD – in 35% of patients [3].

According to the American Society of Thoracic Surgeons, IHD patients with comorbid MS have a 1.4-fold increase in the risk of postoperative complications [3]. A study using the SYNTAX Score II scale showed that the likelihood of unfavorable postoperative complications after CABG doubles in IHD patients with comorbid COPD [4]. Depending on the number of arterial basins damaged by atherosclerosis, the risk of postoperative cardiovascular events increases by 1.64–10.5 times in IHD patients with a comorbid vascular disease, compared with isolated coronary artery disease.

High probability of complications and poor prognosis of surgical intervention in patients with IHD with a comorbidity determine the need for more careful monitoring of the functional state of the circulatory system at all stages of the hospital stay [5]. In routine clinical practice, electrocardiography, echocardiography, pulse oximetry, non-invasive and invasive blood pressure measurement, and registration of central venous pressure are used as hemodynamic monitoring methods. However, the generally accepted methods of hemodynamic research do not always provide a personalized approach to assessing the functional state of the circulatory system, which is especially important in cases with a high risk of complications associated with various comorbidities.

A number of studies have shown that the use of modern technologies of volumetric and hemodynamic monitoring significantly increases the efficiency of early diagnosis of adverse cardiovascular events. These technologies include the transpulmonary thermodilution method, which allows for an objective as-

essment of the patient's volume and hemodynamic statuses and their early correction [6].

The aim of the study was to assess the hemodynamic status by transpulmonary thermodilution in patients with coronary artery disease with different comorbidities before and after CABG.

MATERIALS AND METHODS

A prospective observational clinical study included 66 patients with ischemic heart disease (40 men and 26 women) aged 53 to 77 years (the average age was 66 years) who were admitted to the Medical Center of Far Eastern Federal University for planned CABG. Depending on the prevalence of clinical manifestations of comorbid diseases, the IHD patients were divided into 3 groups according to the comorbidity: cardiovascular, respiratory, and metabolic. The first group included 24 patients with IHD and comorbid multifocal atherosclerosis (chronic lower limb ischemia and an atherosclerotic occlusion of the carotid arteries of $\geq 50\%$). The second group included 20 IHD patients with grade II–III COPD without exacerbation. The third group was represented by 22 patients with IHD and comorbid MS, verified according to the clinical guidelines of the Ministry of Health of the Russian Federation.

Among the patients of the third group, the body mass index was in the range of 31–34 kg / m², which indicated class 1 obesity. The patients of all groups were diagnosed with chronic heart failure (CHF) of II–III functional classes according to the NYHA classification and controlled stage 2–3 hypertension with a very high risk. A written informed consent was obtained from each patient to participate in the study. The research protocols were approved by the local Ethics Committee at Far Eastern Federal University (Protocol No. 12 of 15.11.2017) and complied with the Declaration of Helsinki developed by the World Medical Association “Ethical Principles for Scientific Medical Research with Human Participation” as amended in 2000 and “The Rules of Clinical Practice in the Russian Federation” approved by the Order of the Ministry of Health of the Russian Federation of 19.06.2003, No. 266.

Hemodynamic and volumetric parameters were recorded by transpulmonary thermodilution using the Dreger Delta XL monitor and the Pulsion PiCCO Plus module (Germany) after a catheter from the PV2015L20 kit was inserted into the brachial artery. The duration of its presence in the arterial bed was no more than three days. Arterial line lavage was carried

out with boluses of 0.9% NaCl with the addition of heparin 1 U / ml. When calibrating the module, three consecutive thermodilutions were performed. Hemodynamic and volumetric parameters were analyzed at three stages of the study: immediately after tracheal intubation and the beginning of mechanical ventilation (stage I); after completion of the cardiopulmonary bypass (stage II); and 24 hours after the surgery (stage III).

The following parameters were recorded: stroke index (SI), cardiac index (CI), pulse pressure variability (PPV), stroke volume variability (SVV), systemic vascular resistance index (SVRI), global end-diastolic volume index (GEDI), global ejection fraction (GEF), cardiac function index (CFI), extravascular lung water (EVLW), and extravascular lung water index (EVLWI). In addition, the following parameters were calculated: pulmonary blood volume (PBV) = GEDI – EVLW, pulmonary vascular permeability index (PVPI) = EVLW / PBV, and dynamic arterial elastance (Ea_{dyn}) = PPV / SVV.

Statistical data processing was performed using the STATISTICA 10 software (StatSoft, Inc., USA) and Excel (Microsoft Office 2018) in the Microsoft Windows 10 operating system. The hypothesis of normal distribution of quantitative parameters in the analyzed groups was tested using the Shapiro – Wilk test. Data analysis was performed using descriptive statistics: medians (*Me*) and their 95% confidence intervals (CI). CI was calculated by the bootstrap method. Intergroup differences were assessed using the Kruskal – Wallis test. The differences were considered statistically significant at $p < 0.05$.

RESULTS

The obtained data showed that at the first stage of the study, the SI and CI indices in the IHD patients with comorbid cardiovascular and metabolic disorders corresponded to the standard values (40–60 ml / m² and 3–5 l / min / m²). After cardiopulmonary bypass withdrawal in the IHD patients with comorbid lesions of other arterial regions, a decrease in SI by 18% was recorded against the background of a stable CI level. At stage III, SI reached the initial values in this group, and CI exceeded them by 8.6%. With comorbid metabolic diseases at stage II of the study, the SI and CI indices decreased more significantly (by 37% and 18%, respectively), and a tendency to their increase was noted one day after the surgery. In the IHD patients with comorbid COPD, withdrawal from the cardiopulmonary bypass was accompanied by similar changes

in hemodynamics: a decrease in SI by 34% and in CI – by 9%. At the same time, 24 hours after CABG, this tendency was increasing, which was illustrated by a further decrease in the values of these parameters.

Before the surgery, in the patients of all groups, an increase in GEDI and a decrease in GEF were recorded, compared with their normal values, which indicated systolic and diastolic myocardial dysfunction (Table). 24 hours after CABG, in the IHD patients with a comorbid cardiovascular disease, GEDI decreased by 12.7%, while GEF increased by 2%. In IHD with comorbid MS, the dynamics of these parameters was more pronounced and amounted to 33% and 13.6%, respectively, which indicated a more significant decrease in preload and improvement in the cardiac pump function [5]. In patients with comorbid cardiorespiratory disorders, after withdrawal from the cardiopulmonary bypass, GEDI decreased by only 5%, compared with the initial level, and 24 hours after CABG – by 11%. At the same time, GEF had minimal values at all stages of the study, compared with other groups of the examined, which indicated decreased cardiac reserve in this category of patients.

In our study, the CFI level, which is an integral parameter of myocardial performance, was significantly lower than the standard values in all the patients before surgical treatment. One day after CABG, the patients in groups 1 and 3 showed an increase in this parameter to the reference range, while in the IHD patients with a comorbid respiratory disease, it did not reach it. The SVRI value before the surgery in the patients of all groups significantly exceeded the standard level, but did not have intergroup differences. After CABG, in the patients with comorbid vascular and metabolic diseases, SVRI decreased by 46–48%, and in the IHD patients with comorbid COPD, this parameter did not differ from the initial values.

SVV, PPV, and Ea_{dyn} belong to predictors of the hemodynamic response of patients to infusion load and are indicators of cardiorespiratory interaction during mechanical ventilation [6]. The levels of SVV and PPV depend on the filling of the vascular bed and on the ability of the heart to respond to a sudden increase in preload. At the same time, an increase in the values of these parameters is associated with hypovolemia, and a decrease – with progressing heart failure.

It was found that after withdrawal from the cardiopulmonary bypass, statistically significant changes in SVV and PPV were observed only among the patients with comorbid respiratory diseases, which was manifested through an increase in the values of these

parameters by 28% and 22%, respectively. A day after CABG, a significant increase in these parameters was recorded among the patients of all groups, but with a maximum level in IHD with comorbid COPD. Dynamic arterial elastance in the patients with comorbid vascular and metabolic diseases before CABG was above the threshold value, and after surgery, it reached it. At the same time, in the patients with COPD, a reversed tendency was observed, which was manifested through an increase in this parameter at stages II and III of the study.

EVLWI before surgery exceeded the standard values (3.0–7.0 ml/kg) in the patients of all groups, but was the highest among people with MS and COPD (Table). After withdrawal from the cardiopulmonary bypass, the EVLWI level did not change significantly. One day after the surgery, it decreased, but did not reach the reference values. In the patients with MS, the EVLWI level was the highest, which was associated with the effect of excessive intra-abdominal pressure on pulmonary blood flow [7]. Before the surgery, the PBV index in the patients with COPD was significantly lower than in the comparison groups. After withdrawal from the cardiopulmonary bypass, all the examined patients showed a tendency to its decrease, which persisted 24 hours after CABG. At the first stage of the study, in the patients with a comorbid respiratory disorder, PVPI was significantly higher than in other groups. After withdrawal from the cardiopulmonary bypass, in the patients with comorbid COPD and MS, the increase in PVPI was more noticeable, which could indicate an increasing likelihood of acute lung injury [8].

DISCUSSION

The authors attempted to perform a comprehensive assessment of the hemodynamic and volume status of the IHD patients in order to clarify the phenotypic features of blood circulation in different clinical comorbidities. It was found that initially decreased GEF and CFI levels in patients with comorbid cardiovascular and metabolic diseases and their subsequent growth to the reference level demonstrate significant improvement in the functional state of the myocardium already one day after its surgical revascularization. In the patients with comorbid cardiorespiratory diseases, the absence of positive dynamics of these indicators may be due to the systemic effects of chronic arterial hypoxemia and persistent inflammation, which prevent restoration of myocardial contractile function even with resumed coronary blood flow [1].

The results of SVRI measurement with continuous monitoring significantly complement the hemodynamic “portrait” of IHD patients. Thus, at the first stage of the study, most of them showed an increase in this indicator against the background of normal or decreased CI values, which is typical of elderly people with hypertension [5]. After withdrawal from the cardiopulmonary bypass in individuals with comorbid vascular and metabolic diseases, a decrease in SVRI indicated a decrease in left ventricular (LV) afterload and improvement in the cardiac pump function. In the patients with a comorbid respiratory disorder, the SVRI value in the early postoperative period exceeded the upper limit of the normal values, which may be due to a more noticeable limitation of the dilatation potential of the peripheral arterial bed in a combination of IHD and COPD.

Currently, measurement of GEDI is considered to be one of the most accurate technologies for assessing static cardiac preload [6]. In our study, before transitioning to the cardiopulmonary bypass, this parameter in the IHD patients with COPD was statistically significantly lower than in other groups. In these cases, a decrease in the volume of diastolic filling of the heart chambers may be a consequence of a more noticeable limitation of venous return and an increase in pulmonary vascular resistance induced by remodeling of the lung tissue. This was confirmed by the results of assessing the dynamic cardiac preload using SVV and PPV.

The dynamics of their changes during mechanical ventilation indicated that in the patients with a respiratory comorbidity, a more significant increase in intrathoracic pressure and a decrease in venous return to the heart were observed, compared with other groups. High level of Ea_{dyn} confirmed excessive arterial stiffness in the patients with COPD [5]. According to a number of authors, EVLWI is one of the most accurate parameters characterizing the volume of extravascular lung water. According to the literature, an increase in this parameter of more than 10 ml / kg is a predictor of subclinical pulmonary edema [8, 9].

In our study, the highest values of this parameter were recorded for respiratory and metabolic comorbidity groups, which may be due to more pronounced pulmonary circulatory disorders in this category of patients. Earlier, it was shown that an increase in the pressure in the pulmonary circulation leads to the “sieve effect” of pulmonary capillaries and provokes extravasation of fluid into the interstitial tissue [7]. Important factors in the pathogenesis of extravascular lung water include lung reperfusion.

Parameters of systemic and pulmonary hemodynamics in patients with ischemic heart disease with different comorbidities (Me, 95% CI)										
Parameters	Research stages									
	I			II			III			p-value
	IHD + PAD (n = 24)	IHD + COPD (n = 20)	IHD + MS (n = 22)	IHD + PAD (n = 24)	IHD + COPD (n = 20)	IHD + MS (n = 22)	IHD + PAD (n = 24)	IHD + COPD (n = 20)	IHD + MS (n = 22)	
GEDI, ml (680–800)	989.35 ¹ [911.1; 1,067.5]	948.5 ² [786.2; 1,110.8]	1,193.56 ³ [993.4; 1,393.7]	902.82 ⁴ [824.6; 981.1]	895.2 ⁵ [810.7; 979.7]	917.89 ⁶ [759.2; 1,076.5]	899.06 ⁷ [806.94;991.17]	847.33 ⁸ [745.8; 948.8]	897.56 ⁹ [707.01; 1,088.11]	$p_{1-4} = 0.01$; $p_{4-7} = 0.47$; $p_{1-7} = 0.02$; $p_{2-5} = 0.06$; $p_{5-8} = 0.06$; $p_{2-8} = 0.024$; $p_{3-6} = 0.02$; $p_{6-9} = 0.07$; $p_{3-9} = 0.037$; $p_{2-3} = 0.02$;
GEF, % (25–35)	22 ¹ [20;25]	19 ² [18;22]	22 ³ [20;24]	21 ⁴ [19;24]	18 ⁵ [15;22]	21 ⁶ [18;25]	26 ⁷ [23;30]	19 ⁸ [16;21]	25 ⁹ [22;28]	$p_{1-4} = 0.06$; $p_{4-7} = 0.053$; $p_{1-7} = 0.03$; $p_{2-5} = 0.452$; $p_{5-8} = 0.07$; $p_{2-8} = 0.73$; $p_{3-6} = 0.65$; $p_{6-9} = 0.073$; $p_{3-9} = 0.01$
CFI, l/min (4.5–6.5)	3.9 ¹ [3.2;4.6]	3.98 ² [3.4;4.6]	3.25 ³ [3.1;3.42]	4.32 ⁴ [3.9;4.7]	3.94 ⁵ [3.66;4.23]	4.44 ⁶ [3.62;5.25]	4.75 ⁷ [4.4;5.1]	3.83 ⁸ [3.6;4.09]	5 ⁹ [4.2;5.8]	$p_{1-4} = 0.06$; $p_{4-7} = 0.03$; $p_{1-7} = 0.001$; $p_{2-5} = 0.08$; $p_{5-8} = 0.07$; $p_{2-8} = 0.06$; $p_{3-6} = 0.007$; $p_{6-9} = 0.078$; $p_{3-9} = 0.0056$
SVRI, dyn/sec/ sm ⁻⁵ /m ² (1200–2000)	2,345.6 ¹ [1,942.76; 2,748.41]	2,320.8 ² [1,910.3; 2,731.36]	2,355.89 ³ [1,973.08; 2,739.4]	1,791.5 ⁴ [1,499.1; 2,084.2]	2,359.67 ⁵ [2,125.91; 2,593.42]	1,788.4 ⁶ [1,600.28; 1,976.83]	1,646.4 ⁷ [1,466.4; 1,826.3]	2,294 ⁸ [2,070.7; 2,517.3]	1,589.33 ⁹ [1,383.18; 1,795.48]	$p_{1-4} = 0.02$; $p_{4-7} = 0.07$; $p_{1-7} = 0.002$; $p_{2-5} = 0.45$; $p_{5-8} = 0.09$; $p_{2-8} = 0.06$; $p_{3-6} = 0.005$; $p_{6-9} = 0.07$; $p_{3-9} = 0.015$
SVV, % (< 10)	7.71 ¹ [4.63;10.8]	9.5 ² [7.93;11.1]	7.22 ³ [4.13;10.3]	8.75 ⁴ [5.23;12.3]	11.5 ⁵ [6.57;16.4]	6.33 ⁶ [3.38; 9.39]	12.13 ⁷ [9.4;14.9]	16.8 ⁸ [12.2; 21.5]	14.22 ⁹ [9.8; 18.6]	$p_{1-4} = 0.06$; $p_{4-7} = 0.043$; $p_{1-7} = 0.03$; $p_{2-5} = 0.06$;

[illegible]

Note: PAD – peripheral arterial disease; GEDI – global end-diastolic volume index; GEF – global ejection fraction; CFI – cardiac function index; SVRI – systemic vascular resistance index; SVV – stroke volume variability; PPV – pulse pressure variability; EVLWI – extravascular lung water index; PBV – pulmonary blood volume; PVPI – pulmonary vascular permeability index; p^{1-9} – the level of statistical significance of differences between similar comorbidities at the research stage.

According to randomized clinical trials, its main cause is previous lung hypoperfusion, which develops against the background of the cardiopulmonary bypass [2]. The accumulation of extravascular fluid in the lungs is also facilitated by opening of the pleural cavities, systemic inflammatory response to the cardiopulmonary bypass, excessive transfusion therapy, atelectasis of the basal segments of the lungs, and microangiopathy associated with MS and diabetes mellitus [3]. For early recognition of cardiogenic and non-cardiogenic pulmonary edema, PVPI assessment is important. The latter can significantly increase in acute respiratory distress syndrome, but remain within the reference values in cardiogenic pulmonary edema.

An increase in PVPI indicates capillary leakage of the lungs, which requires timely limitation of infusion therapy and prescription of loop diuretics. Before the surgery, in the IHD patients with comorbid cardiorespiratory diseases, PVPI was significantly higher than in the comparison groups, which indicated initially higher pulmonary capillary permeability due to the influence of the pathogenetic factors of COPD. After withdrawal from the cardiopulmonary bypass in the groups of IHD patients with comorbid COPD and MS, an increase in the value of this parameter was noted. The results obtained indicate that stress factors related to cardiac surgery, including cardiopulmonary bypass, are triggers of pathophysiological reactions that contribute to an increase in pulmonary vascular permeability and the likelihood of respiratory complications in this category of patients.

CONCLUSION

Detailing the circulatory and volume status following TPTD increases the effectiveness of risk stratification and the accuracy of predicting possible complications. The role of these studies increases in IHD with a comorbidity associated with phenotypic features of

blood circulation, which was manifested through a more noticeable increase in extravascular lung water and permeability of pulmonary capillaries in IHD with comorbid respiratory and metabolic diseases.

REFERENCES

1. Lilly L.S. Pathophysiology of the cardiovascular system. Moscow: Binom. Knowledge Laboratory, 2016. 735 p. (in Russ.).
2. Tennyson C., Lee R., Attia R. Is there a role for HbA1c in predicting mortality and morbidity outcomes after coronary artery bypassgraft surgery? *Interactive Cardiovascular and Thoracic Surgery*. 2013; 17 (6): 1000–1008. DOI: 10.1093/icvts/ivt351.
3. Raza S., Sabik J.F., Ainkaran P., Blackstone E.H. Coronary artery bypass grafting in diabetics: a growing healthcare cost crisis. *The Journal of Thoracic and Cardiovascular Surgery*. 2015; 150 (2): 304–312. DOI: 10.1016/j.jtcvs.2015.03.041.
4. Kurniawan E., Ding F.H., Zhang Q., Yang Z.K., Jian H.U., Shen W.F. Predictive value of SYNTAX score II for clinical outcomes in octogenarian undergoing percutaneous coronary intervention. *Journal of Geriatric Cardiology*. 2016; 13 (9): 733–739. DOI: 10.11909/j.issn.1671-5411.2016.09.014.
5. Kirov M. Yu., Lenkin A.I., Kuzkov V.V. Use of volumetric monitoring based on transpulmonary thermodilution during cardiosurgical interventions. *General resuscitation*. 2005; 1 (6): 70–79 (in Russ.).
6. Hilty M.P., Franzen D.P., Wyss C., Biaggi P., Maggiorini M. Validation of transpulmonary thermodilution variables in hemodynamically stable patients with heart diseases. *Ann Intensive Care*. 2017; 7 (1): 86. DOI: 10.1186/s13613-017-0307-0.
7. Tagami T., Ong M.E.H. Extravascular lung water measurements in acute respiratory distress syndrome: why, how, and when? *Current Opinion in Critical Care*. 2018; 24 (3): 209–215. DOI: 10.1097/MCC.0000000000000503.
8. Kozlov I.A., Romanov A.A. Respiration biomechanics, intrapulmonary water and oxygenating function of the lungs during uncomplicated operations with artificial circulation. *General Resuscitation*. 2007; 3 (3): 17–22 (in Russ.).
9. Hilty M.P., Franzen D.P., Wyss C., Biaggi P., Maggiorini M. Validation of transpulmonary thermodilution variables in hemodynamically stable patients with heart diseases. *Ann. Intensive Care*. 2017; 7 (1): 86 DOI: 10.1186/s13613-017-0307-0.

Authors contribution

Geltser B.I. – conception of the study, drafting of the manuscript. Karpov R.S., Kotelnikov V.N. – design of the study, analysis of data, drafting of the manuscript. Sergeev E.A., Silaev – collection of data, interpretation of results, drafting of the manuscript.

Authors information

Geltser Boris I., Dr. Sci. (Med.), Professor, Corresponding Member of the Russian Academy of Sciences, Director of the Clinical Medicine Department, FEFU, Vladivostok, Russian Federation. ORCID 0000-0002-9250-557X.

Sergeev Evgeniy A., Post-Graduate Student, Anesthetist and Resuscitator at the Medical Center, School of Biomedicine, FEFU, Vladivostok, Russian Federation. ORCID 0000-0002-2176-7070.

Kotelnikov Vladimir N., Dr. Sci. (Med.), Professor, Department of Clinical Medicine, School of Biomedicine, FEFU, Vladivostok, Russian Federation. ORCID 0000-0001-5830-1322.

Silaev Andrey A., Cand. Sci. (Med.), Associate Professor, Department of Clinical Medicine, Head of the Center for Anesthesiology and Resuscitation, Medical Center, School of Biomedicine, FEFU, Vladivostok, Russian Federation. ORCID 0000-0003-3701-4038.

Karpov Rostislav S., Dr. Sci. (Med.), Professor, Academician of the Russian Academy of Sciences, Scientific Director of Cardiology Research Institute, Tomsk NRMC, Tomsk, Russian Federation. ORCID 0000-0002-7011-4316.

(✉) **Kotelnikov Vladimir N.**, e-mail: 671235@mail.ru

Received 04.06.2020

Accepted 28.12.2020

Assessment of serum BDNF levels in complex rehabilitation of patients with ischemic stroke using traditional approaches to the restoration of motor functions

Kazakov S.D.¹, Koroleva E.S.¹, Brazovskaya N.G.¹, Zaytsev A.A.², Ivanova S.A.³, Alifirova V.M.¹

¹ Siberian State Medical University
2, Moscow Trakt, Tomsk, 634050, Russian Federation

² Siberian Federal Scientific Clinical Center of Federal Medicobiological Agency
4, Mira Str., Seversk, 63600, Russian Federation

³ Mental Health Research Institute, Tomsk National Research Medical Center (NRMCC), the Russian Academy of Sciences
4, Aleutskaya Str., Tomsk, 634014, Russian Federation

ABSTRACT

Aim. To assess the relationship between changes in serum brain-derived neurotrophic factor (BDNF) level, regression of motor deficiency, and restoration of functional activity in patients with ischemic stroke after stage II of medical rehabilitation.

Materials and methods. The study included 49 patients with ischemic stroke in the middle cerebral artery after stage I of medical rehabilitation. Group I ($n = 32$) went through stage II of rehabilitation in the early recovery period, group II ($n = 17$) was discharged for outpatient monitoring at the place of residence. Observation points: day 14 and day 90. Evaluation scales: National Institute of Health Stroke Scale (NIHSS), Fugle – Meyer Scale (FMA), Modified Rankin Scale (mRS). Serum BDNF levels were determined using a MAGPIX multiplex analyzer (Luminex, USA).

Results. A comparative analysis of the studied population showed that patients who underwent motor rehabilitation in the early recovery period had greater regression of neurologic deficit according to the Δ NIHSS scale ($p_{gr.I-II} = 0.043$), a more pronounced increase in the functional activity on the Δ mRS scale ($p_{gr.I-II} = 0.047$), and positive dynamics according to the FMA scale ($p_{day14-90} = 0.003$) in comparison with patients who received outpatient follow-up. The concentration of BDNF was significantly reduced by the end of the early recovery in the group II ($p_{day14-90_{gr.II}} = 0.002$). On the contrary, there was no decrease in the level of the BDNF ($p_{day14-90_{gr.I}} = 0.613$) in the group of patients undergoing rehabilitation.

Conclusion. The results of the study demonstrated the clinical effectiveness of stage II of the comprehensive rehabilitation of patients in the early period of stroke recovery. We can suggest that the success of neurorehabilitation is closely associated with an increase of the BDNF level against the background of its performance. This makes BDNF a potential marker of evaluating the effectiveness of ongoing rehabilitation treatment

Key words: ischemic stroke, brain-derived neurotrophic factor, rehabilitation, early recovery period, neuroplasticity.

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

Source of financing. The study was supported by the Russian Science Foundation (RSF), grant No. 18-15-00082.

Conformity with the principles of ethics. All the participants of the study signed an informed consent. The study was approved by the local Ethics Committee at Siberian State Medical University (Protocol No. 114 of 22.10.2018).

✉ Kazakov Stanislav D., e-mail: docstastomsk@gmail.com

For citation: Kazakov S.D., Koroleva E.S., Brazovskaya N.G., Zaytsev A.A., Ivanova S.A., Alifirova V.M. Assessment of serum BDNF levels in complex rehabilitation of patients with ischemic stroke using traditional approaches to the restoration of motor functions. *Bulletin of Siberian Medicine*. 2021; 20 (3): 38–45. <https://doi.org/10.20538/1682-0363-2021-3-38-45>.

Оценка уровня сывороточного BDNF при комплексной реабилитации пациентов с ишемическим инсультом с использованием традиционных подходов к восстановлению моторных функций

Казаков С.Д.¹, Королева Е.С.¹, Бразовская Н.Г.¹, Зайцев А.А.², Иванова С.А.³, Алифирова В.М.¹

¹ Сибирский государственный медицинский университет (СибГМУ)
Россия, 634050, Томск, Московский тракт, 2

² Сибирский федеральный научно-клинический центр (СибФНКЦ)
Россия, 636000, г. Северск, ул. Мира, 4

³ Научно-исследовательский институт (НИИ) психического здоровья, Томский национальный исследовательский медицинский центр (НИМЦ) Российской академии наук
Россия, 634014, Томск, ул. Алеутская, 4

РЕЗЮМЕ

Цель – оценить взаимосвязь между изменением уровня сывороточного BDNF, регрессом моторного дефицита и восстановлением функциональной активности у пациентов с ишемическим инсультом после II этапа медицинской реабилитации.

Материалы и методы. В исследовании приняли участие 49 пациентов с ишемическим инсультом в бассейне средней мозговой артерии после I этапа медицинской реабилитации. Группа I (n = 32) прошла II этап реабилитации в ранний восстановительный период, группа II (n = 17) выписана на амбулаторное наблюдение по месту жительства. Точки наблюдения: 14-е и 90-е сут. Оценочные шкалы: шкала инсульта Национального института здоровья (NIHSS), шкала Фугл – Мейера (FMA), модифицированная шкала Рэнкина (mRS). Уровень BDNF в сыворотке крови определяли на мультиплексном анализаторе MAGPIX (Luminex, США).

Результаты. Сравнительный анализ исследуемой популяции показал, что пациенты, получавшие моторную реабилитацию в раннем восстановительном периоде, имеют больший регресс неврологического дефицита по шкале Δ NIHSS ($p_{гр.I-II} = 0,043$), более выраженное повышение функциональной активности по шкале Δ mRS ($p_{гр.I-II} = 0,047$) и положительную динамику по шкале FMA ($p_{сут14-90} = 0,003$) в сравнении с пациентами, находившимися на амбулаторном наблюдении. Концентрация BDNF значительно снижалась к концу раннего восстановительного периода у пациентов на амбулаторном наблюдении в группе II ($p_{сут14-90, гр.II} = 0,002$). В группе пациентов, проходивших реабилитацию, напротив, снижения уровня фактора не наблюдалось ($p_{сут14-90, гр.I} = 0,613$).

Заключение. Результаты исследования демонстрируют клиническую эффективность II этапа комплексной реабилитации пациентов в ранний восстановительный период инсульта и дают основание предположить, что успех нейрореабилитации тесно связан с повышением BDNF на фоне ее проведения. Это делает мозговой нейротрофический фактор потенциальным маркером оценки эффективности проводимого восстановительного лечения.

Ключевые слова: ишемический инсульт, мозговой нейротрофический фактор, реабилитация, ранний восстановительный период, нейропластичность.

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

Источник финансирования. Исследование выполнено при поддержке гранта РНФ «Разработка научных основ роботизированной нейромиеореабилитации» (соглашение № 18-15-00082 от 25.04.2018).

Соответствие принципам этики. Все пациенты подписали информированное согласие на участие в исследовании. Исследование одобрено локальным этическим комитетом ФГБОУ ВО СибГМУ Минздрава России (протокол № 5961 от 18.06.2018).

Для цитирования: Казаков С.Д., Королева Е.С., Бразовская Н.Г., Зайцев А.А., Иванова С.А., Алифирова В.М. Оценка уровня сывороточного BDNF при комплексной реабилитации пациентов с ишемическим инсультом с использованием традиционных подходов к восстановлению моторных функций. *Бюллетень сибирской медицины*. 2021; 20 (3): 38–45. <https://doi.org/10.20538/1682-0363-2021-3-38-45>.

INTRODUCTION

Ischemic stroke is a significant medical and social problem all over the world [1]. More than 50% of patients who have had an ischemic stroke are unable to return to a full life due to the persistence of a disabling neurologic deficit, which in most cases is caused by motor impairment [2]. In this regard, successful rehabilitation of stroke patients is one of the most essential problems of modern medicine [3].

The effectiveness of motor rehabilitation is closely related to the mechanisms of neuroplasticity – the ability of brain neurons to functionally and structurally reorganize themselves [4, 5]. Currently, the role of the brain-derived neurotrophic factor (BDNF) in neurogenesis, plasticity, and neuronal survival induced by damage to brain tissue under conditions of cerebral ischemia is actively studied. BDNF is one of the most studied proteins of the neurotrophin family, which promotes survival, growth, and differentiation of cells in the cerebral cortex and hippocampus [5–9]. In addition, BDNF is involved in the processes underlying cellular mechanisms of memory and motor learning [10–12].

There are two pathways for BDNF expression in the central nervous system. The first is an unregulated constitutive pathway – ischemic stroke induces synthesis of BDNF and expression of its receptors [13]. The second – a dependent pathway – the expression of BDNF increases during motor learning as a result of movement repetition, under the influence of sensory stimulation and activation of the primary motor cortex. The activity-dependent release of BDNF plays a key role in synaptic plasticity in motor rehabilitation [5, 14, 15].

Most of the studies on investigating the relationship between the BDNF levels and post-stroke exercise have been conducted in animals. A number of experimental studies have shown that different intensity and effectiveness of physical activity have different effects on the level of BDNF [16]. Too high intensity and high frequency of physical activity can have a negative therapeutic effect after stroke and slow down

motor recovery [17]. The type of exercise also affects the concentration of BDNF. The results of most experimental studies demonstrate an increase in the level of BDNF against the background of aerobic exercise, at the same time, the data on the effect of anaerobic functional training sessions on the level of BDNF are contradictory [18].

In view of insufficient data on changes in the BDNF concentration during rehabilitation after ischemic stroke, obtained as a result of clinical trials, the study of the dynamics of changes in the level of neurotrophin depending on the type, intensity, and frequency of motor training and assessment of its effect on the outcome of stroke are relevant. Solving these problems will answer the questions if it is possible to use BDNF to predict the outcome of ischemic stroke and assess the effectiveness of rehabilitation methods, as well as the use of which types of neurorehabilitation is the most rational.

The aim of the study was to assess the relationship between changes in serum BDNF level, regression of motor deficiency, and restoration of functional activity in patients with ischemic stroke after stage II of medical rehabilitation.

MATERIALS AND METHODS

A prospective, non-randomized, comparative parallel study was conducted from March 2018 to December 2019. The study included patients over 18 years of age who signed a voluntary informed consent (or a consent was obtained from close relatives). The diagnosis of ischemic stroke was confirmed by CT or MRI of the brain. Exclusion criteria were other lesions of the central and peripheral nervous system; HIV, syphilis, viral hepatitis; oncological diseases; transient ischemic attack, hemorrhagic or recurrent ischemic stroke.

The study was approved by the local Ethics Committee at Siberian State Medical University (Protocol No. 114 of 22.10.2018).

For each study participant, a life history was collected, neurological status was assessed, and venous

blood was taken to determine the level of serum BDNF. Traditional approaches to the restoration of motor functions at the stage II of medical rehabilitation at the Branch of Research Institute of Balneology and Physiotherapy of the Siberian Federal Scientific Clinical Center of Federal Medicobiological Agency include local air cryotherapy applied to the area of spastic muscles, functional electrical stimulation, stimulation of antagonist muscles to spastic muscles, manual classical massage of paretic limbs, and exercise therapy (therapeutic physical gymnastics on the Bobath treatment table, active gymnastics, walking on a treadmill, walking in rehabilitation bars and stairs with an adjustable step height).

Specialists of the multidisciplinary rehabilitation team selected adequate physical activity for the patient, drawing up a plan for their adequate mobilization and rehabilitation, depending on the results of low-load functional tests, assessment of a patient's likelihood of falling on the Morse Fall Scale, post-stroke spasticity patterns, measurement of peripheral muscle strength on the Medical Research Council (MRC) scale, assessment of spasticity in different muscle groups on the Ashworth Scale, and evaluation of violation of the hierarchical control over the motor function by the nervous system. The duration of one training session was 30 minutes, the frequency of sessions was 2 times a day with a break of at least 2 hours, the course consisted of 20 workouts. The functional state of the patient during the rehabilitation measures was controlled by the heart rate, blood pressure level, and oxygen saturation in the blood. The observation period was early recovery period (90 days from the onset of stroke). Rating scales included National Institutes of Health Stroke Scale (NIHSS), Fugl – Meyer Assessment (FMA), and Modified Rankin Scale (mRS) [19]. The BDNF level in the blood serum was determined on a MAGPIX multiplex analyzer (Luminex, USA) using an HND-G3MAG-36K panel manufactured by MILLIPLEX® MAP (Merck, Germany). The results obtained were expressed in pg / ml.

Statistical processing of the results was carried out using the Statistica 13.0 software package. The critical level of significance when testing statistical hypotheses was taken equal to 0.05 (p is the achieved level of significance). Categorical parameters were described in the form of absolute and relative (%) frequencies of occurrence, quantitative and ordinal ones – in the form of the median and the interquartile range $Me (Q_1; Q_3)$. Comparison of two independent

samples was performed using the Mann – Whitney test. To analyze the differences in parameters between the three groups, the nonparametric Kruskal – Wallis test was used, followed by pairwise comparison using the Bonferroni correction for multiple comparisons when the differences were detected. To compare qualitative parameters between the groups, the Pearson's chi-squared test was used. In order to assess the intra-group dynamics of parameters, the Wilcoxon test for dependent samples was used.

RESULTS

The study involved 49 patients (26 men (53.1%) and 23 women (46.9%), average age 65 (59; 68) years) with acute ischemic stroke in the middle cerebral artery, discharged from the Regional Vascular Center of the Tomsk Regional Clinical Hospital after stage I of medical rehabilitation. The population was divided into two groups.

Group 1 ($n = 32$) received rehabilitation treatment at the stage II of rehabilitation at the Branch of Research Institute of Balneology and Physiotherapy of the Siberian Federal Scientific Clinical Center of Federal Medical Biological Agency. Group 2 ($n = 17$) was discharged for outpatient follow-up at the place of residence. After stage I of medical rehabilitation, the total score of the assessment scales in the study population indicated a moderate neurologic deficit ($_{\text{NIHSSday14}} = 4 (3; 8)$ points; $\text{FMA}_{\text{day14}} = 196 (179; 200)$ points) and moderate disability ($\text{mRS}_{\text{day14}} = 3 (2; 4)$ points). To determine the normal values of serum BDNF for a given age population, a comparison group was created, consisting of 50 individuals without stroke, comparable to the study sample by gender, age, and risk factors for developing cerebrovascular diseases (Table 1).

Groups I and II on day 14 after stroke were equivalent in terms of the severity of neurologic deficit and functional independence (Table 2). Group I patients after the second stage of medical rehabilitation using traditional approaches to the restoration of motor functions of the limbs showed pronounced regression of neurologic deficit according to the NIHSS ($p_{\text{day14-90}} = 0.002$) and FMA ($p_{\text{day14-90}} = 0.003$) scales, as well as improved functional independence according to the mRS scale ($p_{\text{day14-90}} = 0.009$). Group II patients discharged for outpatient follow-up also showed a decrease in the total score on the NIHSS ($p_{\text{day14-90}} = 0.025$) and mRS ($p_{\text{day14-90}} = 0.011$) scales, whereas a significant increase in the total score on the FMA scale ($p_{\text{day14-90}} = 0.406$) was not observed (Table 2).

Table 1

Clinical and demographic characteristics of the population				
Parameter	Group I	Group II	Comparison group	<i>p</i>
Average age, years, <i>Me</i> (Q_1 ; Q_3)	62 (57; 67)	66 (61; 68)	63 (56; 65)	0.817
Gender				
Male, abs. (%)	17 (53%)	9 (53%)	29 (58%)	0.876
Female, abs. (%)	15 (47%)	8 (47%)	21 (42%)	0.902
Arterial hypertension (%)	32 (100%)	17 (100%)	37 (74%)	0.914
Duration of hypertension, years, <i>Me</i> (Q_1 ; Q_3)	11 (10; 15)	10 (10; 15)	6 (5; 9)	0.892
Ischemic heart disease (%)	15 (47%)	7 (41%)	11 (22%)	0.789
Myocardial infarction (%)	7 (22%)	4 (23%)	0	0.231
Atrial fibrillation (%)	5 (15%)	4 (23%)	0	0.190
Stenting and artificial heart valves (%)	2 (4%)	1 (6%)	0	0.380
Obesity (%)	21 (65%)	13 (76%)	28 (56%)	0.785
Dyslipidemia (%)	27 (84%)	13 (76%)	43 (86%)	0.872
Diabetes mellitus (%)	8 (25%)	2 (12%)	14 (28%)	0.638
Duration of diabetes mellitus, years, <i>Me</i> (Q_1 ; Q_3)	8 (5; 10)	12 (5; 20)	7 (4; 10)	0.742
Smoking (%)	18 (56%)	9 (53%)	32 (64%)	0.823
BDNF level, day 14, <i>Me</i> (Q_1 ; Q_3)	2,768.0 (2,009.0; 3,652.0)	2,224.0 (1,302.0; 4,497.0)	4,273.0 (2,221.0; 5,251.0)	0.072
BDNF level, day 90, <i>Me</i> (Q_1 ; Q_3)	2,175.0 (1,730.0; 2,739.0)	881.1 (231.9; 1,483.5) * ^{&}	4,273.0 (2,221.0; 5,251.0)	0.000
Stroke subtype according to TOAST criteria				0.204
Large artery atherosclerosis (%)	5 (16%)	2 (12%)		
Cardiac embolism (%)	4 (12%)	5 (29%)		
Small vessel occlusion (%)	0 (0%)	0 (0%)		
Stroke of undetermined etiology (%)	23 (72%)	10 (59%)		
Affected hemisphere of the brain				0.401
right (%)	16 (53%)	11 (65%)		
left (%)	15 (47%)	6 (35%)		

Significant differences in comparison: * with group I; [&] with the comparison group

Table 2

Analysis of clinical parameters between groups I and II according to the NIHSS, FMA, and mRS scales, <i>Me</i> (Q_1 ; Q_3)			
Parameter	Group I	Group II	<i>p</i> _{groups I – II}
NIHSS _{day 14}	4 (3; 4)	3.5 (2; 9)	0.688
NIHSS _{day 90}	3 (2; 4)	3 (2; 9)	0.176
<i>p</i> _{day 14–90}	0.002	0.025	
FMA _{day 14}	191 (177; 201)	201 (169; 203)	0.612
FMA _{day 90}	199 (190; 215)	203 (172; 204)	0.245
<i>p</i> _{day 14–90}	0.003	0.406	
mRS _{day 14}	3 (2; 3)	2 (2; 3)	0.351
mRS _{day 90}	2 (1; 2)	2 (1; 3)	0.143
<i>p</i> _{day 14–90}	0.009	0.011	
ΔmRS	–1 (–2; 0)	0 (–1; 0)	0.047
ΔNIHSS	–2 (–3; –1)	–1 (–2; 0)	0.043
ΔFMA	8 (6; 14)	3 (1; 4)	0.032
ΔFMA _{arm}	6 (4; 9)	2 (1; 3)	0.041
ΔFMA _{leg}	4 (2; 6)	2 (1; 4)	0.015

A comparative analysis of changes in the values of clinical scales in the study groups between the observation points showed that the increase in points on the Δ FMA scale ($p_{\text{groups I-II}} = 0.032$) and regression of total points on the Δ NIHSS ($p_{\text{groups I-II}} = 0.043$) and Δ mRS ($p_{\text{groups I-II}} = 0.047$) scales in the patients undergoing physical rehabilitation significantly exceeded those in the patients receiving outpatient follow-up.

A detailed assessment of upper and lower extremity motor functions according to the FMA scale revealed a more pronounced increase in group I compared with group II ($p_{\text{groups I-II}} = 0.041$ and $p_{\text{groups I-II}} = 0.015$, respectively) (Table 2).

In terms of the BDNF level, no significant differences were found between the serum BDNF level in the stroke patients from groups I and II on day 14 and individuals from the comparison group (Table 1, Fig. 1).

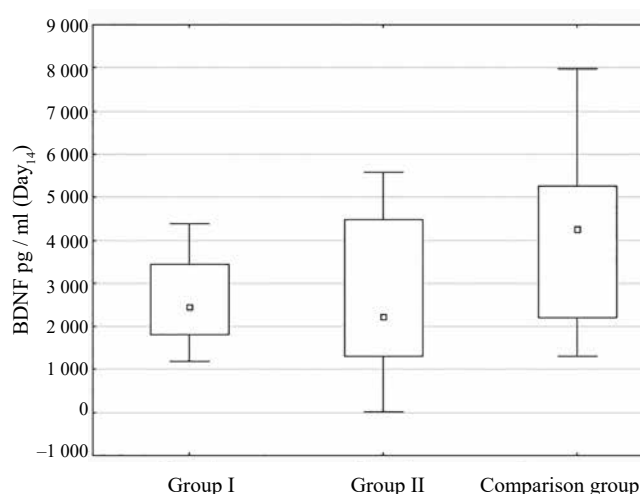


Fig.1. BDNF level in the patients of the comparison group and groups I–II on day 14 after stroke

At the same time, the concentration of the brain plasticity biomarker, which is responsible for neuronal survival and functional recovery after stroke, significantly decreased by the end of the early recovery period in the patients receiving outpatient follow-up in group II ($p_{\text{day14-day90, gr. II}} = 0.002$). In the group of patients undergoing rehabilitation, on the contrary, no decrease in the factor level ($p_{\text{day14-day90, gr. I}} = 0.613$) was noted. In this regard, on day 90 of the study, the concentration of BDNF in group II became significantly lower than in other groups, which still did not differ from one other (Table 1, Fig. 2).

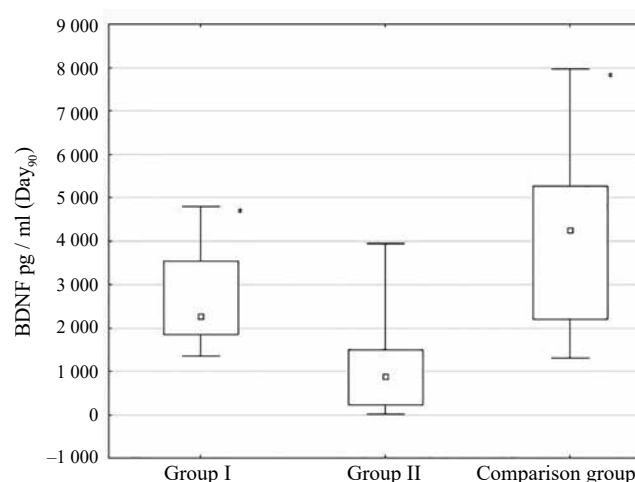


Fig. 2. BDNF level in the patients of the comparison group and groups I–II on day 90 after stroke: * significant differences compared with group II

DISCUSSION

It is known that the level of BDNF in the blood serum increases in response to ischemic damage to the brain tissue and reaches its maximum concentration on average on day 14 from the onset of stroke [14, 15]. The absence of differences between the BDNF level in the study groups on day 14 of the disease and in the comparison group, comparable in terms of sex, age, and risk factors for stroke, can be interpreted as a factor of a favorable prognosis for functional recovery. However, a comparative analysis of the study population in the dynamic follow-up showed that the patients who received motor rehabilitation in the early recovery period had greater regression of neurologic deficit on the NIHSS scale and a positive trend on the FMA scale.

In addition, in group I, there was not only better recovery of the motor function of the limbs, but also a more pronounced increase in the functional activity – Δ mRS ($p_{\text{group I-II}} = 0.047$) in comparison with the patients in group II. The data indicate the clinical effectiveness of rehabilitation measures using traditional approaches to restoring motor functions of the limbs.

The study of neurobiological markers showed that the concentration of BDNF also changes depending on the implementation of rehabilitation procedures or their absence. In the patients in group I, the level of serum BDNF did not significantly change between day 14 and 90 after stroke, maintaining a high concentration and not differing from those in the comparison group. The opposite results were obtained in group II,

where the level of the biomarker steadily decreased and was lower at the end of the early recovery period than in the comparison group.

Numerous fundamental studies have shown the role of BDNF in the implementation of brain plasticity processes due to the activation of neurogenesis, migration of nerve cells, remyelination of axons, as well as prevention of the effect of proinflammatory cytokines and a decrease in neuronal apoptosis [20, 21]. The key role of BDNF in the regulation of memory and motor learning mechanisms through the effect of long-term potentiation was demonstrated [22, 23]. In studies on experimental models of ischemic stroke in animals, it was shown that the expression of this neurotrophin increased during physical rehabilitation due to the activation of the motor cortex and improved the replenishment of motor deficits [24].

We found better recovery of motor function and functional independence in group I, which corresponded to changes in the FMA, NIHSS, and mRS scales. At the same time, in the group of patients who went through the stage II of medical rehabilitation, where a change in the FMA scale was observed, and changes in the NIHSS and mRS scales were more pronounced, the BDNF parameter remained at the same level.

Apparently, the only possible explanation for the improvement of motor function and functional independence was physical rehabilitation in these patients, which, in turn, was also accompanied by maintenance of the BDNF level. In our opinion, this suggests that induction of the activity-dependent pathway of BDNF expression and maintenance of its relatively high concentration during the stage II of medical rehabilitation were the main mechanisms that predetermined a favorable clinical and functional outcome.

CONCLUSION

The results of the study demonstrate the clinical effectiveness of the stage II of comprehensive rehabilitation of patients in the early period of stroke recovery and suggest that successful neurorehabilitation is closely related to an increase in the BDNF level during rehabilitation. This makes BDNF a potential marker for assessing rehabilitation effectiveness. The use of rehabilitation methods that increase the level of BDNF is promising.

REFERENCES

- Kim J., Thayabaranathan T., Donnan G.A., Howard G., Howard V.J., Rothwell P.M., Feigin V., Norrving B., Owolabi M., Pandian J., Liping L., Cadilhac D.A., Thrift A.G. Global stroke statistics 2019. *Int. J. Stroke*. 2020; 15 (8): 819–838. DOI: 10.1177/1747493020909545.
- Hatem S.M., Saussez G., della Faille M., Prist V., Zhang X., Dispa D., Bleyenheuft Y. Rehabilitation of motor function after stroke: a multiple systematic review focused on techniques to stimulate upper extremity recovery. *Front Hum. Neurosci*. 2016; 10: 442. DOI: 10.3389/fnhum.2016.00442.
- Bernhardt J., Hayward K.S., Kwakkel G., Ward N.S., Wolf S.L., Borschmann K., Krakauer J., Boyd L., Carmichael S.T., Corbett D., Cramer S.C. Agreed definitions and a shared vision for new standards in stroke recovery research: the stroke recovery and rehabilitation roundtable taskforce. *Int. J. Stroke*. 2017; 12 (5): 444–450. DOI: 10.1177/1747493017711816.
- Johansson B.B. Current trends in stroke rehabilitation. A review with focus on brain plasticity. *Acta Neurol. Scand*. 2011; 123 (3): 147–159. DOI: 10.1111/j.1600-0404.2010.01417.x.
- Mang C.S., Campbell K.L., Ross C.J.D., Boyd L.A. Promoting neuroplasticity for motor rehabilitation after stroke: considering the effects of aerobic exercise and genetic variation on brain-derived neurotrophic factor. *Phys. Ther.* Dec. 2013; 93 (12): 1707–1716. DOI: 10.2522/ptj.20130053.
- Gusev E.I., Martynov M.Yu., Kostenko E.V. et al. The effectiveness of Semax in the treatment of patients at different stages of ischemic stroke. *Korsakov Journal of Neurology and Psychiatry*. 2018; 118 (2–3): 61–68 (in Russ.). DOI: 10.17116/jnevro20181183261-68.
- Roslavtseva V.V., Salmina A.B., Prokopenko S.V., Kobanenko I.V., Rezvitskaya G.G. Possibilities of using the neurotrophic factor of the brain as a marker of the effectiveness of therapy in degenerative, traumatic, ischemic brain damage. *The Neurological Journal*. 2015; 20 (2): 38–46 (in Russ.).
- Chen S.-D., Wu C.-L., Hwang W.-C., Yang D.-I. More Insight into BDNF against neurodegeneration: anti-apoptosis, anti-oxidation, and suppression of autophagy. *International Journal of Molecular Sciences*. 2017; 18 (3): 545. DOI: 10.3390/ijms18030545.
- Guo W., Nagappan G., Lu B. Differential effects of transient and sustained activation of BDNF-TrkB signaling. *Developmental Neurobiology*. 2018; 78 (7): 647–659. DOI: 10.1002/dneu.22592.
- Liu P.Z., Nusslock R. Exercise-mediated neurogenesis in the hippocampus via BDNF. *Front. Neurosci*. 2018; 12: 52. DOI: 10.3389/fnins.2018.00052.
- Park H., Poo M. Neurotrophin Regulation of neural circuit development and function. *Nat. Rev. Neurosci*. 2013; 14 (1): 7–23 DOI: 10.1038/nrn3379.
- Zagrebelsky M., Korte M. Form follows function: BDNF and its involvement in sculpting the function and structure of synapses. *Neuropharmacology*. 2014; 76: 628–638. DOI: 10.1016/j.neuropharm.2013.05.029.
- Golubev A.M., Petrova M.V., Grechko A.V., Zakharchenko V.E., Kuzovlev A.N., Ershov A.V. Molecular markers of ischemic stroke. *General Reanimatology*. 2019; 15 (5): 11–22 (in Russ.). DOI: 10.15360/1813-9779-2019-5-11-22.
- Coleman E.R., Moudgal R., Lang K., Hyacinth H I., Awosika O.O., Kissela B.M., Feng W. Early rehabilitation after stroke: a narrative review. *Current Atherosclerosis Reports*. 2017; 19 (12): 59. DOI: 10.1007/s11883-017-0686-6.

15. Koroleva E.S., Alifirova V.M., Brazovskaya N.G., Plotnikov D.M., Levchuk L.A., Boyko A.S., Zapekin S.P., Semenenko A.V., Kataeva N.G., Ivanova S.A. Clinical and laboratory assessment of the effectiveness of early rehabilitation of patients with stroke using assisted robotic mechanisms. *Bulletin of Siberian Medicine*. 2019; 18 (4): 55–62 (in Russ.). DOI: 10.20538/1682-0363-2019-4-55–62.
16. Walsh J.J., Bentley R.F., Gurd B.J., Tschakovsky M.E. short-duration maximal and long-duration submaximal effort forearm exercise achieve elevations in serum brain-derived neurotrophic factor. *Front. Physiol.* 2017; 8: 746. DOI: 10.3389/fphys.2017.00746.
17. Mackay C.P., Kuys S.S., Brauer S.G. The effect of aerobic exercise on brain-derived neurotrophic factor in people with neurological disorders: a systematic review and meta-analysis. *Neural Plast.* 2017; 2017: 4716197. DOI: 10.1155/2017/4716197.
18. Alcantara C.C., García-Salazar L.F., Silva-Couto M.A., Santos G.L., Reisman D.S., Russo T.L. Post-stroke BDNF concentration changes following physical exercise: a systematic review. *Frontiers in Neurology*. *Front. Neurol.* 2018; 9: 637. DOI: 10.3389/fneur.2018.00637.
19. Suponeva N.A., Yusupova D.G., Zhirova E.S., Melchenko D.A., Taratukhina A.S., Butkovskaya A.A., Ilyina K.A., Zaitsev A.B.3, Zimin A.A., Klochkov A.S., Lyukmanov R.Kh., Kalinkina M.E., Piradov M.A., Kotov-Smolensky A.M., Khizhnikova A.E. Validation of the Modified Rankin Scale (MRS) in Russia. *Neurology, Neuropsychiatry, Psychosomatics*. 2018; 10 (4): 36–39 (in Russ.). DOI: 10.14412/2074-2711-2018-4-36-39.
20. Himi N., Takahashi H., Okabe N., Nakamura N., Shiromoto T., Narita K., Koga T., Miyamoto O. Exercise in the early stage after stroke enhances hippocampal brain-derived neurotrophic factor expression and memory function recovery. *J. Stroke Cerebrovasc. Dis.* 2016; 25 (12): 2987–2994. DOI: 10.1016/j.strokecerebrovasdis.2016.08.017.
21. Leal G., Bramham C.R., Duarte C.B. BDNF and hippocampal synaptic plasticity. *Neurotrophins*. 2017; 104: 153–195. DOI: 10.1016/bs.vh.2016.10.004.
22. Kotłęga D., Peda B., Zembroń-Łacny A., Gołąb-Janowska M., Nowacki P. The role of brain-derived neurotrophic factor and its single nucleotide polymorphisms in stroke patients. *Neurologia i Neurochirurgia Polska*. 2017; 51 (3): 240–246. DOI: 10.1016/j.pjnns.2017.02.008.
23. Walsh J.J., Tschakovsky M.E. Exercise and circulating BDNF: Mechanisms of release and implications for the design of exercise interventions. *Applied Physiology, Nutrition, and Metabolism*. 2018; 43 (11): 1095–1104. DOI: 10.1139/apnm-2018-0192.
24. Gandolfi M., Smania N., Vella A., Picelli A., Chirumbolo S. Assessed and emerging biomarkers in stroke and training-mediated stroke recovery: state of the art. neural plasticity. *Neural Plast.* 2017; 2017: 1389475. DOI: 10.1155/2017/1389475.

Authors contribution

Kazakov S.D. – collection of material, drafting of the manuscript. Koroleva E.S. – conception and design. Brazovskaya N.G. – statistical processing of data. Zaitsev A.A. – analysis or interpretation of data. Ivanova S.A. – critical revision of the manuscript. Alifirova V.M. – final approval of the manuscript for publication.

Authors information

Kazakov Stanislav D., Post-Graduate Student, Division of Neurology and Neurosurgery, Siberian State Medical University, Tomsk, Russian Federation. ORCID 0000-0001-9818-5549.

Koroleva Ekaterina S., Cand. Sci. (Med.), Associate Professor, Division of Neurology and Neurosurgery, Siberian State Medical University, Tomsk, Russian Federation. ORCID 0000-0003-1911-166X.

Brazovskaya Natalya G., Cand. Sci. (Med.), Associate Professor, Division of Medical and Biological Cybernetics, Siberian State Medical University, Tomsk, Russian Federation. ORCID 0000-0002-0706-9735.

Zaitsev Aleksey A., Cand. Sci. (Med.), Director of the branch of Tomsk Research Institute of Balneology and Physiotherapy, Siberian Federal Scientific Clinical Center of Federal Medicobiological Agency, Tomsk, Russian Federation. ORCID 0000-0003-2601-1739.

Ivanova Svetlana A., Dr. Sci. (Med.), Professor, Head of the Laboratory of Molecular Genetics and Biochemistry, Mental Health Research Institute, Tomsk NRMС, Tomsk, Russian Federation. ORCID 0000-0001-7078-323X.

Alifirova Valentina M., Dr. Sci. (Med.), Professor, Head of the Division of Neurology and Neurosurgery, Siberian State Medical University, Tomsk, Russian Federation. ORCID 0000-0002-4140-3223.

(✉) **Kazakov Stanislav D.**, e-mail: docstastomsk@gmail.com

Received 24.01.2021

Accepted 25.05.2021

Influence of a B16/F10 melanoma variant on the Bcl-2 levels in mitochondria in various organs of female mice

Kit O.I., Frantsiyants E.M., Neskubina I.V., Cheryarina N.D., Shikhlyarova A.I., Surikova E.I., Kaplieva I.V., Nemashkalova L.A.

*National Medical Research Center for Oncology (NMRCO)
63, 14 Liniya Str., Rostov-on-Don, 344037, Rostov-on-Don, Russian Federation*

ABSTRACT

Aim. To study the Bcl-2 level in mitochondria of various organs in female mice with standard and stimulated growth of an experimental B16/F10 melanoma.

Materials and methods. The study included C57BL/6 female mice ($n = 168$). The experimental animals were divided into the following groups: an intact group ($n = 21$), a group with modelled chronic neuropathic pain (CNP) ($n = 21$), an M group with B16/F10 melanoma ($n = 63$), and a CNP + M group ($n = 63$). The Bcl-2 concentration (ng / mg protein) in mitochondrial samples was determined by ELISA (Thermo Fisher Scientific, Austria). Statistical analysis of the results was carried out using Statistica 10.0.

Results. Compared to the Bcl-2 levels in the intact animals, CNP decreased this parameter in the cardiac mitochondria by 1.3 times, while increasing it by 5.9 times in the skin mitochondria. In the dynamics of standard melanoma growth, the Bcl-2 content changed compared with the corresponding intact values in the mitochondria of the brain, heart, and skin, but did not change in the liver and kidneys. In the mitochondria in melanoma, the Bcl-2 levels were high throughout the entire period of standard tumor growth in comparison with the intact skin. The stimulated melanoma growth in CNP was involving more organs into the pathological process as the tumor was growing. Thus, in comparison with the values in the CNP group, the mitochondrial Bcl-2 levels changed in the heart at week 1; in the heart and skin – at week 2; in the heart, skin, and brain – at week 3. The Bcl-2 levels did not change in the liver and kidney mitochondria. In the mitochondria in the CNP-stimulated melanoma, the Bcl-2 levels were lower than in the skin mitochondria in CNP throughout the entire tumor growth period.

Conclusion. The liver and kidney mitochondria are somewhat Bcl-2 stable in both standard and stimulated tumor growth. It is assumed that different Bcl-2 dynamics in the mitochondria in melanoma depending on the variant of tumor development reflects the modulating effect of CNP and the ability to change the Bcl-2 levels according to the growth phase.

Key words: cell mitochondria, Bcl-2, chronic neuropathic pain, experimental B16/F10 melanoma, female mice.

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

Source of financing. The study was carried out as part of the state assignment.

Conformity with the principles of ethics. The study was approved by the Bioethics Committee for Working with Animals of the NMRCO of the Ministry of Health of the Russian Federation (Protocol No. 2 of 31.05.2018).

For citation: Kit O.I., Frantsiyants E.M., Neskubina I.V., Cheryarina N.D., Shikhlyarova A.I., Surikova E.I., Kaplieva I.V., Nemashkalova L.A. Influence of a B16/F10 melanoma variant on the Bcl-2 levels in mitochondria in various organs of female mice. *Bulletin of Siberian Medicine*. 2021; 20 (3): 46–53. <https://doi.org/10.20538/1682-0363-2021-3-46-53>.

Влияние варианта развития меланомы B16/F10 на содержание Bcl-2 в митохондриях клеток различных органов самок мышей

Кит О.И., Франциянц Е.М., Нескубина И.В., Черярина Н.Д., Шихлярова А.И., Сурикова Е.И., Каплиева И.В., Немашкалова Л.А.

Национальный медицинский исследовательский центр (НМИЦ) онкологии
Россия, 344037, г. Ростов-на-Дону, ул. 14-я линия, 63

РЕЗЮМЕ

Цель – изучить содержание Bcl-2 в митохондриях различных органов самок мышей при стандартном и стимулированном росте экспериментальной меланомы B16/F10.

Материалы и методы. Работа выполнена на самках мышей линии C57BL/6 ($n = 168$), которых разделили на группы: интактную ($n = 21$), группу с воспроизведением модели хронической нейрогенной боли (ХНБ) ($n = 21$), группу «М» – меланома B16/F10 ($n = 63$), группу «ХНБ + М» ($n = 63$). В митохондриальных образцах методом иммуноферментного анализа определяли концентрацию Bcl-2 в нг/мг белка (Thermo Fisher Scientific, Австрия). Статистическая обработка результатов проводилась с использованием пакета прикладных программ Statistica 10.0.

Результаты. По сравнению со значениями Bcl-2 у интактных животных, ХНБ способствовала снижению данного показателя в митохондриях сердца в 1,3 раза, а в митохондриях кожи, напротив, повышала в 5,9 раз. В динамике стандартного роста меланомы содержание Bcl-2 изменялось относительно соответствующих интактных величин в митохондриях мозга, сердца, кожи, при этом не менялось в печени и почках. В митохондриях меланомы уровень Bcl-2 по сравнению с интактной кожей был высоким на всем протяжении стандартного роста опухоли. Стимулированный рост меланомы при ХНБ вовлекал в патологический процесс органы, количество которых увеличивалось по мере развития опухоли. Так, по сравнению со значениями в группе ХНБ изменение уровня Bcl-2 на 1-й нед роста фиксировали в митохондриях сердца, на 2-й – в сердце и коже, на 3-й нед – в сердце, коже и мозге. Не изменялся показатель в митохондриях печени и почек. В митохондриях меланомы, стимулированной ХНБ, уровень Bcl-2 на протяжении всего роста опухоли был ниже, чем в митохондриях кожи при ХНБ.

Заключение. Выявлено, что митохондрии клеток печени и почек обладают определенной стабильностью по Bcl-2 как при стандартном развитии опухолевого процесса, так и при стимулированном. Полагаем, что различная динамика Bcl-2 в митохондриях клеток меланомы в зависимости от варианта развития опухоли свидетельствует о модулирующем эффекте ХНБ и способности менять уровень показателя в зависимости от фазы роста.

Ключевые слова: митохондрии клеток, Bcl-2, хроническая нейрогенная боль, экспериментальная меланома B16/F10, самки мышей.

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

Источник финансирования. Исследование выполнено в рамках государственного задания.

Соответствие принципам этики. Исследование одобрено биоэтическим комитетом по работе с животными ФГБУ «НМИЦ онкологии» Минздрава России (протокол № 2 от 31.05.2018).

Для цитирования: Кит О.И., Франциянц Е.М., Нескубина И.В., Черярина Н.Д., Шихлярова А.И., Сурикова Е.И., Каплиева И.В., Немашкалова Л.А. Влияние варианта развития меланомы B16/F10 на содержание Bcl-2 в митохондриях клеток различных органов самок мышей. *Бюллетень сибирской медицины*. 2021; 20 (3): 46–53. <https://doi.org/10.20538/1682-0363-2021-3-46-53>.

INTRODUCTION

Resistance to apoptosis is a confirmed marker of cancer. Different sensitivity of tumors and healthy tissues to apoptosis is a more complex problem than simply the fact that cancer cells are more resistant to death. A deeper understanding of apoptosis, which has

developed over the past decades, now opens up new opportunities for identifying agents capable of triggering apoptosis in carcinogenesis [1].

The concept of selective activation of cell death in malignant tumors has a long history. Programmed cell death can occur through a number of mechanisms,

including apoptosis and necroptosis regulated by various signaling pathways, for instance, the mitochondrial pathway of apoptosis. Several of these signaling pathways for apoptotic cell death have been used with varying success as potential therapeutic targets, which resulted in disappointing early clinical findings due to an incomplete understanding of the fundamentals of biology [2, 3]. The search for and use of agents with selective action on mitochondria is proceeding even more carefully, since it is necessary to take into account the specifics of rearrangement of both mitochondria and mitochondrial metabolism of a transformed cell, as well as (or) activation of energy metabolism by reprogrammed mitochondria [4].

The understanding that Bcl-2 overexpression promoted oncogenesis by inhibiting programmed triggering of cell death helped to identify a family of apoptosis regulators. Moreover, it proved evasion of apoptosis to be the main sign of cancer [5]. Currently, about 20 Bcl-2 family members with pro- and anti-apoptotic features have been found in vertebrates. It was also determined that many interactions between these proteins play a central role in a response of healthy and malignant cells to cytotoxic damage [6].

Apoptotic priming in healthy cells and tissues dynamically changes at several levels. This change can be observed between different tissues of an adult human, within the same tissue at different stages of development, between separate cells in the tissue, and in the same cell at different time, as a cell can be damaged or changed in terms of signaling. The existing fluctuations in the levels of pro- and anti-apoptotic proteins create certain difficulties when acting on neoplastic cells. Moreover, it is necessary to simultaneously minimize the damage to cells of healthy tissues [1].

When apoptosis is activated during embryonic development, changes in the Bcl-2 function are associated with different developmental periods. Thus, in the developing nervous system of mice, the neuronal Bcl-2 levels significantly reduce by the age of 5 months. The Bcl-2 family members, Bcl-XL and Mcl-1, play a key role in the survival of immature B cells during development, while Bcl-2 is necessary for the survival of mature B cells [7]. A recent study showed that minor changes in the balance of pro- and anti-apoptotic Bcl-2 proteins have profound effects during craniofacial development [8]. Many signaling pathways that control cell proliferation and the Bcl-2 protein function also determine heterogeneity of separate cells in their activity [9].

Therefore, it should not be surprising that apoptosis is dependent on intercellular heterogeneity. Separate cells can rapidly shift their apoptotic threshold in response to changes in internal and external signals [10]. One of the ways in which this can happen is related to the fact that Bcl-2 proteins continuously move between cytosol and mitochondria. Under stress, the transmitted signals result in cell buffering with the Bcl-2 protein above the permissible levels [10]. The apoptotic threshold of separate cells is dynamic; the cell population reaches a balance between sensitivity and resistance to proapoptotic stimuli [11]. The level of apoptotic signal stimulation varies considerably within and between cell populations [12]. Thus, affecting malignant cells through apoptosis by disrupting protein interactions of Bcl-2 in mitochondria is complex. Currently, the existing knowledge does not reveal all the intricacies of this interaction, which requires further research in this area.

The aim of this study was to determine the Bcl-2 content in mitochondria of various organs of female mice in standard and stimulated growth of an experimental B16/F10 melanoma.

MATERIALS AND METHODS

The experimental study was carried out on female mice of the C57BL/6 line ($n = 168$), aged 8 weeks and having an initial weight of 21–22 g. The animals were randomly divided into the following experimental groups: an intact group C ($n = 21$), a CNP group with a model of chronic neuropathic pain (CNP) ($n = 21$), an M group with a B16/F10 melanoma ($n = 63$) with standard subcutaneous transplantation of the B16/F10 melanoma, and a CNB + M group ($n = 63$), where the B16/F10 melanoma was transplanted 3 weeks after creating the CNP model.

The experimental animals were obtained from the Scientific Center for Biomedical Technologies of the Federal Medical and Biological Agency (Andreevka Branch, the Moscow Region). The strain of the B16/F10 murine melanoma was provided by N.N.Blokhin National Medical Research Center for Oncology of the Ministry of Health of the Russian Federation. Work with animals was carried out in accordance with the rules of the European Convention for the Protection of Vertebrate Animals Used for Experimental Purposes (Directive 86/609/EEC) and the Declaration of Helsinki. The researchers also adhered to the International Guiding Principles for Biomedical Research Involving Animals and the Order of the Ministry of

Health of the Russian Federation No. 267 “On approval of laboratory practice rules” of 19.06.2003.

The animals were kept under natural lighting with free access to water and food. The manipulations with animals were performed in an isolation room in compliance with the generally accepted rules of asepsis and antiseptics. The study was approved by the Bioethics Committee for Working with Animals of the NMRCO of the Ministry of Health of the Russian Federation (Protocol No. 2 of 31.05.2018).

The B16/F10 melanoma was transplanted into the animals by a standard subcutaneous injection of a 0.5 ml tumor cell suspension diluted 1 : 10 in saline under the right scapula. The model of chronic neuropathic pain (CNP) was reproduced by ligating the sciatic nerve on both sides under Zoletil – xylazine anesthesia [13]. The following anesthesia was used: Zoletil with xylazine administered 10 minutes before the main anesthesia; premedication: xylazine (the drug “Xila”) intramuscularly at a dose of 0.05 ml / kg of body weight (according to the instructions), followed by Zoletil-50 at a dose of 10 mg per 100 g of body weight 10 minutes later.

The animals of the M group and the CNP + M group were decapitated by a guillotine after transplantation of the B16/F10 melanoma according to the following schedule: week 1 – day 7 of the melanoma growth, week 2 – day 14 of the melanoma growth, week 3 – day 21 of the melanoma growth. The animals of the CNP group were sacrificed 3 weeks after the CNP model reproduction, in parallel, the intact animals were decapitated. After decapitation, the skin and tumor were dissected from the animals, their brain, liver, kidneys, and heart were also removed. The apparently healthy

skin was dissected at the maximum distance from the tumor node. The mitochondria were isolated according to the method proposed by M.V. Egorova and S.A. Afanasyev [14] (using cooling agents and differential centrifugation in a high-speed refrigerated Avanti J-E centrifuge (Beckman Coulter Life Sciences, USA).

Before the analysis, the obtained mitochondrial samples (with the protein concentration of 4–6 g / l) were stored at –80 °C in the isolation medium. In the mitochondrial samples, the Bcl-2 concentration in ng / mg protein (Thermo Fisher Scientific, Austria) was determined using an ELISA analyzer (Infinite F50 Tecan, Austria), and the protein concentration in mg / ml was determined by the biuret method (Olvex Diagnosticum, Russia) using a ChemWell automatic analyzer (Awareness Technology Inc., USA).

Statistical analysis of the results was carried out using Statistica 10.0 software. The obtained data were analyzed for the correspondence to the normal distribution law using the Shapiro – Wilk test (for small samples). The comparison of the quantitative data in the groups (independent samples) was performed using the Kruskal – Wallis test (multiple comparisons). The data in the tables were presented as $M \pm m$, where M is the arithmetic mean, and m is the standard error of the mean. The results were considered statistically significant at $p < 0.05$. The obtained results were statistically processed in accordance with the general recommendations for medical studies.

RESULTS

The results of the experiment studying the Bcl-2 level in the mitochondria of various organs and the B16/F10 melanoma are presented in the table.

Table

Dynamics of the Bcl-2 level in organ mitochondria of female mice with standard and stimulated growth of the B16/F10 melanoma, ng / mg protein, $M \pm m$						
Parameter	Organ					Tumor
	brain	liver	heart	kidneys	skin	
Intact group (C)	82.937 ± 2.455	96.335 ± 4.561	62.00 ± 2.85	103.079 ± 4.033	5.56 ± 0.203	–
CNP	107.391 ± 3.738 ¹ $p^1 = 0.00014$	107.348 ± 3.806	46.66 ± 3.87 ¹ $p^1 = 0.00008$	103.573 ± 3.712	32.69 ± 1.37 ¹ $p^1 = 0.00000$	–
M, week 1	107.392 ± 3.787 ¹ $p^1 = 0.00015$	112.189 ± 4.405	36.85 ± 1.92 ¹ $p^1 = 0.00000$	108.786 ± 4.136	49.61 ± 1.71 ¹ $p^1 = 0.00000$	24.22 ± 1.51 ¹ $p^1 = 0.00000$
M, week 2	49.476 ± 2.689 ^{1,3} $p^1 = 0.00000$ $p^3 = 0.00000$	103.349 ± 3.766	21.38 ± 1.56 ^{1,3} $p^1 = 0.00000$ $p^3 = 0.00004$	109.584 ± 4.129	15.46 ± 1.41 ^{1,3} $p^1 = 0.00001$ $p^3 = 0.00000$	28.58 ± 1.95 ¹ $p^1 = 0.00000$
M, week 3	31.688 ± 3.172 ^{1,3} $p^1 = 0.00000$ $p^3 = 0.00107$	103.384 ± 3.413	21.6 ± 1.55 ¹ $p^1 = 0.00000$	113.318 ± 5.042	1.35 ± 0.11 ^{1,3} $p^1 = 0.00000$ $p^3 = 0.00000$	13.88 ± 1.36 ^{1,3} $p^1 = 0.00005$ $p^3 = 0.00004$
CNP + M, week 1	104.265 ± 3.849	102.353 ± 3.805	80.99 ± 1.6 ² $p^2 = 0.00000$	113.928 ± 4.982	27.28 ± 1.77	11.02 ± 1.18 ² $p^2 = 0.00000$

Table (continued)

Parameter	Organ					umor
	brain	liver	heart	kidneys	skin	
CNP + M, week 2	98.069 ± 5.023	98.077 ± 3.501	24.03 ± 1.38 ^{2,3} p ² = 0.00000 p ³ = 0.00000	86.933 ± 13.574	19.08 ± 1.38 ² p ² = 0.00001	20.83 ± 1.42 ^{2,3} p ² = 0.00006 p ³ = 0.00018
CNP + M, week 3	44.994 ± 3.833 ^{2,3} p ² = 0.00000 p ³ = 0.00000	89.653 ± 10.933	19.35 ± 1.09 ^{2,3} p ² = 0.00000 p ³ = 0.00207	103.412 ± 3.715	14.92 ± 1.38 ² p ² = 0.00000	21.65 ± 1.68 ² p ² = 0.00026

Note: C – intact group; CNP – group with the CNP model; M – group with the B16/F10 melanoma; CNP + M – group with the B16/F10 melanoma which was transplanted 3 weeks after the reproduction of the CNP model.

Statistically significant compared with the parameters: ¹ – in the intact group; ² – in the corresponding control group (CNP group); ³ – in the previous study period.

When studying Bcl-2 in the organ mitochondria of the intact mice, it was revealed that the skin mitochondria showed the minimum Bcl-2 level. The same parameter was 11.2 times higher in the heart mitochondria than in the skin mitochondria. The Bcl-2 levels in the mitochondria of the brain, liver, and kidneys were approximately equal and on average 16.9 times higher than in the skin mitochondria.

It was determined that CNP in female mice did not have a significant effect on the change in the Bcl-2 level in the liver and kidney mitochondria in contrast to the corresponding values in the organs of the intact animals (the C group). At the same time, CNP caused a 1.3-fold decrease in Bcl-2 level in the heart mitochondria. In the brain and skin mitochondria, on the contrary, there was a 1.3-fold ($p < 0.05$) and 5.9-fold increase, respectively.

After week 1 of melanoma growth (the M group), the mitochondria of the heart, brain, and skin demonstrated changes in the Bcl-2 level. In the heart mitochondria, the parameter decreased by 1.7 times ($p < 0.05$), while the brain and skin mitochondria showed a 1.3-fold ($p < 0.05$) and 8.9-fold increase compared with the values in the mitochondria of the corresponding organs in the intact animals (the C group). No statistically significant changes were found in the liver and kidney mitochondria. After 2 weeks of tumor growth, the brain mitochondria showed a 1.7-fold decrease ($p < 0.05$) in the Bcl-2 level in contrast to the corresponding parameter in the intact animals and a 2.2-fold decrease compared to the previous study period.

During this period of the study, a 1.7-fold decrease ($p < 0.05$) and a 3.2-fold decrease in the Bcl-2 level in the heart and skin mitochondria were registered in contrast to the corresponding values in the previous study period. No statistically significant changes in the liver and kidney mitochondria were observed. Af-

ter 3 weeks of melanoma growth, the Bcl-2 concentration in the brain mitochondria decreased by 1.5 times ($p < 0.05$) compared with the previous study period and was 2.6 times lower than in the intact mice. In this study period, the Bcl-2 level in the heart mitochondria did not differ from the values of the previous study period and was 2.9 times lower than the values in the intact animals. A drastic drop in the content of Bcl-2 compared with the previous study period was registered in the skin mitochondria after 3 weeks of tumor growth. The Bcl-2 level decreased by 11.5 times and became 4.1 times lower than that in the intact mice. The parameter in the liver and kidney mitochondria did not change.

In the melanoma mitochondria of this group, the Bcl-2 concentration during weeks 1, 2, and 3 was, respectively, 4.4 times, 5.1 times, and 2.5 times higher than in the skin mitochondria of the intact mice. After 1 week of melanoma growth, the mice with CNP (the CNP + M group) demonstrated changes in the Bcl-2 level in the heart mitochondria only – a 1.7-fold increase ($p < 0.05$) in contrast to the corresponding control value (the CNP group). During this study period, no changes in the Bcl-2 level were detected in the brain, liver, kidney, and skin mitochondria. After 2 weeks of melanoma growth in CNP, the Bcl-2 content became 1.9 times lower in the heart mitochondria and 1.7 times ($p < 0.05$) lower in the skin mitochondria than the corresponding values in the CNP group.

Compared with the previous study period, the heart mitochondria demonstrated a 3.4-fold decrease. After 3 weeks of melanoma growth in CNP, the Bcl-2 level decreased by 2.2 times in the brain mitochondria and by 1.24 times ($p < 0.05$) in the heart mitochondria compared with the previous study period. No changes were detected in the Bcl-2 level in the liver and kidney mitochondria. In the mitochondria of the melanoma growing in CNP, the Bcl-2 level after 1 week of tumor

development was 3 times lower than the one in the skin mitochondria of the control CNP mice, after 2 weeks and 3 weeks, it was on average 1.5 times lower than the control values.

Thus, it was shown that CNP, the independent growth of melanoma, and the growth of melanoma in CNP did not affect the Bcl-2 level in the liver and kidney mitochondria of the female mice. At the same time, significant changes were found in the Bcl-2 content in the mitochondria of the brain, heart, non-malignant skin, and melanoma.

DISCUSSION

The Bcl-2 family proteins determine sensitivity of cells to apoptosis, an ancient cell suicide program being essential for body development, tissue homeostasis, and immunity. Low apoptosis rates can lead to cancer and autoimmune diseases, while high apoptosis rates can exacerbate degenerative states in organs and even lead to neurodegeneration. The studies using transgenic mice have elucidated the functions of many Bcl-2 family proteins in both normal physiology and various pathological conditions. There is no doubt that these proteins control survival of all mammalian cells.

Some biochemical findings allow to suggest that members of the Bcl-2 family are also involved in non-apoptotic processes, including mitochondrial fission and fusion, as well as autophagy [15], but the physiological evidence remains limited. It was detected that proteins of the Bcl-2 family regulate permeability of the outer mitochondrial membrane, which allows for release of proapoptotic proteins, including cytochrome c, from the intermembrane space into the cytoplasm [16].

In traditional tumor transplantation, a decrease in the Bcl-2 level in the brain mitochondria was revealed after 2 weeks of tumor growth, and in the melanoma development in CNP – after 3 weeks only. In the heart mitochondria, in case of traditional tumor growth, no increase in the Bcl-2 level was registered throughout the study. On the contrary, its content in the heart mitochondria progressively decreased along with tumor growth progression. In melanoma growth in CNP, the Bcl-2 level increased 1 week after transplantation and then decreased along with tumor growth progression.

Perhaps, this fact can be considered as control over outer mitochondrial membrane permeability to regulate the level of calcium and activate mitochondria following stress caused by tumor growth, since it is the brain and heart that are the organs most susceptible to stress [17, 18]. It is assumed that along with changes in the

outer mitochondrial membrane permeability, Bcl-2 mediates stress signals [19]. It was revealed that various intracellular stress signals act through the Bcl-2 family proteins, causing effector caspase activation [20].

At the same time, no changes were found in the Bcl-2 level in the liver and kidney mitochondria. In the mitochondria of the skin not affected by the malignant process, during independent and CNP-induced melanoma progression, the Bcl-2 level had both common and distinctive features. Thus, at the initial and logarithmic stages of tumor development (weeks 1–2), the skin mitochondria in the independent tumor growth had an increased level of the studied parameter in contrast to the corresponding control. In the stimulated growth, on the contrary, the first period (week 1) did not show significant differences from the control values. Later (week 2), a decrease in the parameter was detected, compared with the corresponding control. And only week 3 (the terminal stage of tumor growth) was characterized by a decrease in the parameter, i.e. unidirectional changes, however, the Bcl-2 values were significantly lower in the standard variant of tumor growth.

In multicellular organisms, cell growth, division, and death are regulated by a variety of signaling pathways that integrate the state and functions of the cell. In healthy tissues, there is a balance between these processes, which allows for existence of tissue homeostasis [21]. The Bcl-2 protein family combines signals that trigger either cell survival or apoptosis. The balance between these processes is important for tissue development and homeostasis. Impaired apoptosis contributes to the development of several pathologies, including cancer. In addition to apoptotic and anti-apoptotic functions, members of the Bcl-2 family play a non-apoptotic role in the regulation of cell migration and invasion through various signaling pathways [22].

The Bcl-2 family controls the integrity of the outer mitochondrial membrane (OMM) and is functionally divided into anti- and pro-apoptotic proteins. Anti-apoptotic Bcl-2 proteins (e.g. Bcl-2/Bcl-xL/MCL-1) maintain the OMM integrity through direct binding of pro-apoptotic proteins that interact and form pores in OMM. The OMM regulation is complex due to many proteins and pathways converging in the Bcl-2 family. Moreover, there are specific patterns of expression and functionality depending on the type of cells and the state of differentiation [21].

In the mitochondria of a tumor growing independently or in CNP, slightly different progression

was noted than in the skin not affected by the malignant process. In the mitochondria of melanoma cells, which grew in a standard manner, the level of Bcl-2 throughout the entire study period was significantly higher than the parameters of the skin in the controls. On the contrary, in the mitochondria of the melanoma cells stimulated by CNP, this level was lower than in the corresponding controls. Thus, CNP promotes changes in the Bcl-2 storage capacity of mitochondria both in cells of the tumor-bearing organ and the tumor itself.

CONCLUSION

Analyzing the obtained results in general, it should be concluded that not only the mitochondria of melanoma cells respond to concomitant chronic neuropathic pain, but also the ones in the skin that is not affected by the malignant process, as it is the target organ for malignant growth. The detected differences in the Bcl-2 level confirm this fact.

We believe that different Bcl-2 level dynamics in the melanoma cell mitochondria, depending on the variant of tumor development, indicates the modulating effect of CNP and the ability to change the parameter level according to the growth phase. Among all the examined internal organs, the mitochondria of the brain and heart cells reacted most vividly to the standard and stimulated growth of the experimental melanoma. At the same time, it was revealed that the mitochondria of two organs, the liver and the kidneys, had certain Bcl-2 stability both in standard and stimulated tumor development.

REFERENCES

- Gilmore A., King L. Emerging approaches to target mitochondrial apoptosis in cancer cells. *F1000Research*. 2019; 8 (1000): 1793. DOI: 10.12688/f1000research.18872.1.
- Fulda S. Smac mimetics to therapeutically target IAP proteins in cancer. *Int. Rev. Cell Mol. Biol.* 2017; 330: 157–169. DOI: 10.1016/bs.ircmb.2016.09.004.
- Von Karstedt S., Montinaro A., Walczak H. Exploring the TRAILs less travelled: TRAIL in cancer biology and therapy. *Nat. Rev. Cancer*. 2017; 17 (6): 352–366. DOI: 10.1038/nrc.2017.28.
- Badrinath N., Yoo S.Y. Mitochondria in cancer: in the aspects of tumorigenesis and targeted therapy. *Carcinogenesis*. 2018; 39 (12): 1419–1430. DOI:10.1093/carcin/bgy148.
- Hanahan D., Weinberg R.A. The hallmarks of cancer. *Cell*. 2000; 100 (1): 57–70. DOI: 10.1016/S0092-8674(00)81683-9.
- Youle R.J., Strasser A. The BCL-2 protein family: opposing activities that mediate cell death. *Nat. Rev. Mol. Cell Biol.* 2008; 9 (1): 47–59. DOI: 10.1038/nrm2308.
- Vikström I.B., Slomp A., Carrington E.M., Moesbergen L.M., Chang C., Kelly G.L., Glaser S.P., Jansen J.H., Leusen J.H., Strasser A., Huang D.C., Lew A.M., Peperzak V., Tarlinton D.M. MCL-1 is required throughout B-cell development and its loss sensitizes specific B-cell subsets to inhibition of BCL-2 or BCL-XL. *Cell Death Dis.* 2016; 7 (8): 2345. DOI: 10.1038/cddis.2016.237.
- Grabow S., Kueh A.J., Ke F., Vanyai H.K., Sheikh B.N., Dengler M.A., Chiang W., Eccles S., Smyth I.M., Jones L.K., De Sauvage F.J., Scott M., Whitehead L., Voss A.K., Strasser F. Subtle changes in the levels of BCL-2 proteins cause severe craniofacial abnormalities. *Cell Rep.* 2018; 24 (12): 3285–3295. DOI: 10.1016/j.celrep.2018.08.048.
- Ankers J.M., Awais R., Jones N.A., Boyd J., Ryan S., Adamson A.D., Harper C.V., Bridge L., Spiller D.G., Jackson D.A., Paszek P., Sée V., White M.R. Dynamic NF-κB and E2F interactions control the priority and timing of inflammatory signalling and cell proliferation. *eLife*. 2016; 5: 10473. DOI: 10.7554/eLife.10473.
- Schellenberg B., Wang P., Keeble J.A., Rodriguez-Enriquez R., Walker S., Owens T.W., Foster F., Tanianis-Hughes J., Brennan K., Streuli C.H., Gilmore A.P. Bax exists in a dynamic equilibrium between the cytosol and mitochondria to control apoptotic priming. *Mol. Cell*. 2013; 49 (5): 959–971. DOI: 10.1016/j.molcel.2012.12.022.
- Flusberg D.A., Roux J., Spencer S.L., Sorger P.K. Cells surviving fractional killing by TRAIL exhibit transient but sustainable resistance and inflammatory phenotypes. *Mol. Biol. Cell*. 2013; 24 (14): 2186–2200. DOI: 10.1091/mbc.e12-10-0737.
- Gascoigne K.E., Taylor S.S. Cancer cells display profound intra- and interline variation following prolonged exposure to antimetabolic drugs. *Cancer Cell*. 2008; 14 (2): 111–122. DOI: 10.1016/j.ccr.2008.07.002.
- Kit O.I., Frantsiyants E.M., Kotieva I.M., et al. Some mechanisms of increasing malignancy of B16/F10 melanoma in female mice with chronic pain. *Russian Journal of Pain*. 2017; 2 (53): 14–20 (in Russ.).
- Egorova M.V., Afanasyev S.A. Isolation of mitochondria from cells and tissues of animals and human: Modern methodical approaches. *Siberian Medical Journal*. 2011; 26 (1-1): 22–28 (in Russ.).
- Czabotar P.E., Lessene G., Strasser A., Adams J.M. Control of apoptosis by the BCL-2 protein family: implications for physiology and therapy. *Nat. Rev. Mol. Cell Biol.* 2014; 15 (1): 49–63. DOI: 10.1038/nrm3722.
- Nakazawa M., Matsubara H., Matsushita Y., Watanabe M., Vo N., Yoshida H., Yamaguchi M., Kataoka T. The human Bcl-2 family member Bcl-rambo localizes to mitochondria and induces apoptosis and morphological aberrations in *Drosophila*. *PLoS One*. 2016; 11 (6): 0157823. DOI: 10.1371/journal.pone.0157823.
- Cenini G., Lloret A., Cascella R. Oxidative stress in neurodegenerative diseases: from a mitochondrial point of view. *Oxidative Medicine and Cellular Longevity*. 2019; 2019: 2105607. DOI: 10.1155/2019/2105607.
- Yin X., Xin H., Mao S., Wu G., Guo L. The role of autophagy in sepsis: protection and injury to organs. *Frontiers in Physiology*. 2019; (10): 1071. DOI: 10.3389/fphys.2019.01071.

19. Edlich F. BCL-2 proteins and apoptosis: Recent insights and unknowns. *Biochemical and Biophysical Research Communications*. 2018; 500 (1): 26–34. DOI: 10.1016/j.bbrc.2017.06.190.
20. Wang J., Feng W., Yuan Z., Weber J.D., Zhang Y. DHX33 Interacts with AP-2 β to regulate Bcl-2 gene expression and promote cancer cell survival. *Molecular and Cellular Biology*. 2019; 39 (17): 17–19. DOI: 10.1128/MCB.00017-19.
21. Elkholi R., Renault T.T., Serasinghe M.N., Chipuk J.E. Putting the pieces together: How is the mitochondrial pathway of apoptosis regulated in cancer and chemotherapy? *Cancer & Metabolism*. 2014; 2: 16. DOI: 10.1186/2049-3002-2-16.
22. Hartman M.L., Czyz M. BCL-w: apoptotic and non-apoptotic role in health and disease. *Cell Death & Disease*. 2020; 11 (4): 260. DOI: 10.1038/s41419-020-2417-0.

Authors contribution

Kit O.I. – final approval of the manuscript for publication. Frantsiyants E.M. – conception and design, analysis and interpretation of data, final approval of the manuscript for publication. Neskubina I.V., Nemashkalova L.A. – analysis and interpretation of data. Cheryarina N.D., Surikova E.I., Kaplieva I.V. – critical revision of the manuscript for important intellectual content. Shikhlyarova A.I. – substantiation of the manuscript.

Authors information

Kit Oleg I., Dr. Sci. (Med.), Professor, Corresponding Member of the Russian Academy of Medical Sciences, Director General of the NMRCO, Rostov-on-Don, Russian Federation. ORCID 0000-0003-3061-6108.

Frantsiyants Elena M., Dr. Sci. (Biology), Professor, Deputy Director General for Science, NMRCO, Rostov-on-Don, Russian Federation. ORCID 0000-0003-3618-6890.

Neskubina Irina V., Cand. Sci. (Biology), Senior Researcher, Laboratory of Study of Malignant Tumor Pathogenesis, NMRCO, Rostov-on-Don, Russian Federation. ORCID 0000-0002-7395-3086.

Cheryarina Natalia D., Laboratory Assistant, Laboratory of Study of Malignant Tumor Pathogenesis, NMRCO, Rostov-on-Don, Russian Federation. ORCID 0000-0002-3711-8155.

Shikhlyarova Alla I., Dr. Sci. (Biology), Professor, Senior Researcher, Laboratory of Study of Malignant Tumor Pathogenesis, NMRCO, Rostov-on-Don, Russian Federation. ORCID 0000-0003-2943-7655.

Surikova Ekaterina I., Cand. Sci. (Biology), Senior Researcher, Laboratory of Study of Malignant Tumor Pathogenesis, NMRCO, Rostov-on-Don, Russian Federation. ORCID 0000-0002-4318-7587.

Kaplieva Irina V., Dr. Sci. (Med.), Senior Researcher, Laboratory of Study of Malignant Tumor Pathogenesis, NMRCO, Rostov-on-Don, Russian Federation. ORCID 0000-0002-3972-2452.

Nemashkalova Lyidmila A., Researcher, Laboratory of Study of Malignant Tumor Pathogenesis, NMRCO, Rostov-on-Don, Russian Federation. ORCID 0000-0003-2713-8598.

(✉) **Surikova Ekaterina I.**, e-mail: sunsur2000@mail.ru

Received 19.06.2020

Accepted 28.12.2020

Neurovisualization features of brain anatomy in children with spastic cerebral palsy revealed by magnetic resonance tractography

Klyuev E.A.¹, Sheiko G.E.¹, Dunaev M.G.², Lobanova E.V.¹, Rasteryaeva M.V.¹, Sharabrin E.D.¹

¹ *Privolzhsky Research Medical University
10/1, Minin and Pozharsky Square, Nizhny Novgorod, 603005, Russian Federation*

² *“Medical and Diagnostic Center of the Dr. Berezin Medical Institute – Nizhny Novgorod” LLC (“MDC MIBS-NN” LLC)
34a, Nesterova Str., Nizhny Novgorod, 603005, Russian Federation*

ABSTRACT

Aim. To perform quantitative evaluation of the degree of white matter tract abnormalities in children with spastic cerebral palsy by magnetic resonance tractography to determine severity of the disease, as well as to carry out a dynamic assessment of treatment effectiveness.

Materials and methods. The study included 46 children (32 males, 14 females; average age 5.4 ± 1.1 years). The participants were divided into two groups. The experimental group consisted of 23 children with spastic cerebral palsy. The control group included 23 children without any neurological disorder. Examination of the brain was performed on the Siemens Essenza 1,5 T system (Siemens, Germany) and included magnetic resonance tractography to reconstruct the major white matter tracts. The number of fibers, average fractional anisotropy value, apparent diffusion coefficient, and coefficient of myelination of major white matter tracts in the brain were calculated and analyzed.

Results. We found a significant difference in the above-stated parameters between the groups. The experimental group showed a decrease in the absolute number of fibers at the central and posterior segments of the corpus callosum, corticospinal tracts, and left inferior longitudinal fasciculus. Besides, we detected a decrease in fractional anisotropy at 2–5 segments of the corpus callosum and right lateral corticospinal tract, an increase in the apparent diffusion coefficient at 2, 4, and 5 segments of the corpus callosum and left lateral corticospinal tract, and a decrease in the myelination coefficient in all the examined tracts, except for superior longitudinal fasciculus. We revealed a positive correlation between the intensity of the motor disturbance and the coefficient of myelination at the anterior corpus callosum and inferior longitudinal fasciculus.

Conclusion. Magnetic resonance tractography is an informative technique for unbiased evaluation of white matter tract anatomy, as well the level and degree of motor tract damage. The most useful characteristics of white matter tract anatomy are the absolute number of fibers in the tract, fractional anisotropy, and coefficient of myelination. Some of them correlated with the intensity of motor disturbance, so they can be regarded as potential predictors of rehabilitation potential.

Key words: cerebral palsy, diffusion tensor imaging, tractography, fractional anisotropy, coefficient of myelination, neuroimaging, motor disturbance.

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

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

Conformity with the principles of ethics. A written informed consent was obtained from parents of the children who participated in the study. The study was approved by the local Ethics Committee at Privolzhsky Research Medical University (Protocol No. 4 of 29.03.2017).

✉ Klyuev Evgeny A., e-mail: eugeneklyuev@yandex.ru

For citation: Klyuev E.A., Sheiko G.E., Dunaev M.G., Lobanova E.V., Rasteryaeva M.V., Sharabrin E.D. Neurovisualization features of brain anatomy in children with spastic cerebral palsy revealed by magnetic resonance tractography. *Bulletin of Siberian Medicine*. 2021; 20 (3): 54–61. <https://doi.org/10.20538/1682-0363-2021-3-54-61>.

Нейровизуализационные особенности строения головного мозга у детей с детским церебральным параличом, полученные методом магнитно-резонансной трактографии

Клюев Е.А.¹, Шейко Г.Е.¹, Дунаев М.Г.², Лобанова Е.В.¹, Растеряева М.В.¹, Шарабрин Е.Г.¹

*Приволжский исследовательский медицинский университет (ПИМУ)
Россия, 603950, г. Нижний Новгород, пл. Минина и Пожарского, 10/1*

² *ООО «Лечебно-диагностический центр Международного института биологических систем – Нижний Новгород» (ООО «ЛДЦ МИБС-НН»)
Россия, 603005, г. Нижний Новгород, ул. Нестерова, 34а*

РЕЗЮМЕ

Цель. Количественная оценка степени нарушений развития проводящих путей головного мозга у детей со спастическими формами детского церебрального паралича (ДЦП) методами магнитно-резонансной (МР) трактографии для определения тяжести заболевания, а также оценка динамики эффективности лечения.

Материалы и методы. Обследованы 46 детей 4–7 лет (средний возраст $(5,4 \pm 1,1)$ лет), из них 14 девочек (33%) и 32 мальчика (66%). Пациенты разделены на две группы. Исследуемую группу составили 23 пациента со спастическими формами ДЦП. В контрольную группу вошли 23 ребенка без неврологического дефицита. Исследование головного мозга проводилось на МР-томографе Siemens Essenza 1,5 Т (Siemens, Германия) и включало метод МР-трактографии. Были рассчитаны и обработаны: количество волокон, средний показатель фракционной анизотропии, коэффициент диффузии, коэффициент миелинизации основных проводящих путей головного мозга.

Результаты. Выявлена достоверная разница указанных выше показателей между пациентами исследуемой и контрольной групп. У детей с ДЦП отмечалось снижение абсолютного количества волокон в области центральных и заднего сегментов мозолистого тела, кортикоспинальных трактов и левого нижнего продольного пучка. Также определялось снижение показателя фракционной анизотропии волокон в области 2–5-го сегментов мозолистого тела, правого кортикоспинального тракта; повышение коэффициента диффузии в области 2, 4, 5-го сегментов и левого кортикоспинального тракта; снижение коэффициента миелинизации во всех исследуемых трактах, за исключением верхних продольных пучков. Выявлена положительная корреляция между выраженностью моторного дефицита и коэффициентом миелинизации в области переднего сегмента мозолистого тела и нижних продольных пучков.

Заключение. МР-трактография является информативным методом объективной оценки организации проводящих путей головного мозга, уровня и степени поражения моторных трактов. Наиболее информативными характеристиками организации проводящих путей являются абсолютное количество волокон в тракте, показатель фракционной анизотропии, а также расчетный показатель – коэффициент миелинизации. Некоторые из выявленных изменений коррелировали с выраженностью моторного дефицита, что позволяет рассматривать их как потенциальные предикторы реабилитационного прогноза.

Ключевые слова: диффузионно-тензорный имиджинг, трактография, фракционная анизотропия, коэффициент миелинизации, детский церебральный паралич, нейровизуализация, моторный дефицит.

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

Источник финансирования. Авторы заявляют об отсутствии финансирования.

Соответствие принципам этики. Исследование проводилось с обязательного письменного информированного согласия родителей. Исследование одобрено локальным этическим комитетом ФГБОУ ВО «ПИМУ» Минздрава России (протокол № 4 от 29.03.2017).

Для цитирования: Ключев Е.А., Шейко Г.Е., Дунаев М.Г., Лобанова Е.В., Растеряева М.В., Шарабрин Е.Г. Нейровизуализационные особенности строения головного мозга у детей с детским церебральным параличом, полученные методом магнитно-резонансной трактографии. *Бюллетень сибирской медицины*. 2021; 20 (3): 54–61. <https://doi.org/10.20538/1682-0363-2021-3-54-61>.

INTRODUCTION

Cerebral palsy (CP) is a neurological syndrome that includes a group of permanent disorders of motor development and posture retention [1]. The prevalence of this disease is quite high and, according to various authors, ranges from 2.2 to 3.3 cases per 1,000 newborns [2, 3]. Due to the severity of clinical presentation, early disability, and social maladaptation, timely diagnosis, treatment, and rehabilitation of patients with CP are of great social importance.

According to most authors, the diagnosis of CP is established on the basis of a combination of clinical and neuroimaging data, such as neurosonography (in the perinatal period) and magnetic resonance imaging [2, 4]. These basic techniques are used to assess the nature and localization of pathological changes in the brain, namely: white matter lesions in the form of periventricular leukomalacia (PVL), basal ganglia lesions, focal strokes, and various types of cerebral dysplasia [5]. Application of new MRI techniques, such as morphometry, tractography, spectroscopy, and functional MRI, has significantly increased the array of diagnostic information [6]. Diffusion tensor imaging (DTI) and magnetic resonance (MR) tractography have made it possible to accurately visualize the brain tracts that, according to some authors, are most often affected in patients with CP [7].

This technology is a mathematical three-dimensional reconstruction of tracts based on the direction of diffusion in each voxel of the brain. One of the main aspects of brain examination by the DTI method is to determine the value of fractional anisotropy (FA), i.e. the degree of co-directional movement of water molecules inside the white matter tracts. This parameter characterizes the degree of myelination of the brain tracts.

Based on the FA maps, a three-dimensional reconstruction of the white matter tracts is performed using software [8]. The reconstruction is estimated taking into account symmetry, the length of tracts, and direction. It allows to identify the predominant side of the dysfunction, even with symmetrical white matter lesion according to structural MRI [9].

The aim of the research was to study disorders in brain white matter tract anatomy by MR tractography in CP patients of preschool age.

MATERIALS AND METHODS

The study encompassed 46 children aged 4–7 years (average age (5.4 ± 1.1) years), including 14 girls (30%) and 32 boys (70%). The experimental group consisted of 23 patients with spastic forms of CP, including 7 girls and 16 boys (the average age 5.4 ± 1.1 years). The diagnosis of cerebral palsy was established in children aged 9 months to 2.5 years.

Spastic diplegia (G80.1 according to ICD-10) was observed in 13 (56%) children, hemiplegic form of the disease (G80.2) – in 10 (44%) children. The average level of motor disorders in the patients with CP according to the Global Motor Function Classification System (GMFCS) [10] was 1.83 ± 0.5 .

The control group consisted of 23 children: 7 girls (30%) and 16 boys (70%). The average age was 5.6 ± 1.3 years. The children were treated for somatic pathology at the departments of the clinic, and had no motor deficits and organic disease of the nervous system, and, therefore, no neurologic deficit was observed. Indications for MRI, according to the referral from the neurologist, were headaches (in 6 patients) and delayed speech development (in 17 patients).

All the patients of the experimental group (with CP) underwent a complete physical examination, as well as a specialized clinical and neurological examination, including an assessment of the condition according to neurological questionnaires and scales, such as the modified Ashworth spasticity scale, the Visual Analogue Scale (VAS), the scale for assessing gross motor functions (Gross Motor Function Measure 88 (GMFM-88)), and the Manual Ability Classification System (MACS) for children with CP.

MRI was performed on the Siemens Essenza 1.5 T system (Siemens, Germany), using an 8-channel surface coil for the head. Structural images of the brain were obtained in the standard T1, T2, and FLAIR sequences in three mutually perpendicular planes. For MR tractography, the T1-weighted MPR (multiplanar reconstruction) sequences in the sagittal plane and the diffusion tensor echo planar pulse sequence in the axial plane were used, the number of diffusion directions was 20, and the diffusion factors were 0.1000 sec/mm^2 .

The data obtained by DT-MRI were processed using the BrainEx 2.2.0 program (NordicNeuroLab, Norway). The software automatically applied motion correction and eddy currents. Echo planar images were correlated with structural ones to perform a three-dimensional reconstruction of the tracts by anatomical landmarks.

The major white matter tracts were identified: segments of the corpus callosum (CC) (5 segments) (CC 1–5), right lateral corticospinal tracts (CSTr) and left lateral corticospinal tracts (CSTl), superior longitudinal fasciculus left (SLFl) and right (SLFr) and inferior longitudinal fasciculus left (ILFl) and right (ILFr) – a total of 11 localizations. The reconstruction was carried out according to the method of deterministic tractography [10].

In order to reconstruct the segments of the corpus callosum, the area of interest was placed on the corresponding segment, based on the scheme proposed by Hofer S. and J. Frahm [11]; for lateral corticospinal tracts, the area of interest was determined in the projection of the precentral gyri, for the superior and inferior longitudinal fasciculi – in the frontal and parietal, temporal and occipital lobes, respectively. For the reconstruction of the white matter tracts, the threshold of the fractional anisotropy index was 0.15, and the minimum fiber length was 20 mm.

For each selected fasciculus, the following parameters were obtained: the number of fibers, the fractional anisotropy index, and the diffusion coefficient. Derivative parameters were also calculated, such as the percentage of the number of fibers in the corpus callosum segments, relative to the total number of fibers in it, the laterality index (for paired tract fibers), as well as the myelination coefficient, which is a product of the number of fibers in the studied tract and the average fractional anisotropy value in it.

Statistical analysis of the obtained quantitative parameters in the tracts was carried out using licensed IBM SPSS v21 software (IBM SPSS, USA). The average values for the independent samples were compared using the Student's *t*-test or the Mann – Whitney test, depending on the normality of the distribution of variables within the samples, to identify statistically significant differences between the groups. The Pearson's correlation coefficient was used to assess the distribution of variables within the group of patients depending on the clinical form of CP and the degree of motor disorders (GMFCS score, GMFM 88 score).

The study was performed at the University clinic of Privolzhsky Research Medical University in the period from 2017 for 2019. The study was approved by the local Ethics Committee at Privolzhsky Research Medical University (Protocol No. 4 of 29.03.2017) and carried out with an obligatory written informed consent obtained from the parents.

RESULTS AND DISCUSSION

According to the results of the MRI study, in the patients of group 1 (with CP), a symptom complex was revealed, including three types of structural changes. The first type included glial changes in the white matter of the brain, predominantly of periventricular localization. Such changes were found in 19 (83%) out of 23 patients, including: minimally pronounced zones of periventricular gliosis in 9 (40%) patients; moderately pronounced zones of periventricular gliosis extending to adjacent structures in 2 (8%) patients; severe glial cysts in 7 (30%) patients; extensive zones of glial cysts combined with atrophic changes in 1 (4%) patient. Porencephalic cysts were observed in 2 (8%) patients. The second type which included changes in the ventricles of the brain was detected in 16 (70%) patients with CP.

Lateral ventricular asymmetry associated with periventricular leukomalacia was found in 6 patients; moderate dilation of the lateral ventricles – in 10 patients, including dilation of the posterior horns – in 7 patients. Signs of glial changes in the white matter of the brain and pathology of the cerebral ventricles were combined in 16 (70%) patients. The third type of the detected changes includes abnormalities of white matter tract anatomy in the brain structures. In the course of post-processing, the main white matter tracts in the brain were reconstructed (Fig. 1). Children from the control group (Fig. 2) had fairly even distribution of fibers over the segments of the corpus callosum (Fig. 2, *b*), as well as a symmetric number of fibers in the corticospinal tracts and superior and inferior longitudinal fasciculi (Fig. 2, *a*).

The patients with CP demonstrated a decrease in the absolute number of fibers in all segments of the corpus callosum (except for the anterior part), corticospinal tracts, as well as in the left inferior longitudinal fasciculus. Fractional anisotropy was significantly decreased in the central and posterior segments of the corpus callosum, the right lateral corticospinal tract, and the left inferior longitudinal fasciculus. The diffusion coefficient was significantly increased in the group of

patients with CP only in 5 out of 11 localizations. From the calculated parameters, the myelination coefficient was significantly reduced in all the studied tracts, except for the superior longitudinal fasciculus.

The percentage of fibers (%) or the laterality index (for symmetric tracts) significantly differed in the three segments of the corpus callosum. The results of statistical processing are presented in the Table.

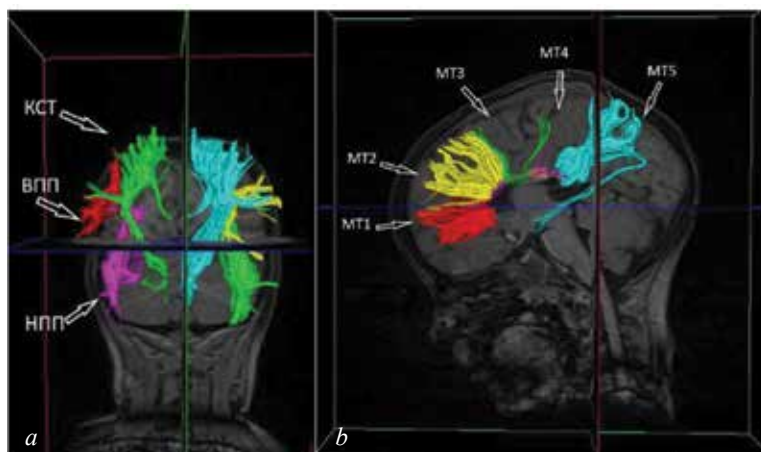


Fig.1. Typical changes in the major white matter tracts identified in the patients with CP: CST – corticospinal tract, SLF – superior longitudinal fasciculus; ILF – inferior longitudinal fasciculus; CC 1, 2, 3,4, 5 – segments of the corpus callosum (here and in Fig. 2). Visually, an asymmetric decrease in the number of fibers in the corticospinal tract and the superior longitudinal fasciculus on the side of the lesion (Fig. 1, *a*) and a decrease in the number of fibers in the central and posterior central segments of the corpus callosum were detected (Fig. 1, *b*).

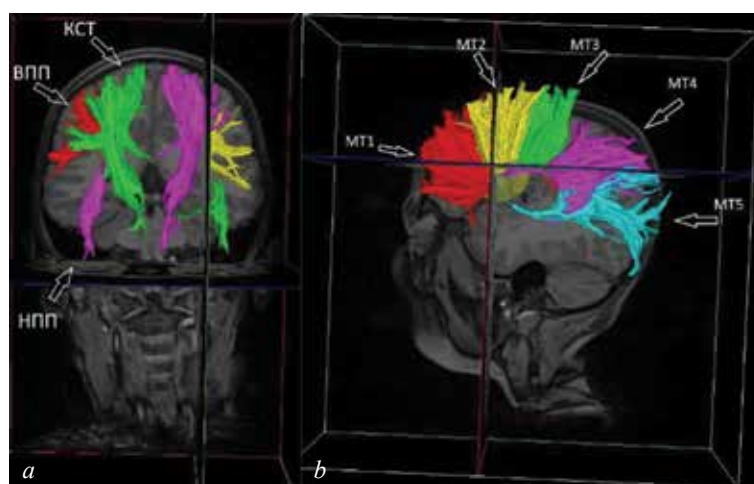


Fig. 2. Reconstructed major white matter tracts of the brain in the patients from the control group: *a* – fibers of the corticospinal tracts, superior and inferior longitudinal fasciculi; *b* – distribution of fibers in the segments of the corpus callosum

Table

Comparison of the characteristics of the white matter tracts between the studied groups.										
Tract	Number of fibers, pc.	<i>p</i>	Fiber percentage (%) / laterality index	<i>p</i>	Fractional anisotropy (FA)	<i>p</i>	Myelination coefficient	<i>p</i>	Apparent diffusion coefficient (ADC)	<i>p</i>
CC1	1,594.2 (148.3)	0.052 (M–W)	0.393 (0.023)	0.000 (St)	0.409 (0.009)	0.07 (M–W)	670.3 (69.8)	0.040 (M–W)	92.0 (1.6)	0.799 (St)
	2,028.4 (144.7)		0.280 (0.013)		0.432 (0.007)		886.5 (66.7)		91.4 (1.2)	

Table (continued)

Tract	Number of fibers, pc.	<i>p</i>	Fiber percentage (%) / laterality index	<i>p</i>	Fractional anisotropy (FA)	<i>p</i>	Myelination coefficient	<i>p</i>	Apparent diffusion coefficient (ADC)	<i>p</i>
CC2	729.4 (102.1)	0.024 (M–W)	0.164 (0.015)	0.258 (M–W)	0.385 (0.01)	0.047 (M–W)	292.1 (43.1)	0.017 (M–W)	101.9 (2.9)	0.036 (St)
	1,101.9 (115.4)		0.143 (0.009)		0.413 (0.007)		463.1 (50.5)		94.8 (1.6)	
CC3	440.9 (99.5)	0.014 (M–W)	0.088 (0.013)	0.362 (M–W)	0.368 (0.013)	0.036 (M–W)	173.2 (40.5)	0.016 (M–W)	112.6 (4.8)	0.052 (M–W)
	817.3 (124.9)		0.097 (0.011)		0.406 (0.009)		344.9 (54.7)		101.8 (2.4)	
CC4	228.7 (56.9)	0.000 (St)	0.041 (0.007)	0.000 (St)	0.332 (0.013)	0.00 (M–W)	86.5 (23.2)	0.000 (St)	129.4 (4.6)	0.000 (St)
	872.7 (140.6)		0.102 (0.012)		0.418 (0.012)		384.6 (66.5)		104.6 (2.8)	
CC5	1,408.3 (157.1)	0.000 (M–W)	0.311 (0.022)	0.027 (M–W)	0.411 (0.012)	0.00 (St)	609.1 (73.5)	0.000 (M–W)	112.1 (2.9)	0.000 (St)
	2,678.1 (159.1)		0.375 (0.015)		0.470 (0.006)		1268.6 (81.4)		97.9 (0.96)	
CSTl	1,559.5 (87.6)	0.000 (M–W)	-0.030 (0.859)¹	0.089 (M–W)	0.381 (0.011)	0.147 (M–W)	598.8 (40.4)	0.000 (M–W)	90.9 (3.4)	0.015 (St)
	2,314.3 (130.2)		0.008 (0.091) ¹		0.391 (0.006)		897.9 (45.9)		82.2 (0.6)	
CSTr	1,590.1 (112.2)	0.001 (M–W)	-0.030 (0.859)¹	0.089 (M–W)	0.379 (0.006)	0.039 (M–W)	608.8 (45.5)	0.000 (M–W)	87.9 (3.0)	0.052 (St)
	2,251.4 (131.5)		0.008 (0.091) ¹		0.400 (0.004)		899.5 (52.5)		81.6 (0.6)	
SLFl	382.1 (44.3)	0.448 (M–W)	0.570 (0.279)¹	0.102 (M–W)	0.320 (0.016)	0.209 (St)	131.2 (16.3)	0.489 (M–W)	79.5 (3.7)	0.582 (M–W)
	421.3 (38.9)		0.683 (0.158) ¹		0.343 (0.004)		144.8 (13.6)		81.6 (0.7)	
SLFr	432.7 (64.8)	0.083 (M–W)	0.570 (0.279)¹	0.102 (M–W)	0.346 (0.009)	0.059 (St)	153.6 (23.9)	0.077 (M–W)	82.0 (1.3)	0.422 (M–W)
	543.9 (54.9)		0.683 (0.158) ¹		0.359 (0.005)		196.5 (20.4)		80.8 (0.9)	
ILFl	455.3 (47.8)	0.002 (M–W)	-0.125 (0.400)¹	0.116 (M–W)	0.354 (0.017)	0.067 (M–W)	172.0 (18.8)	0.001 (M–W)	92.3 (4.3)	0.854 (M–W)
	685.8 (40.9)		-0.079 (0.289) ¹		0.390 (0.005)		268.3 (16.7)		93.1 (0.8)	
ILFr	463.8 (51.6)	0.073 (M–W)	-0.125 (0.400)¹	0.116 (M–W)	0.369 (0.007)	0.052 (M–W)	175.8 (21.3)	0.049 (M–W)	96.1 (1.2)	0.012 (St)
	585.8 (52.9)		-0.079 (0.289) ¹		0.390 (0.005)		229.1 (20.8)		92.3 (0.7)	

Note: italics indicate selected average values in the group of patients with CP; the value of the standard error of the mean is indicated in parentheses; (St) – Student's *t*-test was used, (M–W) – the Mann – Whitney test was used.

¹ indicates the median, the interquartile range is in parentheses.

Glial changes and changes in the cerebral ventricles have been well analyzed by many authors [5, 12, 13]. There is no consensus in the literature on the diagnostic significance of structural changes in the brain matter in patients with CP [14]. A few authors have identified and described changes in the corticospinal (motor) tracts, manifested both through a decrease in the number of fibers and a decrease in the fractional anisotropy index [15]. The authors associated changes in the characteristics of the corticospinal tracts, in particular, a decrease in FA, with motor impairments [6]. However, the degree of damage to the corticospinal tracts, established on the basis of a decrease in the fractional anisotropy index, does not always correspond to the severity of clinical manifestations in patients with CP [15]. No such correlation was found in this study either.

According to our data, abnormalities in the tract anatomy in the patients with CP were detected more often than described in the literature [15]. Changes in the corpus callosum (in all five segments) were observed in all patients with CP, regardless of the damage to other brain structures. Changes in the corpus

callosum in patients with CP were described in the literature. In particular, its delayed development was studied, which was manifested through thinning and reduction of its volume in patients with CP, compared with healthy peers [16].

Also, according to the results of a systematic review by L. Mailloux et al. (2020), separate studies were identified that demonstrate a decrease in fractional anisotropy in patients with CP, mainly in the central regions [6]; similar data were obtained in our study. The nature of changes in the corpus callosum has not been sufficiently studied yet, and their role in the pathogenesis of CP is unclear. However, their association with impaired motor function can be traced quite clearly, because it is the motor areas of the cortex that are connected by commissural tracts of the central corpus callosum.

Among the patients of the CP group, a relationship between MR tractography data and impaired motor function was studied. A moderate positive correlation was found (significant at $p < 0.05$) between the GMFM 88 score and the myelination coefficient in the anterior segment of the corpus callosum (Pearson's

correlation coefficient 0.51), as well as the myelination coefficient in the inferior longitudinal fasciculus (Pearson's correlation coefficient 0.46 in the right fasciculus and 0.47 in the left fasciculus). The myelination coefficient, in our opinion, more accurately characterized disturbances in the tract anatomy, since its calculation takes into account a mutual change in two indicators: the number of fibers and the fractional anisotropy index.

There is a group of patients with CP, where signs of brain changes are not determined by structural MRI, and minor and / or symmetrical white matter lesions cannot fully explain the clinical presentation of the disease [14]. In our MRI study, changes in the white matter were absent in 4 patients (17%) with clinical signs of CP. Abnormalities in the anatomy of the white matter tracts in the corpus callosum were found in all these patients, regardless of the state of the white matter and ventricles of the brain. Thus, in the absence of MRI signs of white matter lesions, MR tractography is the only objective method to confirm CP in a child.

CONCLUSION

MR tractography is an informative method for unbiased assessment of brain white matter tract anatomy and the level and degree of damage to the motor tracts. The most informative characteristics of the tract anatomy are the absolute number of fibers in the tract, the fractional anisotropy index, and the calculated coefficient of myelination. Among the CP patients of the experimental group, a relationship was revealed between the MR tractography data and impaired motor function. A method was developed for assessing the condition of the tracts by calculating the myelination coefficient.

REFERENCES

1. Appleton R.E., Gupta R. Cerebral palsy: not always what it seems. *Arch. Dis. Child.* 2019; 104 (8): 809–814. DOI: 10.1136/archdischild-2018-315633.
2. Michael-Asalu A., Taylor G., Campbell H., Lelea L.-L., Kirby R.S. Cerebral Palsy. *Advances in Pediatrics.* 2019; 66: 189–208. DOI: 10.1016/j.yapd.2019.04.002.
3. Stavsky M., Mor O., Mastrolia S.A., Greenbaum S., Than N.G. and Erez O. Cerebral palsy – trends in epidemiology and recent development in prenatal mechanisms of disease, treatment, and prevention. *Front. Pediatr.* 2017; 5: 21. DOI: 10.3389/fped.2017.00021.
4. Morgan C., Fahey M., Roy B., Novak I. Diagnosing cerebral palsy in full-term infants: cerebral palsy in infants. *Journal of Paediatrics and Child Health.* 2018; 54 (10): 1159–1164. DOI: 10.1111/jpc.14177.
5. Dan B., Mayston M., Paneth N., Rosenbloom L. Cerebral palsy. London; Somerset: Mac Keith Press John Wiley & Sons, Incorporated, 2015: 241–250.
6. Staudt M. Imaging cerebral palsy. In: Handbook of clinical neurology. Elsevier; 2013: 177–181.
7. Maillieux L., Franki I., Emsell L., Peedima M.-L., Fehrenbach A., Feys H., Ortibus E. The relationship between neuroimaging and motor outcome in children with cerebral palsy: A systematic review – Part B diffusion imaging and tractography. *Research in Developmental Disabilities.* 2020 Feb.; 97: 103569. DOI: 10.1016/j.ridd.2019.103569.
8. Ganepola T., Nagy Z., Ghosh A., Papadopoulos T., Alexander D.C., Sereno M.I. Using diffusion MRI to discriminate areas of cortical grey matter. *NeuroImage.* 2018; 182: 456–468. DOI: 10.1016/j.neuroimage.2017.12.046.
9. Cho H.K., Jang S.H., Lee E., Kim S.Y., Kim S., Kwon Y.H., Son S.M. Diffusion tensor imaging –demonstrated differences between hemiplegic and diplegic cerebral palsy with symmetric periventricular leukomalacia. *AJNR Am. J. Neuroradiol.* 2013; 34 (3): 650–654. DOI: 10.3174/ajnr.A3272.
10. Palisano R., Rosenbaum P., Walter S., Russell D., Wood E., Galuppi B. Development and reliability of a system to classify grossmotor function in children with cerebral palsy. *Dev. Med. Child Neurol.* 1997; 39 (4): 214–223 DOI: 10.1111/j.1469-8749.1997.tb07414.x.
11. Hofer S., Frahm J. Topography of the human corpus callosum revisited – comprehensive fiber tractography using diffusion tensor magnetic resonance imaging. *NeuroImage.* 2006; 32 (3): 989–994. DOI: 10.1016/j.neuroimage.2006.05.044.
12. Basser P.J., Pajevic S., Pierpaoli C., Duda J., Aldroubi A. In vivo fiber tractography using DT-MRI data. *Magn. Reson. Med.* 2000; 44 (4): 625–632. DOI: 10.1002/1522-2594(200010)44:4<625::aid-mrm17>3.0.co;2-o.
13. Johnston M.V., Hoon A.H. Cerebral palsy. *NMM.* 2006; 8 (4): 435–450. DOI: 10.1385/NMM:8:4:435.
14. Franki I., Maillieux L., Emsell L., Peedima M.-L., Fehrenbach A., Feys H., Ortibus E. The relationship between neuroimaging and motor outcome in children with cerebral palsy: A systematic review – Part A. Structural imaging. *Research in Developmental Disabilities.* 2020 May; 100: 103606. DOI: 10.1016/j.ridd.2020.103606.
15. Nemanich S.T., Mueller B.A., Gillick B.T. Neurite orientation dispersion and density imaging quantifies corticospinal tract microstructural organization in children with unilateral cerebral palsy. *Hum. Brain Mapp.* 2019; 40 (17): 4888–4900. DOI:10.1002/hbm.24744.
16. Zykin P.A., Yalifimov A.N., Aleksandrov T.A., Krasnoshchekova E.I., Tkachenko L.A., Sereda V.M., Nasyrov R.A. Features of the development of the corpus callosum of the brain of children according to MRI data. *Pediatrician.* 2018; 9 (1): 37–48 (in Russ.). DOI: 10.17816/PED9137-48.

Authors contribution

Klyuev E.A. – conception and design, collection and analysis of material, statistical interpretation of research results, drafting of the manuscript. Sheiko G.E. – collection and analysis of material, drafting of the manuscript, substantiation of the manuscript. Dunaev G.E. – collection and analysis of material, drafting of the manuscript. Lobanova E.V., Rasteryaeva M.V. – critical revision of the manuscript for important intellectual content. Sharabrin G.E. – conception and design, drafting of the manuscript, substantiation of the manuscript, final approval of the manuscript for publication, responsibility for the integrity of all parts of the article.

Authors information

Klyuev Evgeniy A., Post-Graduate Student, Department of Radiation Diagnostics, Privolzhsky Research Medical University, Nizhny Novgorod, Russian Federation. ORCID 0000-0003-2069-1710.

Sheiko Gennady E., Cand. Sci. (Med.), Assistant, Department of Medical Rehabilitation, Privolzhsky Research Medical University, Nizhny Novgorod, Russian Federation. ORCID 0000-0003-0402-7430.

Dunaev Maksim G., Radiologist, MDC MIBS-NN LLC, Nizhny Novgorod, Russian Federation. ORCID 0000-0003-4172-7444.

Lobanova Elena V., Radiologist, University clinic, Privolzhsky Research Medical University, Nizhny Novgorod, Russian Federation. ORCID 0000-0002-9429-5350.

Rasteryaeva Marina V., Cand. Sci. (Med.), Head of the Department of Radiology, University Clinic, Privolzhsky Research Medical University, Nizhny Novgorod, Russian Federation. ORCID 0000-0002-8462-3824.

Sharabrin Evgeniy G., Dr. Sci. (Med.), Professor, Department of Radiation Diagnostics, Privolzhsky Research Medical University, Nizhny Novgorod, Russian Federation. ORCID 0000-0001-5326-7233.

(✉) **Klyuev Evgeny Aleksandrovich**, e-mail: eugeneklyuev@yandex.ru

Received 01.06.2020

Accepted 28.12.2020

The role of sTNFSF14 in the liver mitochondrial dynamics in obese patients

Komar A.A.¹, Skuratovskaia D.A.¹, Vulf M.A.¹, Vu H.Q.¹, Darinskas A.², Gazatova N.D.¹,
Tudosenko N.M.¹, Zatulokin P.A.¹, Kirienkova E.V.¹, Litvinova L.S.¹

¹ Immanuel Kant Baltic Federal University (IKBFU)
14, A. Nevskogo Str., Kaliningrad, 236016, Russian Federation

² National Cancer Institute, Vilnius University
3, Universiteto Str., Vilnius, 01513, Republic of Lithuania

ABSTRACT

Background. The pathogenesis of nonalcoholic fatty liver disease (NAFLD), which develops in obesity and type 2 diabetes mellitus (T2DM), is associated with the effects of inflammatory factors on the liver parenchyma and liver mitochondrial dysfunction.

Aim. To determine the role of sTNFSF14 in the regulation of liver mitochondrial biogenesis in obese patients with and without T2DM.

Materials and methods. The study included 263 obese patients with and without T2DM and 42 apparently healthy donors. Quantitative determination of cytokines in the blood plasma was performed by fluorescence flow cytometry. The level of relative gene expression in the liver biopsy samples was investigated by real-time PCR. Semi-quantitative determination of proteins in the liver biopsy samples was studied by western blotting.

Results. The study showed that the levels of sTNFSF14, interleukin (IL)-10, gp130 / sIL-6Rb, and sIL-6Ra in the blood plasma of the obese patients without T2DM significantly exceeded the similar values in the control patients and obese patients with T2DM. In the liver biopsy samples of the obese patients with T2DM and a body mass index (BMI) > 40 kg / m², the expression level of the dynamin-1-like protein (DRP1 / DNMI1) gene was lower than in the control group, and the expression level of the mitofusin 2 (MFN2) gene tended to be higher. Compared with the control group, an increase in the expression level of the NADH-ubiquinone oxidoreductase chain 4 (MT-ND4) gene was recorded in the liver of all the obese patients. The patients with obesity showed a decrease in the amount of mitochondrial DNA (mtDNA) compared with the control group.

Conclusion. Thus, sTNFSF14, interacting with IL-10 and gp130 / sIL-6Rb in the circulation, positively effects the liver in the obese patients without T2DM. A low level of sTNFSF14 in the blood plasma of the obese patients with T2DM results in decreased mitochondrial division and increased cellular respiration.

Key words: obesity, type 2 diabetes mellitus, sTNFSF14, IL-6, gp130 / sIL-6Rb, sIL-6Ra.

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

Source of financing. The research was supported by the Russian Foundation for Basic Research and Kaliningrad regional government (No. 19-415-393004-r_mol_a, 19-44-390005-r_a); the study was carried out as part of the state assignment in the field of scientific activity (No. FZWM-2020-0010) under the state support of the leading scientific schools of the Russian Federation (No. 2495.2020.7).

Conformity with the principles of ethics. All patients signed an informed consent to participate in the study. The study was approved by the local Ethics Committee at IKBFU (Protocol No. 2 of 06.03.2017).

For citation: Komar A.A., Skuratovskaia D.A., Vulf M.A., Vu H.Q., Darinskas A., Gazatova N.D., Tudosenko N.M., Zatulokin P.A., Kirienkova E.V., Litvinova L.S. The role of sTNFSF14 in the liver mitochondrial dynamics in obese patients. *Bulletin of Siberian Medicine*. 2021; 20 (3): 62–71. <https://doi.org/10.20538/1682-0363-2021-3-62-71>.

Влияние sTNFSF14 на митохондриальную динамику в печени у пациентов с ожирением

Комар А.А.¹, Скуратовская Д.А.¹, Вульф М.А.¹, Ву Х.К.¹, Даринская А.² Газатова Н.Д.¹, Тодосенко Н.М.¹, Затолокин П.А.¹, Кириенкова Е.В.¹, Литвинова Л.С.¹

¹ Балтийский федеральный университет (БФУ) им. И. Канта
Россия, 236016, г. Калининград, ул. А. Невского, 14

² Институт онкологии, Вильнюсский университет
Литовская республика, 01513, г. Вильнюс, ул. Университето, 3

РЕЗЮМЕ

Актуальность. Патогенез неалкогольной жировой болезни печени, формирующейся при ожирении и сахарном диабете (СД) 2-го типа, опосредован воздействием многочисленных воспалительных факторов на паренхиму этого органа, а также развивающейся митохондриальной дисфункцией гепатоцитов.

Цель – определение роли растворимой формы sTNFSF14 в регуляции биогенеза митохондрий в печени у больных ожирением с сахарным диабетом СД 2-го типа и без него.

Материалы и методы. В исследование включены 263 больных ожирением с СД 2-го типа и без него и 42 условно здоровых донора. Количественное определение цитокинов в плазме крови проводили методом проточной флуориметрии. Уровень относительной экспрессии генов в биоптатах печени исследовали методом полимеразной цепной реакции в реальном времени. Полуколичественное определение белков в биоптатах печени проведено методом иммуноблоттинга.

Результаты. Показано, что уровни sTNFSF14, интерлейкина (IL) 10, gp130/sIL-6Rb и sIL-6Ra в плазме крови у больных ожирением без СД 2-го типа значительно превышали аналогичные значения контроля и больных ожирением с СД 2-го типа. В биоптатах печени, полученных у больных ожирением с СД 2-го типа с индексом массы тела более 40 кг/м², уровень экспрессии гена белка, подобного динамину 1 (*DRP1/DNM1L*), был ниже в сравнении с группой контроля, а уровень экспрессии гена митофузина 2 (*MFN2*) имел тенденцию к увеличению. В печени у всех больных ожирением регистрировалось повышение (в сравнении с контролем) уровня экспрессии белка НАДН-убихинона оксидоредуктазы цепи 4 (MT-ND4) и, напротив, снижение количества митохондриальной ДНК (мтДНК).

Заключение. Таким образом, sTNFSF14, взаимодействуя с IL-10 и gp130/sIL-6Rb в циркуляции, оказывает положительное воздействие на печень у больных ожирением без СД 2-го типа. Низкий уровень sTNFSF14 в плазме крови, регистрируемый у больных ожирением с СД 2-го типа, приводит к снижению деления митохондрий и увеличению клеточного дыхания у этой категории больных.

Ключевые слова: ожирение, СД 2-го типа, sTNFSF14, IL-6, gp130/sIL-6Rb, sIL-6Ra.

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

Источник финансирования. Исследование выполнено при поддержке РФФИ и Правительства Калининградской области (№ 19- 415-393004-г_mol_a, 19-44-390005- г_a); в рамках Государственного задания в области научной деятельности (№ FZWM-2020-0010); при государственной поддержке ведущих научных школ Российской Федерации (№ 2495.2020.7).

Соответствие принципам этики. Все пациенты подписали информированное согласие на участие в исследовании. Исследование одобрено локальным этическим комитетом БФУ им. И. Канта (протокол № 2 от 06.03.2017).

Для цитирования: Комар А.А., Скуратовская Д.А., Вульф М.А., Ву Х.К., Даринская А., Газатова Н.Д., Тодосенко Н.М., Затолокин П.А., Кириенкова Е.В., Литвинова Л.С. Влияние sTNFSF14 на митохондриальную динамику в печени у пациентов с ожирением. *Бюллетень сибирской медицины*. 2021; 20 (3): 62–71. <https://doi.org/10.20538/1682-0363-2021-3-62-71>.

INTRODUCTION

Obesity is a multifactorial disease that affects more than one third of the world population [1]. Obesity significantly increases the risk of developing chronic diseases such as type 2 diabetes mellitus (T2DM), cardiovascular diseases (atherosclerosis, hypertension), nonalcoholic fatty liver disease (NAFLD), and some types of cancer [1]. The complications of obesity often lead to disability and death. In 2017, 8% of deaths worldwide were associated with obesity [1]. NAFLD is one of the most common complications of obesity and occupies the leading position in hepatology [2, 3].

Changes in the function and structure of mitochondria are a hallmark in NAFLD formation. The hyperactive tricarboxylic acid cycle overloads the electron transport chain (ETC) in hepatocyte mitochondria, which intensifies production of reactive oxygen species (ROS) [4]. The experimental models of NAFLD (with choline deficiency) showed that mitochondrial compensatory mechanisms were activated at early stages of the disease. However, these reserves become depleted over time [5]. Despite the relevance of the problem and many works on the etiological factors and aspects of the NAFLD pathogenesis, protective factors in the liver steatosis and inflammation in obesity are still poorly studied and understood.

Tumor necrosis factor ligand superfamily member (TNFSF) 14 (also known as LIGHT) has a beneficial effect in treatment of various diseases [6, 7]. For instance, high level of sTNFSF14 in the circulation promotes formation of antitumor immunity in colon [6] and liver cancer [7].

Currently, the role of TNFSF14 in the development of metabolic syndrome is being actively discussed. Studies by J. Bassols et al. (2010) demonstrated a high level of TNFSF14 in the plasma of obese patients compared with healthy controls [8].

The *Tnfsf14* $-/-$ mice receiving a diet high in fat develop obesity, glucose intolerance, and impaired insulin sensitivity [9]. Besides, TNFSF14 deficiency in the experimental animals led to dysregulation of mitochondrial respiration in the liver, which contributed to increased oxygen consumption by the respiratory ETC in the mitochondria [9]. Given that the mitochondria play an important role in NAFLD development in obesity [4], changes in the mitochondrial dynamics (fission and fusion) and biogenesis may significantly contribute to the progression of steatosis in obese patients.

Thus, the study was aimed at determining the role of sTNFSF14 in the regulation of mitochondrial biogenesis in the liver of obese patients with and without T2DM.

MATERIALS AND METHODS

The study included 263 patients suffering from alimentary-constitutional obesity with abdominal localization hypertrophic in morphology. Among them, there were 44 obese patients without T2DM with a body mass index (BMI) $< 40 \text{ kg} / \text{m}^2$ (group 2), 88 obese patients without T2DM with a BMI $> 40 \text{ kg} / \text{m}^2$ (group 3). Groups 4 and 5 included obese patients with T2DM – 42 patients with a BMI $< 40 \text{ kg} / \text{m}^2$ and 89 patients with a BMI $> 40 \text{ kg} / \text{m}^2$, respectively (Table).

Table

The main clinical parameters of the studied groups					
Parameters	Group 1	Group 2	Group 3	Group 4	Group 5
Males / Females	17/25	11/33	18/70	18/24	21/68
Age, years, M $\pm$ SD	38.69 $\pm$ 7.57	41.59 $\pm$ 8.72	44.19 $\pm$ 9.11	45.1 $\pm$ 9.13	48.47 $\pm$ 8.17
BMI, kg / m^2 , M $\pm$ SD	23.11 $\pm$ 3.33	35.69 $\pm$ 2.62	48.38 $\pm$ 7.31	36.1 $\pm$ 2.73	49.53 $\pm$ 7.59

Plasma factors (TNFSF14 / LIGHT, IL-6, gp130 / sIL-6Rb, sIL6-Ra, and IL-10) were evaluated in the venous blood taken in the fasted state in the morning into vacuum tubes Vacuette (Greiner Bio-One, Austria) with EDTA (BD Vacutainer, Russia). The expression levels of gene mRNA, absolute mitochondrial DNA copy number (mtDNA-CN), and protein production levels were analyzed in the liver biopsy samples (1 ml each) obtained during elective laparoscopic surgeries.

According to the data that we published earlier, the histological analysis revealed steatosis in 72.7% of the obese patients without T2DM and in 93.3% of the obese patients with T2DM. Morphological manifestations of steatohepatitis were found in 63.6% of the patients without T2DM and in 86.6% of the patients with T2DM [10].

The control group (group 1) consisted of 42 apparently healthy donors with normal anthropometric and biochemical parameters. The main clinical character-

istics of the studied groups are presented in the table. These groups were comparable in terms of age and gender characteristics.

The quantitative determination of TNFSF14 / LIGHT, IL-6, gp130 / sIL-6Rb, sIL6-Ra, and IL-10 in the blood plasma was carried out by fluorescence flow cytometry using commercial test systems (Bio-Plex Pro Human Inflammation Panel 1, 37-Plex, Bio-Rad, USA) on a dual-laser automated analyzer (Bio-Plex 200 Systems, Bio-Rad, USA) and Bio-PlexManager software (Bio-Rad, USA).

Isolation of total RNA from the liver biopsy samples was carried out using the ExtractRNA reagent (Evrogen, Russia). Isolation of mitochondria for the analysis of the NADH-ubiquinone oxidoreductase chain 4 (MT-ND4) protein gene was carried out in a buffer containing sucrose and was followed by centrifugation. To determine the relative gene expression level of dynamin-1-like protein (DRP1 / DNMI1L), mitofusin 2 (MFN2), and MT-ND4, quantitative PCR was performed. To do that, qPCR mix-HS reagents (Evrogen, Russia) and a CFX96 detection system (Bio-Rad, USA) were used.

Isolation of protein molecules from the liver biopsy samples was carried out using the RIPA buffer (RIPA Buffer, Thermo Fisher Scientific, USA), followed by measuring the protein concentration with the help of the Bradford protein assay (BCA Protein Assay Kit, Thermo Fisher Scientific, USA). To confirm the gene expression results, semi-quantitative analysis of protein production in the liver biopsy samples was performed by western blotting. It was carried out using specific monoclonal antibodies: DNMI1L (Invitrogen, USA), MFN2 (Invitrogen, USA), GAPDH (Thermo Fisher Scientific, USA) and blotting systems (Mini-PROTEAN Tetra Systems, Trans-Blot Turbo, Bio-Rad, USA). The target proteins were detected by a ChemiDoc MP Imaging System (Bio-Rad, USA).

To better investigate the mitochondrial biogenesis in the liver samples, the analysis of the absolute mitochondrial DNA copy number (mtDNA-CN) was carried out. The Droplet Digital PCR (ddPCR) method and a QX200 Droplet Digital PCR System (Bio-Rad, USA) were used.

The data were analyzed for normal distribution using the Kolmogorov – Smirnov test. For normal distribution, the differences were assessed using the Student's t-test (two groups, a parametric test). For non-normal distribution, the Mann – Whitney test was used (two groups, a non-parametric test). Quantitative variables were presented as the mean and standard de-

viation ($M \pm SD$) and the median and the interquartile range $Me (Q_{25} - Q_{75})$. The relationship between the two variables was analyzed using the Spearman's correlation coefficient. The results were considered significant at $p < 0.05$.

RESULTS

The level of pro- and anti-inflammatory cytokines in the blood plasma. In the obese patients without T2DM with a BMI $> 40 \text{ kg/m}^2$, the values of sLIGHT / TNFSF14 in the blood plasma significantly exceeded the same parameters in the control and obese patients without T2DM with a BMI $< 40 \text{ kg/m}^2$ ($p = 0.0408$), obese patients with T2DM with a BMI $< 40 \text{ kg/m}^2$ ($p = 0.0017$), and obese patients with T2DM with a BMI $> 40 \text{ kg/m}^2$ ($p < 0.0001$). The level of sLIGHT / TNFSF14 in the blood plasma was lower in the obese patients with T2DM with a BMI $> 40 \text{ kg/m}^2$ than in the controls ($p = 0.0003$) and in the group of the obese patients without T2DM with a BMI $< 40 \text{ kg/m}^2$ ($p < 0.0001$) (Fig. 1, a).

In the obese patients with T2DM with a BMI $> 40 \text{ kg/m}^2$, the level of IL-10 in the blood plasma was significantly higher than in the controls ($p = 0.0002$) and lower than in the obese patients without T2DM with a BMI $< 40 \text{ kg/m}^2$ ($p < 0.0001$) and a BMI $> 40 \text{ kg/m}^2$ ($p < 0.0001$). In the obese patients with T2DM with a BMI $< 40 \text{ kg/m}^2$, the level of IL-10 in the plasma was significantly lower in comparison with the obese patients without carbohydrate metabolism disorders with a BMI $< 40 \text{ kg/m}^2$ ($p = 0.0019$) and with a BMI $> 40 \text{ kg/m}^2$ ($p = 0.0003$). The plasma levels of IL-10 in both groups of the obese patients without T2DM significantly exceeded those in the control group ($p < 0.0001$) (Fig. 1, b).

In all the studied groups, the plasma levels of IL-6 were higher than in the control group ($p < 0.05$). In the obese patients with T2DM with a BMI $> 40 \text{ kg/m}^2$, the levels of IL-6 in the blood plasma decreased significantly, compared with the group of the obese patients without carbohydrate metabolism disorders with a BMI $< 40 \text{ kg/m}^2$ ($p = 0.0416$) (Fig. 1, c).

The plasma values of gp130 / sIL-6Rb in all the studied groups differed from the control group significantly. Thus, in both groups of the obese patients without T2DM, the level of gp130 / sIL-6Rb in the plasma was remarkably higher than in the control group ($p = 0.0079$ and $p < 0.0001$, respectively). Both groups of the obese patients with T2DM showed lower plasma levels of gp130 / sIL-6Rb in contrast to the control group ($p < 0.0001$) (Fig. 1, d).

The groups with and without T2DM demonstrated different gp130 / sIL-6Rb levels. Plasma gp130 / sIL-6Rb levels were significantly lower in the obese patients with T2DM with a BMI < 40 kg / m² than in the obese patients without T2DM ($p < 0.0001$). Similar differences were found in the obese patients with T2DM with a BMI > 40 kg / m² in contrast to the obese patients without T2DM ($p < 0.0001$) (Fig. 1, d).

In the obese patients without T2DM with a BMI < 40 kg / m² and a BMI > 40 kg / m², the plasma sIL-6Ra level exceeded similar values in the control group

($p = 0.0026$ and $p = 0.0002$, respectively). However, the obese patients with T2DM with a BMI > 40 kg / m² had lower sIL-6Ra level than the control group ($p = 0.0024$) as well as both groups of the obese patients without T2DM with a BMI < 40 kg / m² ($p < 0.0001$) and with a BMI > 40 kg / m² ($p < 0.0001$). In the obese patients with T2DM with a BMI < 40 kg / m², the plasma sIL-6Ra levels were significantly lower than in the obese patients without T2DM with a BMI < 40 kg / m² ($p = 0.0005$) and with a BMI > 40 kg / m² ($p < 0.0001$) (Fig. 1, e).

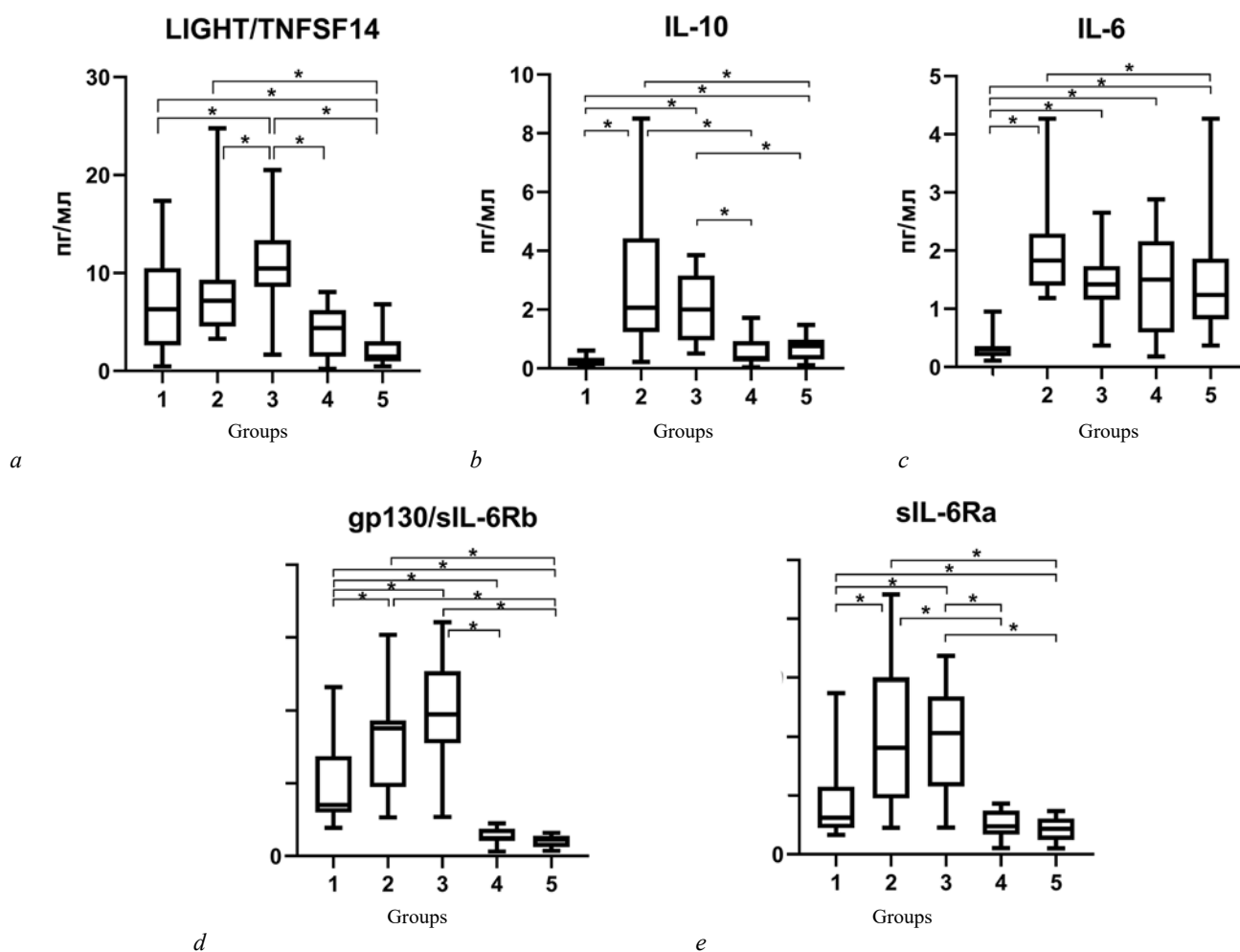


Fig. 1. Changes in the levels of pro- and anti-inflammatory cytokines in the blood plasma. 1 – control group; group of the obese patients: 2 – without T2DM with a BMI < 40 kg / m²; 3 – without T2DM with a BMI > 40 kg / m²; 4 – with T2DM with a BMI < 40 kg / m²; 5 – with T2DM with a BMI > 40 kg / m². The level in the blood plasma: a – of sLIGHT / TNFSF14; b – IL-6; c – gp130 / sIL-6Rb; d – sIL-6Ra; e – IL-10. Here and in Fig. 2: * significant differences between the groups ($p < 0.05$)

Production of components of mitochondrial dynamics and biogenesis in the liver biopsy samples. In the obese patients with T2DM with a BMI > 40 kg / m², a decrease in the DRP1 gene expression in the liver was noted, compared with the controls ($p = 0.04$)

(Fig. 2, a). The relative amount of staining of the DN-M1L protein was measured using densitometry and was equal to 0.47 relative units (r.u.) in the control group; in the obese patients without T2DM, it was 0.2 r.u., in the obese patients with T2DM – 0.14 r.u.

The groups demonstrated no significant differences in the MFN2 gene expression in the liver biopsy samples. Despite this fact, the MFN2 gene expression tended to be higher in the liver biopsy samples of the obese patients with and without T2DM with a BMI < 40 kg / m² than in the controls (Fig. 2, *c*). The relative amount of staining of the MFN2 protein in the control group was 0.18 r.u.; in the obese patients without T2DM – 0.23 r.u.; in the obese patients with T2DM – 0.35 r.u. (Fig. 2, *d*).

The MT-ND4 gene expression in the liver biopsy samples in all the groups significantly exceeded similar control parameters ($p = 0.0094$; $p < 0.0001$; $p = 0.0001$; $p = 0.0023$) (Fig. 2, *e*). The amounts of mtDNA in the liver biopsy samples of the obese patients without carbohydrate metabolism disorders with a BMI > 40 kg / m² was significantly lower than in the control group and in the obese patients with T2DM with a BMI < 40 kg / m² ($p = 0.0045$) and with a BMI > 40 kg / m² ($p = 0.0007$) (Fig. 2, *f*).

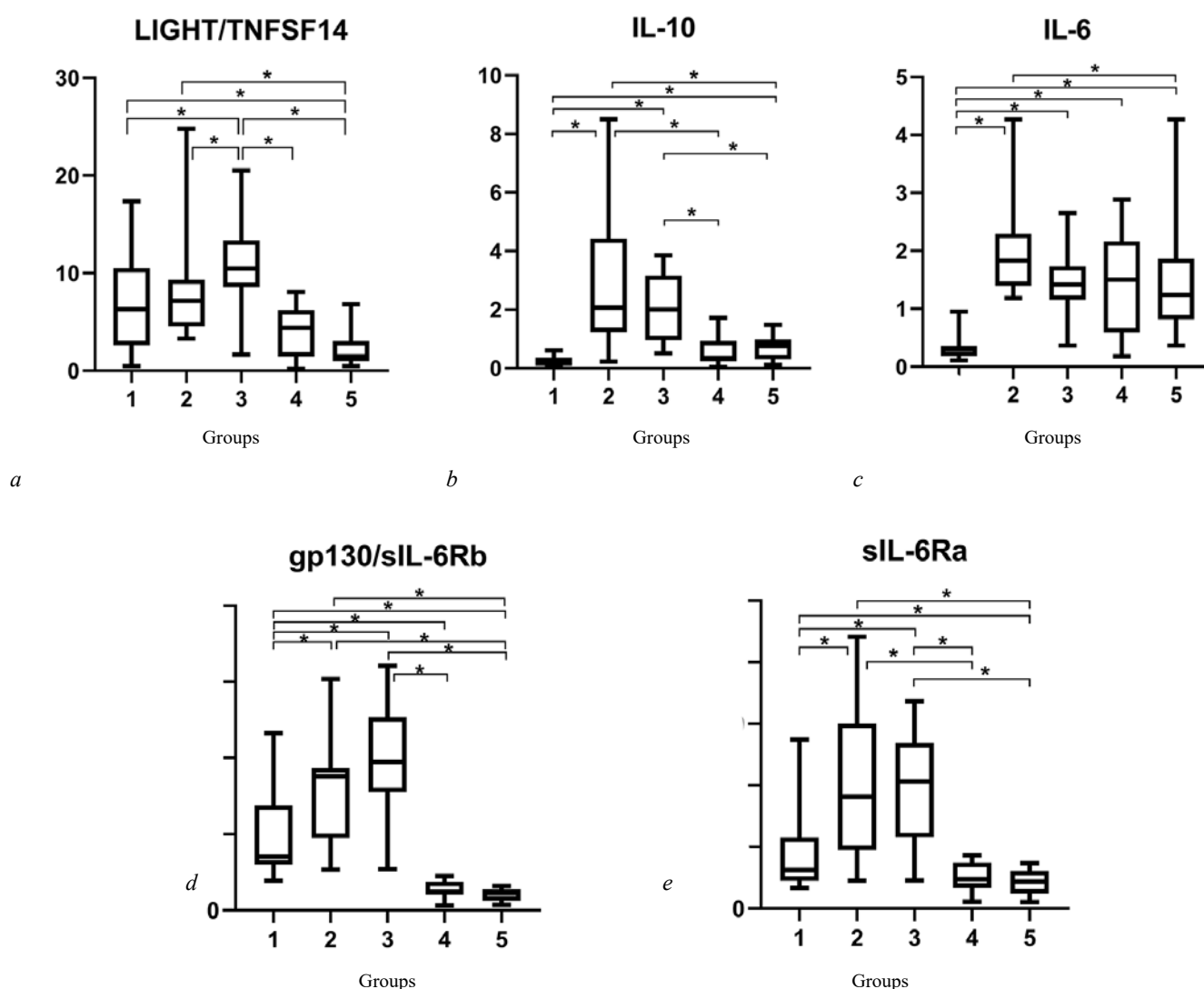


Fig. 2. Changes in the expression levels of the genes and corresponding proteins of liver mitochondrial dynamics and bioenergetics: *a* – the expression level of the DRP1 gene; *b* – an image of chemiluminescence of the DNM1L protein with respect to the reference GAPDH protein; *c* – the expression level of the MFN2 gene; *d* – the MFN2 protein; *e* – the expression level of the MT-ND4 gene; *f* – the amount of mitochondrial DNA. *a*, *c*, *e*, *f*: 1 – control group; group of the obese patients: 2 – without T2DM with a BMI < 40 kg / m²; 3 – without T2DM with a BMI > 40 kg / m²; 4 – with T2DM with a BMI < 40 kg / m²; 5 – with T2DM with a BMI > 40 kg / m²; *b*, *d*: 1 – control group; group of the obese patients: 2 – without T2DM; 3 – with T2DM

DISCUSSION

In obesity, an excessive amount of triglycerides enter the liver, which results in steatosis [11]. Progression of the latter is facilitated by an increase in the secretion of proinflammatory cytokines (TNF α , IL-1 α and β) by immune cells [12]. Once in the hepatic portal vein, proinflammatory cytokines can aggravate the already existing high level of ROS in hepatocytes [4]. Increased β -oxidation in the mitochondria leads to accumulation of toxic lipid intermediates (ceramides and diacylglycerol) [4].

These toxins and high ROS levels trigger production of proinflammatory cytokines by immune cells in the liver [4, 12]. Chronic liver inflammation aggravates the progression of NAFLD and leads to fibrosis and cirrhosis [4]. In this regard, the search for protective molecules aimed at reducing liver inflammation and preventing complications of steatosis is becoming more relevant.

As it was mentioned earlier, some secreted factors are involved in the pathogenesis of obesity and insulin resistance [4, 11, 12]. One of these factors is LIGHT / TNFSF14. Our data on the increase in the plasma level of sTNFSF14 in all the obese patients without T2DM in contrast to the parameters in the apparently healthy donors and patients with obesity and T2DM are consistent with other studies [8].

However, the reason for an increase in sLIGHT / TNFSF14 levels in obesity has not been found. It is known that many immune cells, including resting and activated T cells, B cells, monocytes and macrophages, produce TNFSF14 [13]. TNFSF14 transmits a signal through the receptors – lymphotoxin- β (LT β R) and herpes virus entry mediator (HVEM). The latter is highly expressed in visceral adipose tissue and, along with LT β R, in β -cells of the pancreas [13].

The study by G. Tiller et al. (2011) on 3T3-L1 preadipocyte cell lines and primary human preadipocytes demonstrated that LIGHT / TNFSF14 prevents the differentiation of preadipocytes into adipocytes in mice and humans without changing their metabolic functions [14]. There are assumptions that sTNFSF14 may potentially contribute to the pathogenesis of complications in obesity.

It was previously shown that the effects of sTNFSF14 could be mediated by its interaction with other cytokines, in particular, IL-6 and IL-10. It was found that IL-10 helps to suppress inflammation by inhibiting synthesis and effect of proinflammatory cytokines, including TNF α , IL-1 β , and IL-6, as well as by reducing activation of macrophages [15]. B.M. Saunders et al. (2018) showed that the Tnfsf14 $-/-$ mice re-

ceiving a diet high in fat developed insulin resistance. Moreover, the IL-10 production in the liver of such mice sharply increased in comparison with the wild-type mice [9]. The authors considered this increase in the mediator as a compensatory mechanism to reduce inflammation in the liver tissue.

In support of the above-mentioned, the study found a significant increase in plasma IL-10 in the obese patients without T2DM and, on the contrary, its significant decrease in the obese patients with T2DM (Fig. 1, *b*). Furthermore, the study revealed a positive relationship between sTNFSF14 and IL-10 in the obese patients without and with T2DM ($r = 0.43$ and $r = 0.4$, respectively). Probably, the decrease in TNFSF14 and IL-10 in the blood plasma of the obese patients with T2DM plays a crucial role in the progression of complications in obesity.

Cytokines of the IL-6 family regulate liver damage / regeneration and maintain a balance between regulatory and effector T cells [16]. IL-6 is one of the main factors in chronic subclinical inflammation, as it promotes insulin resistance and impairs glucose homeostasis [16]. In our study, an increase in plasma IL-6 was detected in all the obese patients compared with the group of apparently healthy donors. The obese patients with T2DM showed a positive relationship between IL-6 and sTNFSF14 ($r = 0.43$).

It is known that IL-6 on the cell surface binds to the receptor complex, which contains two subunits of the gp130 signaling receptor [17]. We found a significant increase in the amount of sIL-6Ra and gp130 / sIL-6Rb in the plasma of the obese patients without T2DM compared with the control group and obese patients with T2DM.

Hepatocytes and immune cells are the main sources of sIL-6R [16]. Having bound to IL-6R or sIL-6R, IL-6 initiates subsequent signaling through interaction with gp130 [17]. The IL-6 / sIL-6R complex acts as an agonist of the gp130-mediated IL-6 signaling and is called trans-signaling [17]. This type of signaling broadens the array of potential IL-6 targets for any cell type due to the ubiquitous expression of gp130. However, gp130 can also be in a soluble form; because of this, the IL-6 signaling is inhibited while the classical type of signaling is not affected [17]. Soluble gp130 (sgp130) is found in the bloodstream of healthy donors, inhibiting the systemic response to circulating IL-6 [17]. IL-6R can be in the soluble form of sIL-6Ra and form a complex with IL-6.

The complex of IL-6 and sIL-6Ra implements trans-signaling effects by binding to surface gp130

receptors [15, 16]. gp130 activates the yes-associated protein 1 (YAP) and Notch pathways and controls tissue growth and regeneration independently of the signal transducer and activator of transcription 3 (STAT3) protein [18]. A positive relationship was found between sIL-6Ra and gp130 / sIL-6Rb in the groups of the obese patients without T2DM ($r = 0.88$) and with T2DM ($r = 0.94$). It may indicate a protective role of these receptors regarding complications in obesity. Plasma sTNFSF14 in both groups of the obese patients with and without T2DM, regardless of their BMI, positively correlated with sIL-6Ra ($r = 0.34$ and $r = 0.43$, respectively) and gp130 / sIL-6Rb ($r = 0.37$ and $r = 0.44$, respectively). It should be noted that in the patients with obesity and T2DM, a positive correlation was found between gp130 / sIL-6Rb and IL-10 ($r = 0.3$).

Several studies demonstrated that TNFSF14 directly induces IL-6 [16], which supports the hypothesis that IL-6 production is regulated by TNFSF14. Thus, the *Tnfsf14* $-/-$ mice had a low level of IL-6 production in the liver, compared with the wild-type mice [9]. Therefore, it can be assumed that a decrease in the production of sTNFSF14 and IL-6 may be one of the key factors involved in the progression of NAFLD in obesity.

Hepatic mitochondria control energy metabolism through ATP synthesis and fatty acid oxidation [4]. It was previously shown that insulin resistance is associated with mitochondrial dysfunction in the liver [4]. Thus, TNFSF14 deficiency in the mice that received a diet rich in fat contributed to a significant increase in liver oxygen consumption with the use of substrates for complexes I, II, and III, compared with the wild-type mice [9].

The conducted study of liver mitochondrial dynamics in obesity, depending on the absence / presence of T2DM, revealed the following patterns. The expression of the DRP1 gene responsible for mitochondrial fission was significantly lower in the obese patients with T2DM than in the control group. Regarding the MFN2 gene responsible for the mitochondrial fusion, the groups demonstrated no differences. However, this parameter tended to be higher in the obese patients with T2DM than in the control group. This is consistent with the data of quantitative determination of mtDNA: a decrease (compared with the control group) in the mtDNA level in the liver of the obese patients with and without T2DM with a BMI < 40 kg / m². However, such a rise in the mtDNA level increases respiration in the hepatocyte mitochondria, thus making ROS production higher.

Consequently, the liver biopsy samples from the obese patients with and without T2DM demonstrated a high (compared with the control group) expression level of the MT-ND4 gene encoding the subunit of the respiratory chain complex I. Complex I is responsible for the first step in electron transfer from nicotinamide adenine dinucleotide (NADH) to ubiquinone. The electrons are then transferred from ubiquinone through complexes II, III, and IV to provide energy during ATP synthesis. A negative relationship between the level of sTNFSF14 in the blood plasma and the expression of the MT-ND4 gene ($r = -0.3$) in the liver was found in the obese patients without T2DM. Thus, the level of sTNFSF14 in the blood plasma can influence the liver mitochondrial biogenesis through interaction with the genes of fission (DRP1), fusion (MFN2), and mitochondrial respiration (MT-ND4).

CONCLUSION

The following conclusions can be made.

A high sTNFSF14 level in the plasma of the obese patients without T2DM (compared with the controls and obese patients with T2DM) has a protective effect on the liver through the interaction of gp130 / sIL-6Rb and IL-10.

A unidirectional decrease in the levels of sTNFSF14 and IL-6 in the plasma of the obese patients with T2DM is one of the key factors involved in the NAFLD progression in obesity.

In the obese patients with T2DM, a reduced (compared with the controls and obese patients without T2DM) sTNFSF14 level in the plasma mediates a decrease in the DRP1 gene expression in the liver, and, on the contrary, an increase in the MT-ND4 gene expression, which leads to a decrease in mitochondrial fission and a compensatory increase in mitochondrial respiration.

REFERENCES

1. Hruby A., Hu F.B. The epidemiology of obesity: a big picture. *Pharmacoeconomics*. 2015; 33 (7): 673–689. DOI: 10.1007/s40273-014-0243-x.
2. Byrne C.D., Targher G. NAFLD: a multisystem disease. *J. Hepatol*. 2015; 62 (Suppl. 1): S47–64. DOI: 10.1016/j.jhep.2014.12.012.
3. Ivashkin V.T., Drapkina O.M., Mayev I.V., Trukhmanov A.S., Blinov D.V., Palgova L.K., Tsukanov V.V., Ushakova T.I. Prevalence of non-alcoholic fatty liver disease in outpatients of the Russian Federation: DIREG 2 study results. *Russian Journal of Gastroenterology, Hepatology, Coloproctology*. 2015; 25 (6): 31–41 (in Russ.).

4. Simões I.C.M., Fontes A., Pinton P., Zischka H., Wiecekowsk M.R. Mitochondria in non-alcoholic fatty liver disease. *Int. J. Biochem. Cell Biol.* 2018; 95: 93–99. DOI: 10.1016/j.biocel.2017.12.019.
5. Ikawa-Yoshida A., Matsuo S., Kato A., Ohmori Y., Higashida A., Kaneko E., Matsumoto M. Hepatocellular carcinoma in a mouse model fed a choline-deficient, L-amino acid-defined, high-fat diet. *Int. J. Exp. Pathol.* 2017; 98 (4): 221–233. DOI: 10.1111/ieip.12240.
6. Qiao G., Qin J., Kunda N., Calata J.F., Mahmud D.L., Gann P., Fu Y.X., Rosenberg S.A., Prabhakar B.S., Maker A.V. LIGHT Elevation enhances immune eradication of colon cancer metastases. *Cancer Res.* 2017; 77 (8): 1880–1891. DOI: 10.1158/0008-5472.CAN-16-1655.
7. Zhang H., Chen X., Li D., Cui L., Li X., Ye X., Wan X. DcR3 promotes hepatoma cell migration by downregulating E-cadherin expression. *Oncol. Rep.* 2017; 38 (1): 377–383. DOI: 10.3892/or.2017.5685.
8. Bassols J., Moreno-Navarrete J.M., Ortega F., Ricart W., Fernandez-Real J.M. LIGHT is associated with hypertriglyceridemia in obese subjects and increased cytokine secretion from cultured human adipocytes. *Int. J. Obes.* 2010; 34 (1): 146–156. DOI: 10.1038/ijo.2009.199.
9. Saunders B.M., Rudnicka C., Filipovska A., Davies S., Ward N., Hricova J., Schlaich M.P., Matthews V.B. Shining LIGHT on the metabolic role of the cytokine TNFSF14 and the implications on hepatic IL-6 production. *Immunol. Cell Biol.* 2018; 96 (1): 41–53. DOI: 10.1111/imcb.1002.
10. Vulf M.A., Kirienkova E.V., Skuratovskaia D.A., Levada E.V., Volkova L.V., Zatolokin P.A., Gazatova N.D., Litvinova L.S. Factors governing development of non-alcoholic fatty liver disease and insulin resistance in obesity. *Biomedical Chemistry.* 2018; 64 (5): 444–450 (in Russ.). DOI: 10.18097/PBMC20186405444.
11. Kaila B., Raman M. Obesity: a review of pathogenesis and management strategies. *Can. J. Gastroenterol.* 2008; 22 (1): 61–68. DOI: 10.1155/2008/609039.
12. Tzanavari T., Giannogonas P., Karalis K.P. TNF-alpha and obesity. *Curr. Dir. Autoimmun.* 2010; 11: 145–156. DOI: 10.1159/000289203.
13. Kwon B.S., Tan K.B., Ni J., Oh K.O., Lee Z.H., Kim K.K., Kim Y.J., Wang S., Gentz R., Yu G.L., Harrop J., Lyn S.D., Silverman C., Porter T.G., Truneh A., Young P.R. A newly identified member of the tumor necrosis factor receptor superfamily with a wide tissue distribution and involvement in lymphocyte activation. *J. Biol. Chem.* 1997; 272 (22): 14272–14276. DOI: 10.1074/jbc.272.22.14272.
14. Tiller G., Laumen H., Fischer-Posovszky P., Finck A., Skurk T., Keuper M., Brinkmann U., Wabitsch M., Link D., Hauner H. LIGHT (TNFSF14) inhibits adipose differentiation without affecting adipocyte metabolism. *Int. J. Obes.* 2011; 35 (2): 208–216. DOI: 10.1038/ijo.2010.126.
15. de Waal Malefyt R., Abrams J., Bennett B., Figdor C.G., de Vries J.E. Interleukin 10 (IL-10) inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes. *J. Exp. Med.* 1991; 174 (5): 1209–1220. DOI: 10.1084/jem.174.5.1209.
16. Rose-John S. Interleukin-6 Family Cytokines. *Cold Spring Harb. Perspect. Biol.* 2018; 10 (2): a028415. DOI: 10.1101/cshperspect.a028415.
17. Reeh H., Rudolph N., Billing U., Christen H., Streif S., Bullinger E., Findeisen R., Schaper F., Huber H.J., Dittrich A. Response to IL-6 trans- and IL-6 classic signalling is determined by the ratio of the IL-6 receptor α to gp130 expression: fusing experimental insights and dynamic modelling. *Cell Commun. Signal.* 2019; 17 (1): 46. DOI: 10.1186/s12964-019-0356-0.
18. Garbers C., Aparicio-Siegmund S., Rose-John S. The IL-6/gp130/STAT3 signaling axis: recent advances towards specific inhibition. *Curr Opin. Immunol.* 2015; 34: 75–82. DOI: 10.1016/j.coi.2015.02.008.

Authors contribution

Komar A.A., Darinskas A. – analysis and interpretation of data, conception and design. Skuratovskaia D.A., Vulf M.A., Gazatova N.D. – substantiation of the manuscript, analysis and interpretation of data. Todosenko N.M., Vu H.K. – analysis of data. Zatolokin P.A. – provision of materials for the study. Kirienkova E.V. – critical revision of the manuscript for important intellectual content. Litvinova L.S. – conception and design, final approval of the manuscript for publication.

Authors information

Komar Aleksandra A., Post-Graduate Student, Basic Laboratory of Immunology and Cellular Biotechnology, IKBFU, Kaliningrad, Russian Federation. ORCID 0000-0002-1922-3592.

Skuratovskaia Daria A., Cand. Sci. (Biology), Senior Researcher, Basic Laboratory of Immunology and Cellular Biotechnology, IKBFU, Kaliningrad, Russian Federation. ORCID 0000-0002-8679-1135.

Vulf Maria A., Cand. Sci. (Biology), Senior Researcher, Basic Laboratory of Immunology and Cellular Biotechnology, IKBFU, Kaliningrad, Russian Federation. ORCID 0000-0002-4989-045X.

Vu Hung Q., Post-Graduate Student, Basic Laboratory of Immunology and Cellular Biotechnology, IKBFU, Kaliningrad, Russian Federation. ORCID 0000-0002-4067-4718.

Darinskas Adas, Dr. Sci. (Med.), Senior Researcher, Laboratory of Immunology, National Cancer Institute, Vilnius University, Vilnius, Republic of Lithuania. ORCID 0000-0002-1916-7535.

Gazatova Natalia D., Cand. Sci. (Biology), Head of the Laboratory of Experimental Blood Products, IKBFU, Kaliningrad, Russian Federation. ORCID 0000-0002-4646-3436.

Todosenko Natalia M., Cand. Sci. (Biology), Senior Researcher, Basic Laboratory of Immunology and Cellular Biotechnology, IKBFU, Kaliningrad, Russian Federation. ORCID 0000-0001-7468-4861.

Zatolokin Pavel A., Cand. Sci. (Med.), Senior Researcher, Basic Laboratory of Immunology and Cellular Biotechnology, IKBFU, Kaliningrad, Russian Federation. ORCID 0000-0002-8631-7361.

Kirienkova Elena V., Cand. Sci. (Med.), Senior Researcher, Basic Laboratory of Immunology and Cellular Biotechnology, IKBFU, Kaliningrad, Russian Federation. ORCID 0000-0002-5980-3321.

Litvinova Larisa S., Dr. Sci. (Med.), Head of the Basic Laboratory of Immunology and Cellular Biotechnology, IKBFU, Kaliningrad, Russian Federation. ORCID 0000-0001-5231-6910.

(✉) **Komar Aleksandra A.**, e-mail: alexandkomar@gmail.com

Received 10.02.2021

Accepted 25.05.2021

Fibrosis biomarkers and echocardiographic parameters in patients with type 2 diabetes depending on the degree of albuminuria

Lebedev D.A., Laevskaya M.Yu., Babenko A.Yu.

*Almazov National Medical Research Center
15, Parkhomenko Str., 194156, Saint Petersburg, Russian Federation*

ABSTRACT

Background. Diabetic nephropathy (DN) in patients with type 2 diabetes mellitus (T2DM) is associated with a risk of developing chronic heart failure (CHF). The degree of albuminuria is a marker of DN and is associated with an increased risk of chronic heart failure (CHF).

Aim. To evaluate fibrosis biomarkers and echocardiographic parameters in patients with T2DM without CHF, depending on urinary albumin excretion.

Materials and methods. The study included 42 patients with T2DM without verified CHF. The patients were divided into two groups: 1) a group with normoalbuminuria and 2) a group with a moderate increase in albuminuria (albumin / creatinine ratio of 30–300 mg / g). Echocardiography was performed and galectin-3, ST-2, P1CP, MMP-9, and TIMP-1 concentrations were measured.

Results. The groups did not differ by age, sex, body mass index (BMI), glycated hemoglobin (HbA1c), and glomerular filtration rate (GFR). Galectin-3 concentrations were significantly higher in the group with a moderate increase in albuminuria than in the group of patients without albuminuria – 13.6 (11.2; 15.1) ng / ml and 7.4 (6.7; 7.9) ng / ml, respectively, $p = 0.002$. The groups also did not differ in the values of biomarkers, such as P1CP, TIMP-1, and MMP-9. Besides, the group with normoalbuminuria had lower E/e' values than the group with a moderate increase in albuminuria – 8 (7; 9) and 10 (9; 12.5), $p = 0.02$.

Conclusion. The patients with type 2 diabetes and a moderate increase in albuminuria have higher values of galectin-3 and a more pronounced diastolic dysfunction. The identified changes may reflect a higher risk of chronic heart failure in this group of patients.

Key words: type 2 diabetes mellitus, chronic heart failure, albuminuria, fibrosis, galectin-3.

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

Source of financing. The study was financed by the Russian Science Foundation grant No. 17–75–30052 “Development of personalized therapy for obesity and type 2 diabetes mellitus in order to decrease cardiovascular risks”.

Conformity with the principles of ethics. All the participants signed an informed consent to take part in the study. The study was approved by the local Ethics Committee at Almazov National Medical Research Center (Protocol No. 020419 of 08.04.2019).

For citation: Lebedev D.A., Laevskaya M.Yu., Babenko A.Yu. Fibrosis biomarkers and echocardiographic parameters in patients with type 2 diabetes depending on the degree of albuminuria. *Bulletin of Siberian Medicine*. 2021; 20 (3): 72–78. <https://doi.org/10.20538/1682-0363-2021-3-72-78>.

Маркеры фиброза и эхокардиографические параметры у пациентов с сахарным диабетом 2-го типа в зависимости от уровня альбуминурии

Лебедев Д.А., Лаевская М.Ю., Бабенко А.Ю.

Национальный медицинский исследовательский центр им. В.А. Алмазова
Россия, 194156, г. Санкт-Петербург, пр. Пархоменко, 15

РЕЗЮМЕ

Введение. Наличие диабетической нефропатии (ДН) у пациентов с сахарным диабетом 2-го типа (СД2) ассоциировано с риском развития хронической сердечной недостаточности (ХСН). Степень альбуминурии является маркером прогрессирования ДН и связана с повышенным риском ХСН.

Цель. Оценка биомаркеров фиброза и эхокардиографических параметров у пациентов с СД2 без ХСН в зависимости от экскреции альбумина с мочой.

Материалы и методы. В исследование были включены 42 пациентов с СД2 без установленного диагноза ХСН. Пациенты были разделены на группы: с нормальной альбуминурией и с умеренным повышением альбуминурии (альбумин/креатининовое соотношение 30–300 мг/г). Пациентам была выполнена эхокардиография, определялась концентрация галектина-3, ST-2, P1CP, MMP-9 и TIMP-1.

Результаты. Группы не различались между собой по возрасту, полу, индексу массы тела (ИМТ), значениям гликированного гемоглобина (HbA1c), скорости клубочковой фильтрации (СКФ). Концентрации галектина-3 были значимо выше в группе с умеренным повышением альбуминурии – 13,6 (11,2; 15,1) нг/мл и 7,4 (6,7; 7,9) нг/мл соответственно, $p = 0,002$. Группы также не различались между собой по значениям таких биомаркеров, как P1CP, TIMP-1 и MMP-9. Кроме того, группа с нормоальбуминурией имела меньшие значения E/e', чем группа с умеренным повышением альбуминурии – 8 (7; 9) и 10 (9; 12,5), $p = 0,02$.

Заключение. Пациенты с СД2 и умеренным повышением альбуминурии имеют большие значения галектина-3 и более выраженное нарушение диастолической функции сердца, чем пациенты с нормоальбуминурией. Выявленные изменения могут отражать более высокий риск развития хронической сердечной недостаточности в данной группе пациентов.

Ключевые слова: сахарный диабет 2-го типа, хроническая сердечная недостаточность, альбуминурия, фиброз, галектин-3.

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

Источник финансирования. Исследование выполнено при поддержке Российского научного фонда (грант № 17–75–30052 «Разработка персонализированной терапии ожирения и сахарного диабета 2-го типа в целях снижения сердечно-сосудистых рисков»).

Соответствие принципам этики. До включения в исследование у всех участников было получено письменное информированное согласие. Исследование одобрено этическим комитетом ФГБУ НМИЦ им. В.А. Алмазова (протокол № 020419 от 08.04.2019).

Для цитирования: Лебедев Д.А., Лаевская М.Ю., Бабенко А.Ю. Маркеры фиброза и эхокардиографические параметры у пациентов с сахарным диабетом 2-го типа в зависимости от уровня альбуминурии. *Бюллетень сибирской медицины*. 2021; 20 (3): 72–78. <https://doi.org/10.20538/1682-0363-2021-3-72-78>.

INTRODUCTION

The morbidity rate with type 2 diabetes mellitus (T2DM) is increasing worldwide [1] along with the number of patients with microvascular complications caused by T2DM, including diabetic nephropathy (DN), which is one of the main causes of end-stage renal disease (ESRD) in T2DM patients [2]. DN is

characterized not only by a decrease in the glomerular filtration rate (GFR) but also by the development of albuminuria that can be observed even in patients with preserved GFR. Patients with T2DM and albuminuria have a higher risk of chronic heart failure (CHF) in comparison with patients with normal albumin excretion [3]. T2DM is associated with the development of structural and functional changes in the heart, includ-

ing left ventricular hypertrophy (LVH), concentric remodeling of the left ventricle, and the development of diastolic dysfunction [3].

CHF in patients with T2DM has common pathogenetic mechanisms with DN consisting in the development of low-grade systemic inflammation and acceleration of fibrosis. Recently, several biomarkers involved in the fibrosis processes have been discovered. These biomarkers include galectin-3, ST-2, procollagen type I C-peptide (PICP), as well as matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMP) [4–7]. The aim of the study was to evaluate biomarkers of fibrosis and echocardiographic parameters in patients with T2DM without CHF depending on the excretion of albumin in urine.

MATERIALS AND METHODS

Before the inclusion into the study, all patients signed an informed consent. The study included 42 patients with T2DM without verified CHF who had received regular hypoglycemic and antihypertensive therapy for 3 months before the inclusion in the study. To exclude CHF, medical records of the included patients were analyzed, and the patients were examined by a cardiologist. The inclusion criteria were as follows: female or male patients aged 40–75 years with T2DM, disease duration of more than 1 year, glycated hemoglobin (HbA1c) 7.0–11.0%, regular hypoglycemic therapy for at least 12 weeks before the inclusion in the study, therapy with angiotensin-converting enzyme (ACE) inhibitors or angiotensin II receptor blockers (ARB), duration of arterial hypertension (AH) for more than 1 year.

The study did not include patients with chronic renal disease of non-diabetic genesis, reduced GFR < 60 ml / min / 1.73 m², spot urine albumin-to-creatinine ratio (ACR) > 300 mg / g, significant cardiovascular (hemodynamically significant valve disorders, ischemic heart disease, and congenital heart disorders) and liver pathology, arterial hypertension (BP $> 140/90$ mm Hg), and class 3 obesity. The patients were divided into two groups by the ACR value: a group with normal albuminuria (ACR < 30 mg / g; $n = 24$) and a group with a moderate increase in albuminuria (ACR, 30–300 mg / g; $n = 18$). During the study, clinical data of the studied population were registered, including age, sex, duration of T2DM, blood pressure, body weight, height, and BMI. The patients underwent transthoracic echocardiography on the Vivid 7 system (GE, USA) using a 3.25-MHz sensor in M- and two-dimensional modes in standard echocardiographic positions.

The following parameters were measured: Simpson's ejection fraction (EF), left ventricular end-diastolic volume (LVEDV), left ventricular end-systolic volume (LVESV), left ventricular myocardium mass index (LVMMI), and the ratio of the peak early diastolic LV filling velocity to early diastolic mitral ring motion (E/e'), which indicates diastolic dysfunction. The samples of venous blood and urine were collected after fasting. Tubes with ethylenediaminetetraacetic acid (EDTA) were used for taking samples of venous blood for testing. The concentrations of glycated hemoglobin (HbA1c), C-reactive protein (CPR), low-density lipoproteins (LDL), high-density lipoproteins (HDL), triglycerides (TG), and creatinine with calculation of GFR by the CKD-EPI equation were measured. Urine samples were used to evaluate ACR. HbA1c was determined using the method of affinity chromatography (Bio-Rad, USA) (normal value $< 6\%$); biochemical parameters (creatinine (normal values 53–106 μ mol / l), lipid profile (LDL (normal value < 3.0 μ mol / l), HDL (normal value > 1.2 mmol / l), TG (normal value < 1.77 mmol / l)), CRP (normal value < 5.0 mg / l)) were measured using a reagent kit and analyzer Cobas Integra 400 plus (Roche, France). The immunoenzyme technique was used to estimate galectin-3, MMP-9, TIMP-1, and ST-2 using the reagent kit produced by R&D System (USA). PICP was detected using the reagents from the assay kit Cloud-Clone Corporation. To detect galectin-3, ST-2, PICP, MMP-9, and TIMP-1, the blood samples were centrifuged for 10 minutes at 2,000g after a 30-minute waiting period. The plasma samples had been stored at -80 °C for 3 months before assessing the concentration of biomarkers.

SPSS software (version 21.0, IBM Corp, New York, USA) was used for statistical analysis of the data. The sampling size was calculated by the formula $n = [A+B]^2 \times 2 \times SD^2 / DIFF^2$. Galectin-3 was chosen as the main variable, because there are data on changes in its level in patients with albuminuria. Each group required 16 patients to detect differences at a 5% significance level and 80% statistical power (standard deviation (SD) = 1.5). The comparison of quantitative variables between the samples was performed using the Mann – Whitney test. The evaluation of a relationship between the studied variables was performed using the Spearman's rank correlation coefficient. Quantitative variables were presented as the median and the interquartile range ($Me (Q_{25}-Q_{75})$), nominal variables – as $n (\%)$. The differences were statistically significant at $p < 0.05$.

RESULTS

We performed the analysis of the data obtained from 42 patients: 24 patients with normal albumin excretion and 18 patients with a moderate increase in albumin excretion. All patients received metformin therapy in combination with DPP-4 inhibitors and / or sulfonylureas. Demographic and clinical characteristics and laboratory data of the patients are presented in the Table. There were no significant differences between the groups in terms of age, sex, BMI, glycated hemoglobin, GFR, and lipid profile. There were no differences between the groups in such biomarkers as PICP, TIMP-1, and MMP-9 either.

The concentration of galectin-3 was significantly higher in the group with a moderate increase in albuminuria. NT-pro-BNP and ST-2 levels did not differ significantly between the groups. Echocardiographic parameters did not differ between the groups, with the exception of E/e' values, which were significantly higher in the group with a moderate increase in albuminuria.

There was a positive correlation between galectin-3 and ACR ($r = 0.783$, $p < 0.001$), as well as between galectin-3 and E/e' ($r = 0.59$, $p = 0.001$). There was no significant correlation between the biomarkers and such parameters as glycated hemoglobin, duration of T2DM, sex, age, and BMI.

Table

Characteristics of the patients			
Parameter	Group with albuminuria, $n = 18$	Group without albuminuria, $n = 24$	p
Age, years, $Me (Q_{25}-Q_{75})$	57 [50; 63]	51 [46; 60]	0.17
Females, n (%)	12 (66.7)	15 (62.5)	0.56
Smoking, n (%)	6 (33.3)	5 (20.8)	0.23
Retinopathy, n (%)	8 (44.4)	11 (45.8)	0.39
Diabetes duration, years, $Me (Q_{25}-Q_{75})$	6 [4; 8]	5 [2; 7]	0.62
Arterial hypertension (AH) duration, years, $Me (Q_{25}-Q_{75})$	8 [5; 11]	9 [5; 11]	0.45
ACE-inhibitors/ARB, n (%)	15 (83.3)	18 (75)	0.79
BMI, kg / m^2 , $Me (Q_{25}-Q_{75})$	31.4 [29.6; 34.9]	33 [30.7; 35.1]	0.33
HbA1c, %, $Me (Q_{25}-Q_{75})$	8.2 [7.65; 8.7]	8.6 [7.25; 9.9]	0.75
Systolic arterial pressure, mm Hg., $Me (Q_{25}-Q_{75})$	134.5 [126.1; 142.0]	129.2 [117.5; 135.9]	0.66
Diastolic arterial pressure, mm Hg., $Me (Q_{25}-Q_{75})$	78.2 [75.2; 83.6]	75.6 [72.1; 79.2]	0.73
GFR, $ml / min / 1.73m^2$, $Me (Q_{25}-Q_{75})$	95.3 [78.3; 100.1]	101.1 [87.4; 106.0]	0.46
CRP, $Me (Q_{25}-Q_{75})$	1.82 [0.44; 4.8]	1.65 [0.31; 6.51]	0.88
NT-pro-BNP, $Me (Q_{25}-Q_{75})$	48.53 (24.53; 73.24)	30.8 [18.73; 65.23]	0.11
Galectin-3, ng / ml , $Me (Q_{25}-Q_{75})$	13.6 [11.2; 15.1]	7.4 [6.7; 7.9]	< 0.001
PICP, ng / ml , $Me (Q_{25}-Q_{75})$	164.7 [137.8; 188.8]	118.8 [105.3; 139.7]	0.2
TIMP-1, ng / ml , $Me (Q_{25}-Q_{75})$	240.8 [155.7; 298.2]	208 [186.5; 239.5]	0.47
MMP-9, ng / ml , $Me (Q_{25}-Q_{75})$	505.7 [382.3; 672.0]	410.5 [342.1; 535.4]	0.75
ST-2, ng / ml , $Me (Q_{25}-Q_{75})$	16.4 [13.8; 20.9]	17.9 [14.7; 22.3]	0.71
LDL, $mmol / l$, $Me (Q_{25}-Q_{75})$	2.38 (1.85; 2.79)	3.45 [2.64; 3.79]	0.08
HDL, $mmol / l$, $Me (Q_{25}-Q_{75})$	1.1 [0.86; 1.28]	1.16 [0.91; 1.30]	0.68
TG, $mmol / l$, $Me (Q_{25}-Q_{75})$	2.58 [1.76; 2.98]	2.34 [1.65; 3.14]	0.28
EF, %, $Me (Q_{25}-Q_{75})$	61 [55; 63]	61 [57; 63]	0.59
E/e', $Me (Q_{25}-Q_{75})$	10 [9; 12.5]	8 [7; 9]	0.006
Left atrial volume index, ml / m^2 , $M (Max-Min)$	49.4 [39.2-56.2]	42.8 [35.0-49.5]	0.03
EDV, ml , $Me (Q_{25}-Q_{75})$	110 [98; 129]	119 [100; 133]	0.28
ESV, ml , $Me (Q_{25}-Q_{75})$	40 [31; 48.5]	47 [28.5; 55.5]	0.21
LV MMI, g / m^2 , $Me (Q_{25}-Q_{75})$	105 [89; 120]	105 [84; 115]	0.65

Note: LV MMI – left ventricular myocardial mass index.

DISCUSSION

The study results showed that the concentration of galectin-3 was significantly higher in the patients with a moderate increase in albuminuria than in the patients

with normoalbuminuria. The synthesis of galectin-3 is observed in different cells and tissues of the body, but the level of its expression varies significantly. Active expression of galectin-3 is observed in cells, such as macrophages, mast cells, neutrophils, T-cells,

eosinophils, fibroblasts, and cardiomyocytes. Galectin-3 is involved in cell differentiation, apoptosis, angiogenesis, and fibrogenesis. Galectin-3 causes fibroblast proliferation and macrophage activation. The activation of macrophages leads to an increase in the expression of interleukin-4 (IL-4) and interleukin-13 (IL-13) and formation of the extracellular matrix. In addition, galectin-3 increases synthesis of the transforming growth factor TGF- β and different types of collagen [8].

The obtained results suggest that an increase in the circulating galectin-3 may contribute to the development and progression of DN. An independent correlation between the levels of galectin-3 and the degree of albuminuria, especially in the patients with preserved filtration function of the kidneys, can indicate the involvement of galectin-3 in the pro-fibrotic processes observed during DN. The obtained data are consistent with the results obtained by M. Iacoviello et al. (2015) that showed that the levels of galectin-3 were associated with increased albumin excretion with urine in patients with T2DM and CHF [9].

Taking part in the development of fibrotic changes in the kidneys, galectin-3 can influence the progression of both renal and cardiac dysfunctions. This study revealed a correlation between an increased level of galectin-3 and a higher E/e' ratio, which could reflect the impact of galectin-3 on the development of diastolic dysfunction, but does not prove the presence of cause-and-effect relationship. Similar data were obtained in the patients with heart failure, and the level of galectin-3 was positively correlated with the degree of diastolic dysfunction [10, 11]. In this study, the patients did not have CHF. However, higher levels of galectin-3 in the patients with increased albuminuria could indirectly reflect a greater risk of developing CHF in the future [12]. Nevertheless, to date, the role of galectin-3 remains controversial and the exact mechanism of elevated serum levels of galectin-3 in patients with DN remains unclear [13]. Further research should be carried out to determine whether serum levels of galectin-3 reflect its expression in the kidneys, or in the heart, or whether these are concurrent processes.

Another biomarker studied was soluble plasma protein ST-2. This study did not reveal an increased concentration of ST-2 in patients with moderate albuminuria compared with normoalbuminuria. This could be associated with the fact that the study did not include patients with severe albuminuria. One of the prospective studies in Sweden showed that patients

with DM had significantly increased levels of ST2 depending on the degree of albuminuria. A relationship was revealed between higher ST2 levels in the early stages of DM and the development of DN within 10 years [14]. It is advisable to further study the role of ST2 in the pathogenesis of DN to determine its prognostic value at later stages of DN.

DN is associated with progressive accumulation of extracellular matrix components. A number of studies showed that plasma concentrations of MMP-9 and TIMP-1 in patients with T2DM were higher than in the control group without T2DM [15, 16]. However, in this study, no significant increase in MMP-9 and TIMP-1 levels in the patients with a moderate increase in albuminuria was revealed compared with patients with normoalbuminuria. Similar results were obtained in the study by G. Cakirca, F. Turgut (2018) that analyzed the differences in the concentrations of MMP-9 and TIMP-1 in patients with T2DM depending on the degree of albuminuria [16]. Additionally, this study did not reveal statistically significant differences in the concentration of PICP between the studied groups. Earlier studies showed that type I collagen was involved in the process of glomerular sclerosis [17], and the levels of PICP were significantly higher in patients with pronounced albumin excretion (ACR > 300 mg / g) compared with patients with normoalbuminuria [18]. A significant increase in the level of PICP in patients with pronounced albuminuria and no increase in PICP in the group of patients with moderately elevated albuminuria could be associated with a less widespread nature of glomerular sclerosis in the patients included in the study.

According to the studies, higher ACR is associated with the development of cardiac dysfunction in patients with T2DM [19, 20]. In this study, the patients with increased excretion of albumin had a significantly higher E/e' ratio and left atrial volume index (LAVI) than the patients with normoalbuminuria, which may reflect a potential risk of CHF in the future. No significant differences in LVMMI, LVEDV, and LVESV were revealed in the studied groups. However, according to some studies, albuminuria is associated with the development of LV hypertrophy both in patients with arterial hypertension without T2DM [21] and with T2DM [22].

This study had a number of limitations, so the data obtained should be interpreted with caution. Firstly, a small number of patients were included in the study. Secondly, the study did not include a group of patients with pronounced excretion of albumin in urine

that could show a significant increase in other fibrosis markers. In addition, the patients received different combinations of antihyperglycemic and antihypertensive medications with different dosages and duration of therapy, which may affect the absolute values of the studied fibrosis markers.

The results obtained indicate that the patients with preserved glomerular filtration of the kidneys and a moderate increase in albuminuria have higher levels of galectin-3, E/e' ratio, and LVMMI than patients with normoalbuminuria. The identified changes may indicate a higher risk of developing CHF in this group of patients. The applicability of galectin-3 measurement in assessing the development and progression of both DN and CHF requires further prospective studies.

REFERENCES

- Satirapoj B., Adler S. Prevalence and management of diabetic nephropathy in Western countries. *Kidney Dis. (Basel)*. 2015;1 (1): 61–70. DOI: 10.1159/000382028.
- De Boer I., Rue T., Hall Y. et al. Temporal trends in the prevalence of diabetic kidney disease in the United States. *JAMA*. 2011; 305 (24): 2532–2539. DOI: 10.1001/jama.2011.861
- Gerstein H., Mann J., Yi Q. et al. Albuminuria and risk of cardiovascular events, death, and heart failure in diabetic and nondiabetic individuals. *JAMA*. 2001; 286 (4): 421–426. DOI: 10.1001/jama.286.4.421
- Shalenkova M.A., Trebunsky K.S., Kolosova K.S. New biomarker galectin-3: application possibilities for diagnostics and prognosis of the course of cardiovascular and kidney diseases. *Disease Treatment and Prevention*. 2019; 9 (3): 47–52 (in Russ.).
- Garcia-Fernandez N., Jacobs-Cachá C., Mora-Gutiérrez J. et al. Matrix metalloproteinases in diabetic kidney disease. *Journal of Clinical Medicine*. 2020; 9 (2): 472. DOI: 10.3390/jcm9020472.
- Bansal N., Zelnick L., Go A. et al. Cardiac Biomarkers and Risk of Incident Heart Failure in Chronic Kidney Disease: The CRIC (Chronic Renal Insufficiency Cohort) Study. *Journal of the American Heart Association*. 2019; 8 (21): e012336. DOI: 10.1161/JAHA.119.012336.
- Tan K., Cheung C., Lee A. et al. Galectin-3 is independently associated with progression of nephropathy in type 2 diabetes mellitus. *Diabetologia*. 2018; 61 (5): 1212–1219. DOI: 10.1007/s00125-018-4552-z.
- Zhong X., Qian X., Chen G., Song X. The role of galectin-3 in heart failure and cardiovascular disease. *Clin. Exp. Pharmacol. Physiol.* 2019; 46 (3): 197–203. DOI: 10.1111/1440-1681.13048.
- Iacoviello M., Aspromonte N., Leone M. et al. Galectin-3 serum levels are independently associated with microalbuminuria in chronic heart failure outpatients. *Res. Cardiovasc. Med*. 2015; 5 (1): e28952. DOI: 10.5812/cardiovascmed.28952.
- Shah R., Chen-Tournoux A., Picard M. et al. Galectin-3, cardiac structure and function, and long-term mortality in patients with acutely decompensated heart failure. *Eur. J. Heart Fail*. 2010; 12 (8): 826–832. DOI: 10.1093/eurjhf/hfq091.
- Ansari U., Behnes M., Hoffmann J. et al. Galectin-3 reflects the echocardiographic grades of left ventricular diastolic dysfunction. *Ann. Lab. Med*. 2018; 38 (4): 306–315. DOI: 10.3343/alm.2018.38.4.306.
- Ho J.E., Liu C., Lyass A., Courchesne P., Pencina M.J., Vasan R.S., Larson M.G., Levy D. Galectin-3, a marker of cardiac fibrosis, predicts incident heart failure in the community. *Journal of the American College of Cardiology*. 2012; 60 (14): 1249–1256. DOI: 10.1016/j.jacc.2012.04.053.
- O'Seaghdha C., Hwang S., Ho J. et al. Elevated galectin-3 precedes the development of CKD. *J. Am. Soc. Nephrol*. 2013; 24 (9): 1470–1477. DOI: 10.1681/ASN.2012090909.
- Samuelsson M., Dereke J., Svensson M. et al. Soluble plasma proteins ST2 and CD163 as early biomarkers of nephropathy in Swedish patients with diabetes, 15–34 years of age: a prospective cohort study. *Diabetol. Metab. Syndr*. 2017; 9: 41. DOI: 10.1186/s13098-017-0240-2.
- Abreu B.J., de Brito Vieira W.H. Metalloproteinase changes in diabetes. *Adv. Exp. Med Biol*. 2016; 920: 185–190. DOI: 10.1007/978-3-319-33943-6_17.
- Cakirca G., Turgut F. Serum matrix metalloproteinase-9, tissue inhibitor of metalloproteinase-1 and matrix metalloproteinase-9/ neutrophil gelatinase-associated lipocalin complex levels in patients with early-stage diabetic nephropathy. *Iranian Journal of Kidney Diseases*. 2018; 12 (5): 299–304.
- Duprez D.A., Gross M.D., Ix J.H. et al. Collagen biomarkers are associated with decline in renal function independently of arterial pressure and other cardiovascular risk factors: the Multi-Ethnic Study of Atherosclerosis Study. *J. Hypertens*. 2019; 37 (12): 2398–2403. DOI: 10.1097/HJH.0000000000002207.
- Matsushita K., van der Velde M., Astor B. et al. Association of estimated glomerular filtration rate and albuminuria with all-cause and cardiovascular mortality in general population cohorts: a collaborative meta-analysis. *Lancet*. 2010; 375 (9731): 2073–2081. DOI: 10.1016/S0140-6736(10)60674-5.
- Duman D., Demirtunc R., Karadag B. et al. Association of albuminuria with impaired aortic elasticity and left ventricular diastolic dysfunction in type 2 diabetes. *Anadolu Kardiyol. Derg*. 2008; 8 (1): 10–15.
- Swoboda P., McDiarmid A., Erhayiem B. et al. Diabetes mellitus, microalbuminuria, and subclinical cardiac disease: identification and monitoring of individuals at risk of heart failure. *J. Am. Heart Assoc*. 2017; 6 (7): e005539. DOI: 10.1161/JAHA.117.005539.
- Wang T., Zhong H., Lian G. et al. Low-grade albuminuria is associated with left ventricular hypertrophy and diastolic dysfunction in patients with hypertension. *Kidney Blood Press. Res*. 2019; 44 (4): 590–603. DOI: 10.1159/000500782.
- Jørgensen P., Biering-Sørensen T., Mogelvang R. et al. Presence of micro- and macroalbuminuria and the association with cardiac mechanics in patients with type 2 diabetes. *Eur. Heart J. Cardiovasc. Imaging*. 2018; 19 (9): 1034–1041. DOI: 10.1093/ehjci/jex231.

Authors information

Lebedev Denis A., Endocrinologist, Junior Researcher, Laboratory of Diabetology, Institute of Endocrinology, Almazov Medical Research Center, Saint Petersburg, Russian Federation. ORCID 0000-0003-1808-1331.

Laevskaya Mariya Yu., Cand. Sci. (Med.), Senior Researcher, Laboratory of Diabetology, Institute of Endocrinology, Almazov Medical Research Center, Saint Petersburg, Russian Federation. ORCID 0000-0003-0837-8385.

Babenko Alina Yu., Dr. Sci. (Med.), Head of the Laboratory of Diabetology, Institute of Endocrinology, Almazov National Medical Research Center, Saint Petersburg, Russian Federation. ORCID 0000-0002-0559-697X.

(✉) **Lebedev Denis A.**, e-mail: doctorlebedev@yandex.ru

Received 08.05.2020

Accepted 28.12.2020

The structure of affective fluctuations in a non-clinical sample

Osipova N.N.¹, Bardenstein L.M.¹, Aleshkina G.A.¹, Dmitrieva E.V.²

¹ *Moscow State University of Medicine and Dentistry named after A.I. Evdokimov
20/1, Delegatskaya Str., Moscow, 127473, Russian Federation*

² *Smolensk State Medical University
28, Krupskoy Str., Smolensk, 214019, Russian Federation*

ABSTRACT

Aim. To study the structure of mood fluctuations at the preclinical stage based on the findings of screening methods.

Materials and methods. A total of 129 students participated in the study. The average age was 18.95 ± 0.08 years. We used the clinical and psychopathological method, the Mini-International Neuropsychiatric Interview (MINI), and screening methods, such as the diagnostic questionnaire for bipolar disorder (Hypomania Checklist-32 (HCL-32)), and Hamilton Depression Scale (HAMD-17).

Results. Upon a clinical and psychopathological examination according to ICD-10 criteria, class V, mental and behavioral disorders (F00-F99), including affective pathology, were not detected. According to the findings of the HCL-32 screening technique, 61.2% ($n = 79$) of respondents had a cumulative score above the threshold. Analysis of individual items on the HCL-32 scale across the entire sample revealed that the majority of examinees positively assessed the impact of mood elevations on the family sphere (63.57%; $n = 82$), social activities (68.99%; $n = 89$), work (75.19%; $n = 91$), and recreational sphere (82.17%; $n = 106$). Positive (36.43%; $n = 47$; 95% confidence interval (CI) 28.13–44.74) and neutral (37.21%; $n = 48$; 95% CI 33.35–50.37) assessments of mood elevations were also detected by the respondents' immediate circle, which, in general, significantly complicates recognition of hypomania symptoms and delays seeking specialized care. In the structure of mood elevation episodes irritability ($r = -0.684$), conflict ($r = -0.665$), risk-taking behavior ($r = -0.550$), increased sexual desire ($r = 0.527$), increased energy and activity ($r = 0.431$), distractibility ($r = -0.467$), stimulant use ($r = -0.467$), and decreased need for sleep ($r = 0.408$) dominated. These signs are very similar to the clinical manifestations of a hypomanic episode in bipolar II disorder.

Signs of mild depression revealed according to the HAMD-17 scale in 34.8% ($n = 45$) of respondents included sleep disorders ($r = 0.693$), decreased ability to work ($r = 0.520$), depressive mood ($r = 0.579$), hypochondria ($r = 0.466$), general somatic symptoms ($r = -0.508$), and gastrointestinal disorders ($r = 0.513$). These signs did not result in chief complaints and were not the reason for seeking specialized care.

Conclusion. In the non-clinical sample, in the structure of mood swings, mood elevations dominated, which were not subjectively identified as illness symptoms and did not appear as complaints in clinical and psychopathological examinations. Low mood was accompanied by general somatic symptoms, which may indicate subsequent formation of comorbid pathology. The identified subsyndromal signs of hypomania and depression in the non-clinical sample in the absence of complaints and psychiatric care-seeking are of clinical significance as predictors of a bipolar affective disorder and require further clinical and dynamic monitoring.

Key words: hypomania, subdepression, bipolar affective disorder, screening methods.

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

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

Conformity with the principles of ethics. A written informed consent was obtained from all the participants prior to inclusion in the study. The study was approved by the Ethics Committee at Moscow State University of Medicine and Dentistry named after A.I. Evdokimov (Protocol No. 3 of 02.12.2018).

✉ Osipova Natalia N., e-mail: natinen@yandex.ru

For citation: Osipova N.N., Bardenstein L.M., Aleshkina G.A., Dmitrieva E.V. The structure of affective fluctuations in a non-clinical sample. *Bulletin of Siberian Medicine*. 2021; 20 (3): 79–87. <https://doi.org/10.20538/1682-0363-2021-3-79-87>.

Структура аффективных колебаний в неклинической выборке

Осипова Н.Н.¹, Барденштейн Л.М.¹, Алешкина Г.А.¹, Дмитриева Е.В.²

¹ Московский государственный медико-стоматологический университет имени А.И. Евдокимова
Россия, 127473, г. Москва, ул. Делегатская, 20/1

² Смоленский государственный медицинский университет
Россия, 214019, г. Смоленск, ул. Крупской, 28

РЕЗЮМЕ

Цель. Изучение структуры колебаний настроения на доклиническом этапе по результатам скрининговых методов.

Материалы и методы. В исследовании приняли участие 129 студентов. Средний возраст $18,95 \pm 0,08$ лет. Применялись клинко-психопатологический метод, краткий международный нейропсихиатрический опросник Mini-International Neuropsychiatric Interview [34], а также скрининговые методы: диагностический опросник по биполярному расстройству (Hypomania Checklist (HCL-32)), шкала депрессии Гамильтона (HAM-D-17).

Результаты. При клинко-психопатологическом исследовании в соответствии с критериями МКБ-10, класс V: психические расстройства и расстройства поведения (F00-F99) психические расстройства, в том числе аффективная патология выявлены не были. По результатам скрининговой методики HCL-32, у 61,2% ($n = 79$) респондентов суммарный балл превышал пороговое значение. Анализ отдельных пунктов шкалы HCL-32 на всей выборке выявил, что большинство исследуемых положительно оценивали влияние подъемов настроения на семейную сферу (63,57%; $n = 82$), общественную деятельность (68,99%; $n = 89$), работу (75,19%; $n = 91$), сферу досуга (82,17%; $n = 106$). Положительная (36,43%; $n = 47$; 95%-й ДИ 28,13–44,74) и нейтральная (37,21%; $n = 48$; 95%-й ДИ 33,35–50,37) оценка подъемов настроения также выявлена со стороны ближайшего окружения респондентов, что в целом существенно затрудняет распознавание гипоманиакальных симптомов и задерживает обращение за специализированной помощью. В структуре эпизодов подъемов настроения преобладали раздражительность ($r = -0,684$), конфликтность ($r = -0,665$), рискованное поведение ($r = -0,550$), усиление сексуальных побуждений ($r = 0,527$), повышение энергии и активности ($r = 0,431$), отвлекаемость ($r = -0,467$), употребление стимулирующих веществ ($r = -0,467$), снижение потребности во сне ($r = 0,408$). Эти признаки во многом сходны с клиническими проявлениями гипоманиакального эпизода при биполярном расстройстве II типа. Выявленные по шкале HAM-D-17 у 34,8% ($n = 45$) респондентов признаки легкой депрессии были представлены нарушениями сна ($r = 0,693$), снижением работоспособности ($r = 0,520$), депрессивным настроением ($r = 0,579$), ипохондрией ($r = 0,466$), общесоматическими симптомами ($r = -0,508$), нарушением функции желудочно-кишечного тракта ($r = 0,513$). Данные признаки не носили характер активных жалоб и не служили поводом для обращения за специализированной помощью.

Выводы. В неклинической выборке в структуре колебаний настроения преобладали подъемы, которые субъективно не идентифицировались как болезненные симптомы и не выступали в качестве жалоб при клинко-психопатологическом исследовании. Сниженный фон настроения сочетался с общесоматическими симптомами, что может указывать на последующее формирование коморбидной патологии. Выявленные субсиндромальные признаки гипомании и депрессии в неклинической выборке при отсутствии активных жалоб и обращений за психиатрической помощью представляют клиническую значимость как предикторы аффективной патологии биполярного спектра и требуют дальнейшего клинко-динамического наблюдения.

Ключевые слова: гипомания, субдепрессия, биполярное аффективное расстройство, скрининговые методы.

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

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

Соответствие принципам этики. До включения в исследование у всех участников было получено письменное информированное согласие. Исследование одобрено этическим комитетом Смоленского государственного медицинского университета (протокол № 3 от 02.12.2018).

Для цитирования: Осипова Н.Н., Барденштейн Л.М., Алешкина Г.А., Дмитриева Е.В. Структура аффективных колебаний в неклинической выборке. *Бюллетень сибирской медицины*. 2021; 20 (3): 79–87. <https://doi.org/10.20538/1682-0363-2021-3-79-87>.

INTRODUCTION

Bipolar disorder (BD) is a relapsing, chronic, and disabling disease. Risk prediction and early diagnosis and treatment for this disorder have been the main clinical and research goals over the past decades [1–10].

Since the first manifestations of the disease are detected before the age of 18 in almost two thirds of patients [1, 2], efforts for early recognition and treatment should be focused specifically on adolescent and juvenile age [1, 3–8].

The fact that bipolar disorder, as well as schizophrenia, has a prodromal phase that can be identified before the full clinical presentation of the disease has developed, is becoming more recognized. Unlike schizophrenia, where the presence of a prodrome is confirmed by criteria predicting the onset of psychosis from six months to three years in 10–40% of cases [9, 11], the existence of a specific prodrome for BD that would allow for targeted therapeutic intervention requires further investigation [2, 5–11].

In most cases, an early onset of the disease is associated with longer treatment delays, which are risk factors for an adverse outcome of bipolar disorder in adults [12, 13].

The typology of clinical BD includes a considerably larger number of variants than presented in international classifications of diseases [14, 15].

The classification of mental disorders by the American Psychiatric Association (DSM-IV-TR (APA), 2000; DSM-V (APA), 2013) highlights two main variants of the course of bipolar disorder: bipolar I disorder and bipolar II disorder [16, 17]. Bipolar I disorder is diagnosed in a patient when at least one manic or mixed episode is detected. In bipolar II disorder (296.89 – DSM-V, APA, 2013), at least one hypomanic episode and at least one episode of major depressive disorder (current or in history) are required. In 2019, the World Health Organization approved the

ICD-11 project that also includes division of BD into types I and II [18].

In the Russian Federation, diagnosis of bipolar II disorder in accordance with ICD-10 (class V: mental and behavioral disorders (F00-F99)) is complicated by the absence of diagnostic criteria for this disorder. There is an instruction to include bipolar II disorder in the heading F31.8 within the category “Other bipolar affective disorders”, thus complicating identification and statistical accounting of this BD variant in the Russian Federation [19].

The problems of early BD diagnosis in adolescent and juvenile age are largely associated with atypical clinical presentation of subdepressive, depressive, hypomanic, manic, and mixed episodes, as well as with polymorphism and incompleteness of the clinical presentation due to characteristics of the developing adolescent psyche [20, 21]. This leads to the fact that a significant part of adolescents suffering from BD do not receive timely adequate treatment or do not come to the attention of a psychotherapist or psychiatrist at all. The study by K.R. Merikangas et al. [22] noted that patients with the onset of BD in childhood on average remain untreated for more than 16 years.

Significant issues in bipolar II disorder diagnosis are hypomanic episodes that are difficult to recognize both by patients themselves and their relatives. Most patients do not consider such conditions as illness and, consequently, do not seek medical help [23]. This is especially true of adolescents who may like the state of elevated mood and increased energy. They may exacerbate this condition by taking psychoactive substances followed by risky behavior [24].

It should also be noted that in the temporal aspect of BD dynamics, depressive episodes are predominant, and their differentiation from recurrent unipolar depression poses a clinical problem [25]. Depression is initially considered unipolar in over 40% of patients who are later diagnosed with bipolar disorder [36]. There is also evidence that diagnosis and adequate

treatment of BD are delayed by 6–8 years or more, especially with the onset of the disease in adolescence [26–29].

An early onset of the disease is also associated with a high suicidal risk, the addition of a comorbid pathology, and a quick-cycle course [30–32].

Timely diagnosis and treatment of BD play an important role in slowing down the development of mood disorder episodes, which will enable to reduce the likelihood of cognitive impairment and deterioration in the quality of life, as well as to prevent premature mortality caused by suicide and cardiovascular diseases [33].

The aim of this study was to analyze the structure of affective fluctuations in a non-clinical sample using screening methods.

MATERIALS AND METHODS

The study involved 129 students enrolled in academic programs of higher education at a medical university. The average age was 18.95 ± 0.08 years. The students included 25.6% of men ($n = 33$), and 74.4% of women ($n = 96$). All the respondents had previously given their written informed consent to participate in this study. The clinical and psychopathological method and the Mini-International Neuropsychiatric Interview (MINI) were used in the study [34]. The Hypomania Checklist-32 (HCL-32) for BD was used to diagnose hypomania [35, 36]. The Hamilton Rating Scale for Depression (HAM-D-17) was used to assess the severity of depression [37].

Statistical processing of the data was carried out by methods of descriptive statistics. The 95% confidence interval (CI) was constructed according to the formula for fractions and frequencies by the Wald test. To study the factorial structure of the episodes of mood swings according to the HCL-32 and the HAM-D-17 scales, factor analysis (principal component analysis) was applied. The decision regarding the number of factors was made based on the most profound explanation of the sample variance. Statistical analysis of the results was performed in Microsoft Excel 16 using the Data Analysis and AtteStat 12.0.5 add-ins.

RESULTS

During the clinical and psychopathological examination of the respondents with application of the MINI, mental disorders, including affective pathology, were not identified. When using the HCL-32 questionnaire, the corresponding recommendations of the developer (J. Angst 2005) were taken into account to

confirm the tendency to hypomania (total score ≥ 14). In the studied sample, the mean value according to the HCL-32 scale was 14.80 ± 0.44 (min = 4, max = 30). The total score in 79 respondents (61.2%; 95% CI 52.83–69.65) exceeded the threshold value.

Analysis of separate items on the HCL-32 scale across the entire sample revealed that when comparing their condition with that of other people, 44.9% ($n = 58$; 95% CI 36.38–53.55) of the respondents called their condition stable; 27.13% ($n = 35$; 95% CI 19.46–34.80) of the respondents noted the alternation of ups and downs in their mood. Analysis of separate items on the HCL-32 scale across the entire sample revealed that most of the participants positively assessed the effect of mood elevations on the family sphere (63.57%; $n = 82$), social activities (68.99%; $n = 89$), work (75.19%; $n = 91$), and leisure (82.17%; $n = 106$). Additionally, the subjective assessment of the reaction of others to the mood elevation episodes was, in most cases, interpreted by the respondents as positive (36.43%; $n = 47$; 95% CI 28.13–44.74) and neutral (37.21%; $n = 48$; 95% CI 33.35–50.37). Only 3.18% ($n = 17$; 95% CI –0.55–7.21) of the respondents reported negative assessment of their mood elevation episodes by people around them.

The subjective assessment of the duration of mood elevation episodes ranged from 1 month ($n = 5$; 3.88%; 95% CI 0.55–7.21) to 1 day ($n = 30$; 23.26%; 95% CI 15.97–30.59). 23.26% ($n = 30$; 95% CI 15.97–30.53) of the respondents were unable to estimate the duration of a mood elevation episode. The presence of mood elevation episodes over the last 12 months was reported by all the respondents (100%; 95% CI 100.0–100.0).

The next stage of the study was aimed at investigating the structure of mood elevation episodes according to the HCL-32 questionnaire using factor analysis among the respondents ($n = 79$), whose total score exceeded the screening threshold (≥ 14 points). In total, 11 factors were identified in the structure of the mood elevation episode, the aggregate variance percentage of which was equal to 66.9%. The most significant values were noted in 8 factors. The first factor (Factor I) was formed by the following scales presented in Table 1:

Table 1

The structure of Factor I according to the HCL-32 questionnaire findings in the non-clinical sample		
Scale No.	HCL-32 items	Factor I
9.	In everyday life, I take risks more often	–0.550
21.	I'm more easily distracted	–0.467

Table 1 (continued)

Scale No.	HCL-32 items	Factor I
25.	I am more impatient and / or irritated faster	-0.684
26.	I can be tiresome and irritating	-0.585
27.	I find myself in conflict situations more often	-0.665

Table 1 shows that the parameters associated with irritability ($r = -0.684$), conflict behavior ($r = -0.665$), risky behavior ($r = -0.550$), and increased distraction ($r = -0.467$) had the most significant values in Factor I.

Significant values of Factor II parameters are presented in Table 2.

Table 2

The structure of Factor II according to the HCL-32 questionnaire findings in the non-clinical sample		
Scale No.	HCL-32 items	Factor II
2.	I'm more energetic and active	0.556
16.	I have an increased interest in sex and / or increased sexual desire	0.527
18.	I am more talkative	0.468
20.	In conversations, I often joke and pun	0.418
28.	I'm in high spirits and more optimistic	0.481
29.	I drink more coffee	0.514

Factor II showed significant values for such parameters as increased energy and activity ($r = 0.556$), increased sexual desire ($r = 0.527$), drinking more coffee ($r = 0.514$), elevated mood ($r = 0.481$), and increased talkativeness ($r = 0.468$).

Factor III with its significant values is presented in Table 3.

Table 3

The structure of Factor III according to the HCL-32 questionnaire findings in the non-clinical sample		
Scale No.	HCL-32 items	Factor III
3.	I'm more self-confident	0.509
4.	I enjoy my work more	0.540
11.	I make more plans and projects	0.528
12.	I have more creative ideas	0.511
22.	I find many new activities to do	0.506
32.	I take more medications (sedatives, anxiolytics, stimulants)	0.444

The contribution of Factor III is associated with getting more pleasure in the performed activities ($r = 0.540$), emergence of plans and projects ($r = 0.528$), creative ideas ($r = 0.511$), parameters of increased self-confidence ($r = 0.509$), and the use of stimulating substances ($r = 0.444$).

The contribution of factor IV included the following parameters: an increase in the number of trips associated with the desire to travel ($r = 0.535$), risky driving style ($r = 0.523$), racing thoughts ($r = -0.480$), and spending large amounts of money ($r = 0.441$).

Factor V is represented by a parameter associated with increased swiftness and ease of performing habitual activities ($r = 0.441$). In Factor VI, parameters of increased physical activity ($r = 0.668$) and decreased need for sleep ($r = 0.668$) had the most significant values. The significant value ($r = 0.539$) of Factor VIII is represented by the parameter associated with a desire to dress brightly and extravagantly.

The Hamilton Scale (HAMD-17) was used to study depressive mood swings. In the studied sample, the mean value on the scale was 6.51 ± 0.39 (min = 0, max = 13), indicating the absence of depression symptoms in the general sample. For 84 respondents (65.1%), the total score did not exceed the threshold values and ranged from 0 to 7. 45 respondents (38.4%) scored from 8 to 13 points.

The next stage of the study was analyzing the structure of mood drop episodes according to the HAMD-17 scale using factor analysis among the respondents ($n = 45$), whose total score exceeded the normal values and ranged from 8 to 13 corresponding to manifestations of mild depression. While identifying the maximum number of the factors, taking into account the Kaiser criterion, the structure of depressive states was represented by 6 factors and explained 60.99% of the variance of the sample features. The factor analysis of the structure of depression episodes is presented in Table 4.

Table 4

Factor analysis of the structure of depressive mood according to the HAMD-17 scale (factor loadings; variance)						
HAMD-17 factors	Factor I	Factor II	Factor III	Factor IV	Factor V	Factor VI
Depressive mood	-0.367	-0.356	0.161	0.579	0.103	0.102
Feeling of guilt	0.209	-0.504	0.287	-0.191	-0.475	0.014
Suicidal tendencies	-0.082	0.682	0.257	0.281	0.130	0.305
Early insomnia (difficulty with falling asleep)	0.693	0.028	0.050	0.010	-0.025	-0.253
Middle-of-the-night insomnia (interrupted sleep)	0.663	0.292	0.085	0.190	-0.021	-0.190
Late insomnia (early awakenings)	0.537	0.030	0.115	0.028	0.272	0.249
Work and interests	-0.388	0.112	0.290	0.520	-0.252	-0.214

Table 4 (continued)

HAMD-17 factors	Factor I	Factor II	Factor III	Factor IV	Factor V	Factor VI
Inertia	–0.299	0.154	–0.247	0.053	–0.524	–0.041
Tension	–0.378	0.032	–0.642	–0.362	0.401	0.069
Somatic symptoms in the gastrointestinal tract	0.001	0.513	0.164	–0.158	–0.185	0.644
General somatic symptoms	–0.050	0.415	–0.508	–0.160	–0.143	–0.411
Genital disorder	–0.290	–0.068	–0.098	–0.153	–0.484	0.403
Hypochondria	0.010	–0.453	–0.359	0.466	0.114	0.142
Weight loss	–0.447	0.399	0.194	0.245	0.238	–0.363
Criticism of the disease	0.266	0.315	–0.326	0.229	–0.099	0.180
% of accumulated variance	60.99					

The four factors contributed the most to the overall variance. Parameters associated mainly with dyssomnia had significant Factor I values: difficulty with falling asleep ($r = 0.693$), interrupted sleep ($r = 0.663$), and early awakening ($r = 0.537$).

Factor II had significant values in terms of guilt feelings ($r = -0.504$), suicidal tendencies ($r = 0.682$), general somatic and gastrointestinal dysfunction symptoms, hypochondria and weight loss.

The contribution of Factor III is associated with the tension parameter ($r = -0.642$) and general somatic symptoms ($r = -0.508$). The contribution of Factor IV to the total variance was 9.73% and included the following: decreased performance ($r = 0.520$), depressive mood ($r = 0.579$), and hypochondria ($r = 0.466$).

DISCUSSION

Diagnosis of hypomanic and subdepressive states at the preclinical stage presents difficulties associated with the subjective assessment of the condition by the respondents themselves and with the interpretation of their state by their immediate circle. The subclinical mood elevations identified in 61.2% ($n = 79$) of the respondents were, in most cases, assessed by the respondents and their immediate circle as positive and advantageously affecting family relationships, social activities, and leisure. A qualitative analysis showed that the structure of mood elevation episodes was dominated by such signs of hypomania as increased energy and general activity, irritability, risky behavior, decreased need for sleep, use of stimulating substances, increased sexual desire, and increased talkativeness, which are identical to the clinical manifestations of a BD hypomanic episode.

J. Angst et al. (2020) conducted a comparative analysis of diagnostic criteria for BD using the DSM-V (APA, 2013) and the ICD-11 Project Plan. They noted that, unlike the DSM-IV-TR and ICD-10, in the DSM-V and the ICD-11 Project Plan, the main

criteria of the diagnosis of a hypomanic episode, in addition to changes in mood (euphoria, irritability), emphasize constantly increasing activity, a surge of strength, and a subjective feeling of energy increase. This, according to the authors, is essential for describing more complete and accurate clinical presentation of the disorder [38].

Low mood and associated manifestations of dyssomnia, general somatic symptoms, mental and somatic anxiety, as well as deterioration in working capacity were detected in 34.8% of the subjects only upon careful questioning in the absence of active complaints from the respondents and corresponded to the degree of mild depression according to the HAMD-17 Scale. Moreover, the detected signs of depressive conditions at the preclinical stage tended to be associated with general somatic symptoms, which can indirectly indicate formation of comorbid pathology. The chronological sequence study of the occurrence of comorbid conditions in BD can also facilitate early diagnosis and timely treatment. According to many authors, most comorbid disorders occur long before the onset of the underlying disease.

Studies by E. A. Frazier, J. I. Hunt et al. (2020) addressed comorbid disorders preceding BD. It was found that alcohol and cannabis abuse in men preceded bipolar I disorder, while anxiety and eating disorders were more often identified before the onset in women. According to the authors, timely treatment of comorbid disorders preceding the BD onset can act as a measure of secondary prevention of the underlying disease [39].

The findings are consistent with the concept of subthreshold depression, which has high prevalence in adolescent age and is associated with concomitant somatic pathology and functional impairment. In a study of 2,022 Chilean adolescents in grades 9–11, it was found that subthreshold depression in girls, apart from low mood, manifested itself through sleep problems. Boys, on the other hand, showed more pronounced

anhedonia, impaired concentration, and psychomotor retardation or agitation [40].

The studies by A.R. van Meter et al. (2019) identified prodromal symptoms preceding the first episode of mood disorder. In more than half of the participants (51%), the affective episode was preceded by a symptom of increased energy. The researchers identified more than 40 different prenosological symptoms, which generally indicated heterogeneity of premorbid manifestations. This study also showed that the onset of BD was gradual [41].

The existing data on the presence of more than one prodromal symptom in the majority of patients may further help to identify more accurate clusters of signs that could serve as convincing criteria for the prognosis, prevention, and early intervention in bipolar disorder.

CONCLUSION

During this clinical and psychopathological study, in accordance with the criteria of ICD-10 (class V: mental and behavioral disorders (F00–F99)), affective pathology was not identified. According to the HCL-32 screening technique, 61.2% ($n = 79$) of the respondents exceeded the threshold value with their total score. Analysis of individual items on the HCL-32 scale across the entire sample revealed that most of the subjects positively assessed the influence of mood elevations on the family sphere (63.57%; $n = 82$), social activities (68.99%; $n = 89$), work (75.19%; $n = 91$), and leisure (82.17%; $n = 106$). Positive (36.43%; $n = 47$) and neutral (37.21%; $n = 48$) assessments of mood elevations were also reported by respondents' immediate circle, which, in general, significantly complicates recognition of hypomanic symptoms and delays seeking specialized care.

The structure of mood elevation episodes was represented by 11 factors, with the largest contribution to the total variance made by such signs as irritability ($r = -0.684$), conflicts ($r = -0.665$), risky behavior ($r = -0.550$), increased sexual desire ($r = 0.527$), increased energy and activity ($r = 0.431$), distraction ($r = -0.467$), use of stimulants ($r = -0.467$), and decreased need for sleep ($r = 0.408$), which are similar to the clinical manifestations of a hypomanic episode in bipolar II disorder.

The signs of mild depression identified by the HAMD-17 scale in 34.8% ($n = 45$) of the respondents were represented by sleep disorders ($r = 0.693$), decreased performance ($r = 0.520$), depressive mood ($r = 0.579$), hypochondria ($r = 0.466$), general so-

matic symptoms ($r = -0.508$), and gastrointestinal dysfunction ($r = 0.513$), were not active complaints in nature, and did not serve as a reason for seeking specialized care.

Therefore, in terms of mood swings within the non-clinical sample, elevations that were not subjectively identified as illness symptoms and did not take the form of complaints during the clinical and psychopathological examination prevailed. Low mood was accompanied by general somatic symptoms, which may indicate subsequent formation of comorbid pathology. The identified subsyndromal signs of hypomania and depression in the non-clinical sample in the absence of active complaints or psychiatric help-seeking are of clinical significance as predictors of bipolar affective pathology and require further clinical and dynamic monitoring for early diagnosis and timely therapeutic intervention.

REFERENCES

1. Post R.M., Goldstein B.I., Birmaher B., Findling R.L., Frey B.N., DelBello M.P., Miklowitz D.J. Toward prevention of bipolar disorder in at-risk children: potential strategies ahead of the data. *J. Affect. Disord.* 2020; 272: 508–520. DOI: 10.1016/j.jad.2020.03.025.
2. Perlis R.H., Miyahara S., Marangell L.B., Wisniewski S.R., Ostacher M., DelBello M.P., Bowden C.L., Sachs G.S., Nierenberg A.A., STEP-BD Investigators. Long-term implications of early onset in bipolar disorder: data from the first 1000 participants in the systematic treatment enhancement program for bipolar disorder (STEP-BD). *Biol. Psychiatry.* 2004; 55 (9):875–881. DOI: 10.1016/j.biopsych.2004.01.022.
3. Correll C.U., Hauser M., Auther A.M., Cornblatt B.A. Research in people with psychosis risk syndrome: a review of the current evidence and future directions. *J. Child Psychol. Psychiatry.* 2010; 51 (4):390–431. DOI: 10.1111/j.1469-7610.2010.02235.x.
4. Fusar-Poli P., Bonoldi I., Yung A.R., Borgwardt S., Kemp-ton M.J., Valmaggia L., Barale F., Caverasi E., McGuire P. Predicting psychosis: meta-analysis of transition outcomes in individuals at high clinical risk. *Arch. Gen. Psychiatry.* 2012; 69 (3): 220–229. DOI: 10.1001/archgenpsychiatry.2011.1472.
5. Luby J.L., Navsaria N. Pediatric bipolar disorder: evidence for prodromal states and early markers. *J Child Psychol. Psychiatry.* 2010; 51 (4): 459–471. DOI: 10.1111/j.1469-7610.2010.02210.x.
6. Skjelstad D.V., Malt U.F., Holte A. Symptoms and signs of the initial prodrome of bipolar disorder: a systematic review. *J. Affect. Disord.* 2010; 126 (1-2): 1–13. DOI: 10.1016/j.jad.2009.10.003.
7. Pavuluri M.N. Effects of early intervention on the course of bipolar disorder: theories and realities. *Curr. Psychiatry Rep.* 2010; 12 (6):490–498. DOI: 10.1007/s11920-010-0155-1.
8. McNamara R.K., Nandagopal J.J., Strakowski S.M., DelBello M.P. Preventative strategies for early-onset bipolar disorder:

- towards a clinical staging model. *CNS Drugs*. 2010; 24 (12): 983–996. DOI: 10.2165/11539700-000000000-00000.
9. Leopold K., Ritter P., Correll C.U., Marx C., Özgürdal S., Juckel G., Bauer M., Pfennig A. Risk constellations prior to the development of bipolar disorders: rationale of a new risk assessment tool. *J. Affect. Disord.* 2012; 136 (3): 1000–1010. DOI: 10.1016/j.jad.2011.06.043.
 10. Brietzke E., Mansur R.B., Soczynska J.K., Kapczinski F., Bressan R.A., McIntyre R.S. Towards a multifactorial approach for prediction of bipolar disorder in at risk populations. *J. Affect. Disord.* 2012; 140 (1): 82–91. DOI: 10.1016/j.jad.2012.02.016.
 11. Correll C.U., Penzner J.B., Frederickson A.M., Richter J.J., Auther A.M., Smith C.W., Kane J.M., Cornblatt B.A. Differentiation in the preonset phases of schizophrenia and mood disorders: evidence in support of a bipolar mania prodrome. *Schizophr. Bull.* 2007; 33 (3): 703–714. DOI: 10.1093/schbul/sbm028.
 12. Post R.M., Altshuler L., Kupka R., McElroy S.L., Frye M.A., Rowe M., Grunze H., Suppes T., Keck P., Leverich G., Nolen W. More childhood onset bipolar disorder in the United States than Canada or Europe: implications for treatment and prevention. *Neurosci. Biobehav. Rev.* 2017; 74 (A): 204–213. DOI: 10.1016/j.neubiorev.2017.01.022.
 13. Post R.M., Leverich G.S., Kupka R.W., Keck P.E. Jr., McElroy S.L., Altshuler L.L., Frye M.A., Luckenbaugh D.A., Rowe M., Grunze H., Suppes T., Nolen W.A. Early-onset bipolar disorder and treatment delay are risk factors for poor outcome in adulthood. *J. Clin. Psychiatry*. 2010; 71 (7): 864–872. DOI: 10.4088/JCP.08m04994yel.
 14. Akiskal H.S., Pinto O. The evolving bipolar spectrum: prototypes I, II, III, and IV. *Psychiatr. Clin. North Am.* 1999; 22 (3): 517–534. DOI: 10.1016/s0193-953x(05)70093-9.
 15. Yong R.C., Klerman G.L. Mania in late life: focus on age at onset. *Am. J. Psychiatry*. 1992; 149 (7): 867–876. DOI: 10.1176/ajp.149.7.867.
 16. American Psychiatric Association. Diagnostic and statistical manual of mental disorders; 4th ed. Text revision (DSM-IV-TR). 2000: 943.
 17. American Psychiatric Association. Diagnostic and statistical manual of mental disorders: DSM-5TM; 5th ed. Arlington: American Psychiatric Association, 2013.
 18. Reed G.M., First M.B., Kogan C.S., Hyman S.E., Gureje O., Gaebel W. et al. Innovations and changes in the ICD-11 classification of mental, behavioural and neurodevelopmental disorders. *World Psychiatry*. 2019; 18 (1):3–19. DOI: 10.1002/wps.20611.
 19. Mental and behavioral disorders (F00–F99). ICD-10 Class V adapted for use in the Russian Federation; edited by B.A. Kazakovtseva, V.B. Holland. M.: Ministry of Health of Russia; 1998: 512 (in Russ.).
 20. Kovalev V.V. Child psychiatry (a guide for doctors). M.: Medicine, 1979: 608 (in Russ.).
 21. Lichko A.E. Psychopathies and character accentuations in adolescents; 2nd ed., suppl. and revised. L.: Medicine, 1983: 255 (in Russ.).
 22. Merikangas K.R., He J.P., Burstein M., Swanson S.A., Avenevoli S., Cui L., Benjet C., Georgiades K., Swendsen J. Lifetime prevalence of mental disorders in U.S. adolescents: results from the national comorbidity survey replication–adolescent supplement (NCS-A). *J. Am. Acad. Child Adolesc. Psychiatry*. 2010; 49 (10): 990–989. DOI: 10.1016/j.jaac.2010.05.017.
 23. Wium-Andersen I.K., Vinberg M., Kessing L.V., McIntyre R.S. Personalized medicine in psychiatry. *Nord J. Psychiatry*. 2017; 71 (1):12–19. DOI: 10.1080/08039488.2016.1216163.
 24. Goldstein B.I., Birmaher B., Carlson G.A., DelBello M.P., Findling R.L., Fristad M., Kowatch R.A., Miklowitz D.J., Nery F.G., Perez-Algorta G., Van Meter A., Zeni C.P., Correll C.U., Kim H.W., Wozniak J., Chang K.D., Hillegers M., Youngstrom E.A. The international society for bipolar disorders task force report on pediatric bipolar disorder: knowledge to date and directions for future research. *Bipolar Disord.* 2017; 19 (7): 524–543. DOI: 10.1111/bdi.12556.
 25. Bardenshteyn L.M., Slavgorodsky Y.M., Beglyankin N.I., Kekelidze D., Aleshkina G.A. Early recognition of bipolar depression. *International Journal of Neuropsychopharmacology*. 2016; 19 (S1): 21–22. DOI: 10.1093/ijnp/pyw043.062.
 26. Baldessarini R.J., Tondo L., Violi C. First-episode types in bipolar disorder: predictive associations with later illness. *Acta Psychiatr. Scand.* 2014; 129 (5): 383–392. DOI: 10.1111/acps.12204.
 27. Yildiz A., Nemeroff C., Ruiz P. (ed.). The bipolar book: history, neurobiology, and treatment. New York: Oxford University Press, 2015: 686.
 28. Baldessarini R.J., Tondo L., Vázquez G.H. Unmet needs in psychiatry: bipolar depression. In: Pompili M., McIntyre R.S., Fiorillo A., Sartorius N. (ed.). New directions in psychiatry. New York: Springer Press, 2020.
 29. Vöhringer P.A., Perlis R.H. Discriminating between bipolar disorder and major depressive disorder. *Psychiatr. Clin. N. Am.* 2016; 39 (1): 1–10. DOI: 10.1016/j.psc.2015.10.001.
 30. Goldstein B.I., Blanco C., He J.P., Merikangas K. Correlates of overweight and obesity among adolescents with bipolar disorder in the national comorbidity survey-adolescent supplement (NCS-A). *J. Am. Acad. Child Adolesc. Psychiatry*. 2016; 55 (12): 1020–1026. DOI: 10.1016/j.jaac.2016.08.010.
 31. Mota N.B., Copelli M., Ribeiro S. Computational tracking of mental health in youth: Latin American contributions to a low-cost and effective solution for early psychiatric diagnosis. *New Directions for Child and Adolescent Development*. 2016; 2016 (152): 59–69. DOI: 10.1002/cad.20159.
 32. Jamison K.R. Suicide and Bipolar Disorder. *J. Clin. Psychiatry*. 2000; 61 (19): 47–51.
 33. Vieira D.C., de Azevedo Cardoso T., Mondin T.C., Jansen K., da Silva R.A., de Mattos Souza L.D., Kapczinski F., Magalhães P.V.S. Mood disorders and prospective suicidality in young adults: a population-based cohort study. *Acta Psychiatr. Scand.* 2018; 137 (2):109–115. DOI: 10.1111/acps.12846.
 34. Sheehan D.V., Lecrubier Y., Sheehan K.H., Amorim P., Janavs J., Weiller E. The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *J. Clin. Psychiatry*. 1998; 59 (20): 22–33.
 35. Angst J., Adolfsson R., Bennazzi F., Gamma A., Hantouche E., Meyer T.D., Skeppar P., Vieta E., Scott J. The HCL-32: Towards a self-assessment tool for hypomanic symptoms in

- outpatients. *J. Affect. Disord.* 2005; 88 (2):217–233. DOI: 10.1016/j.jad.2005.05.011.
36. Mosolov S.N., Ushkalova A.V., Kostukova E.G., Shafarenko A.A., Alfimov P.V., Kostyukova A.B., Angst J. Validation of the Russian version of the Hypomania Checklist (HCL-32) for the detection of bipolar II disorder in patients with a current diagnosis of recurrent depression. *J. Affect. Disord.* 2014; 155: 90–95. DOI: 10.1016/j.jad.2013.10.029.
 37. Hamilton M. Development of a rating scale for primary depressive illness. *Br. J. Soc. Clin. Psychol.* 1967; 6 (4): 278–296. DOI: 10.1111/j.2044-8260.1967.tb00530.x.
 38. Angst J., Ajdacic-Gross V., Roessler W. Bipolar disorders in ICD-11: current status and strengths. *Int. J. Bipolar Disord.* 2020; 8 (1): 3. DOI: 10.1186/s40345-019-0165-9.
 39. Frazier E.A., Hunt J.I., Hower H., Jones R.N., Birmaher B., Strober M., Goldstein B.I., Keller M.B., Goldstein T.R., Weinstock L.M., Dickstein D.P., Diler R.S., Ryan N.D., Gill M.K., Axelson D., Yen S., Loftus J., Scott J., Vorspan F., Icick R., Henry C., Gard S., Kahn J.P., Leboyer M., Bellivier F., Etain B. Psychiatric comorbidities in bipolar disorders: An examination of the prevalence and chronology of onset according to sex and bipolar subtype *J. Affect. Disord.* 2020; 267: 258–263. DOI: 10.1016/j.jad.2020.02.035.
 40. Crockett M.A., Martínez V., Jiménez-Molina Á. Sub-threshold depression in adolescence: Gender differences in prevalence, clinical features, and associated factors. *J. Affect. Disord.* 2020; 272: 269–276. DOI: 10.1016/j.jad.2020.03.111.
 41. Van Meter A.R., Moreira A.L., Youngstrom E.A. Meta-analysis of epidemiologic studies of pediatric bipolar disorder. *J. Clin. Psychiatry.* 2011; 72 (9): 1250–1256. DOI: 10.4088/JCP.10m06290.

Authors information

Osipova Natalia N., Cand. Sci. (Med.), Associate Professor, Department of Psychiatry and Narcology, Moscow University of Medicine and Dentistry n.a. A.I. Evdokimov, Moscow, Russian Federation. ORCID 0000-0002-8034-4457.

Bardenstein Leonid M., Dr. Sci. (Med.), Professor, Honored Doctor of the Russian Federation, Head of the Department of Psychiatry and Narcology, Moscow University of Medicine and Dentistry n.a. A.I. Evdokimov, Moscow, Russian Federation. ORCID 0000-0002-1171-5517.

Aleshkina Galina A., Dr. Sci. (Med.), Professor, Department of Psychiatry and Narcology, Moscow University of Medicine and Dentistry n.a. A.I. Evdokimov, Moscow, Russian Federation. ORCID 0000-0001-7028-8669.

Dmitrieva Elena V., Senior Lecturer, Department of Physics, Mathematics and Medical Informatics, Smolensk State Medical University, Smolensk, Russian Federation. ORCID 0000-0003-0375-0870.

(✉) **Osipova Natalia N.**, e-mail: natinen@yandex.ru

Received 18.12.2020

Accepted 27.05.2021

The role of the autonomic nervous system in stress cardiomyopathy

Prokudina E.S., Maslov L.N., Naryzhnaya N.V., Kurbatov B.K., Mukhomedzyanov A.V., Shipulin V.V., Krylatov A.V., Tsibulnikov S.Yu., Voronkov N.S.

Cardiology Research Institute, Tomsk National Research Medical Center (NRMС), Russian Academy of Sciences 111A, Kievskaya Str., Tomsk, 634012, Russian Federation

ABSTRACT

Aim. To identify the role of the autonomic nervous system in stress cardiomyopathy in an experimental model of Takotsubo syndrome.

Materials and methods. The study was carried out on 120 female Wistar rats. Stress modeling was performed by immobilizing animals on the back for 24 hours. Intact rats were used as controls. The rats were decapitated after termination of immobilization under general anesthesia with ether. Stress cardiomyopathy (SCM) was quantified by accumulation of ^{99m}Tc pyrophosphate radiopharmaceutical (^{99m}Tc PP) in the myocardium. The pharmacological agents used included the ganglionic blocker hexamethonium, administered five times at a dose of 20 mg / kg; guanethidine (50 mg / kg) administered subcutaneously once a day for three days, the last injection was performed 24 hours before immobilization; the muscarinic receptor antagonist atropine methyl nitrate (1 mg / kg); the α_1 -AR (adrenergic receptor) antagonist prazosin (2 mg / kg); the α_2 -AR antagonist yohimbine, administered at a dose of 2 mg / kg; the β_1 -AR antagonist nebivolol (1.2 mg / kg); the β_2 -AR antagonist ICI 118,551 (0.3 mg / kg); and the β_3 -AR antagonist L-748337 (0.1 mg / kg).

Results. Three-day administration of guanethidine caused a decrease in the degree of ^{99m}Tc -PP accumulation in the heart by 35.9%. Hexamethonium did not affect the degree of SCM. The blockade of the muscarinic receptor caused an increase in accumulation of ^{99m}Tc -PP by 26.5%. Inhibition of α_1 -AR did not affect SCM. The blockade of α_2 -AR caused a 2.2-fold increase in the accumulation compared with stress control. The blockade of β_1 -AR reduced ^{99m}Tc -PP accumulation by 2.5 times. The blockade of β_2 -AR by ICI 118,551 increased the degree of ^{99m}Tc -PP accumulation by 34.6%. Inhibition of β_3 -AR had no effect on SCM.

Conclusion. The adrenergic system and β_1 -adrenergic receptor play an important role in the development of SCM. The parasympathetic nervous system ensures resistance of the heart to stress.

Key words: stress, heart, autonomic nervous system, Takotsubo syndrome, adrenergic nervous system.

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

Source of financing. This work was performed within the state assignment AAAA-A15-115120910024-0.

Conformity with the principles of ethics. The study was approved by the Ethics Committee of Cardiology Research Institute, Tomsk NRMС (Protocol No. 192 of 18.12.2019).

For citation: Prokudina E.S., Maslov L.N., Naryzhnaya N.V., Kurbatov B.K., Mukhomedzyanov A.V., Shipulin V.V., Krylatov A.V., Tsibulnikov S.Yu., Voronkov N.S. The role of the autonomic nervous system in stress cardiomyopathy. *Bulletin of Siberian Medicine*. 2021; 20 (3): 88–94. <https://doi.org/10.20538/1682-0363-2021-2-88-94>.

Роль вегетативной нервной системы в стресс-индуцированном повреждении сердца

Прокудина Е.С., Маслов Л.Н., Нарыжная Н.В., Курбатов Б.К., Мухомедзянов А.В., Шипулин В.В., Крылатов А.В., Цибульников С.Ю., Воронков Н.С.

Научно-исследовательский институт (НИИ) кардиологии, Томский национальный исследовательский медицинский центр (НИМЦ) Российской академии наук
Россия, 634012, г. Томск, ул. Киевская, 111А

РЕЗЮМЕ

Цель. Оценка роли вегетативной нервной системы в стресс-индуцированном повреждении сердца в экспериментальной модели синдрома такотсубо.

Материалы и методы. Исследование выполнено на 120 самках крыс линии Вистар. Каждая группа животных состояла из 12 особей. Моделирование стресса осуществляли с помощью иммобилизации животных на спине в течение 24 ч. В качестве контроля использовали интактных особей. Крыс декапитировали после прекращения иммобилизации под общим эфирным наркозом. Количественную оценку стресс-индуцированного повреждения сердца (СИПС) осуществляли по аккумуляции радиофармпрепарата ^{99m}Tc -пирофосфата (^{99m}Tc -ПФ) в миокарде.

Фармакологические агенты вводили внутривенно: ганглиоблокатор гексаметоний вводили пятикратно в дозе 20 мг/кг; гуанетидин (50 мг/кг) – подкожно 1 раз/сут в течение 3 сут, последнюю инъекцию делали за 24 ч до иммобилизации. Остальные препараты (антагонист М-холинорецепторов атропина метилнитрат (1 мг/кг); антагонист α_1 -адренорецепторов (АР) празозин (2 мг/кг); антагонист α_2 -АР йохимбин (2 мг/кг); антагонист β_1 -АР небиволол (1,2 мг/кг); антагонист β_2 -АР ICI 118,551 (0,3 мг/кг); антагонист β_3 -АР L-748337 (0,1 мг/кг)) вводили 2 раза/сут с интервалом 12 ч.

Результаты. Трехдневное введение гуанетидина вызвало уменьшение степени аккумуляции ^{99m}Tc -ПФ в сердце на 35,9%. Гексаметоний не оказал влияния на степень СИПС. Блокада М-холинорецепторов вызвала усиление аккумуляции ^{99m}Tc -ПФ на 26,5%. Ингибирование α_1 -АР не оказало влияния на СИПС. Блокада α_2 -АР вызвала усиление аккумуляции в 2,2 раза по сравнению со стресс-контролем. Блокада β_1 -АР снизила степень аккумуляции ^{99m}Tc -ПФ в 2,5 раза. Блокада β_2 АР ICI 118,551 увеличила степень аккумуляции ^{99m}Tc -ПФ на 34,6%. Ингибирование β_3 -АР не оказало эффекта на СИПС.

Заключение. Симпатoadренальная система и, в частности, β_1 -адренорецепторы играют важную роль в развитии СИПС. Парасимпатическая нервная система обеспечивает устойчивость сердца к стрессу.

Ключевые слова: стресс, сердце, вегетативная нервная система, синдром такотсубо, адренорецепторы.

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

Источник финансирования. Исследование выполнено в рамках государственного задания ААА-А-А15-115120910024-0.

Соответствие принципам этики. Исследование одобрено этическим комитетом НИИ кардиологии, Томский НИМЦ (протокол № 192 от 18.12.2019).

Для цитирования: Прокудина Е.С., Маслов Л.Н., Нарыжная Н.В., Курбатов Б.К., Мухомедзянов А.В., Шипулин В.В., Крылатов А.В., Цибульников С.Ю., Воронков Н.С. Роль вегетативной нервной системы в стресс-индуцированном повреждении сердца. *Бюллетень сибирской медицины*. 2021; 20 (3): 88–94. <https://doi.org/10.20538/1682-0363-2021-3-88-94>.

INTRODUCTION

In 1974, G. Johansson et al. revealed the existence of stress cardiomyopathy (SCM) in a study carried out on pigs [1]. However, it was only in 1990 that a group of Japanese cardiologists first verified SCM

in humans, calling it Takotsubo syndrome (TS), because the heart of these patients resembled an octopus trap – “takotsubo” [2]. This condition is characterized by dilatation of the left ventricle of the heart, chest pain, an increase in the level of myocardial necrosis markers, contractile dysfunction of the left ventricle,

and ECG changes (prolongation of the QTc interval, T wave inversion, elevation or depression of the ST segment) [3].

Takotsubo syndrome is a serious illness with mortality comparable to that of acute ST-segment elevation myocardial infarction (STEMI) [4]. This is explained by insufficient knowledge about the mechanism of SCM formation and, consequently, a lack of pathogenetically substantiated therapy. It is assumed that the autonomic nervous system plays an important role in the pathogenesis of SCM [5]. The literature indicates the important role of hyperactivation of the sympathoadrenal system in the pathogenesis of TS. In the study by A. Vaccaro et al., it was shown that in TS patients in the subacute period, the activity of the sympathetic nervous system is increased [6]. The level of adrenaline in the blood plasma of patients with TS in the subacute period is higher than 100 days or 12 months after hospitalization [7, 8]; in TS patients, the level of norepinephrine in the blood serum is also elevated [9]. The results of the above-mentioned studies confirm that the development of TS is accompanied by an increase in the activity of the sympathetic system. It is important to note that TS is characterized by higher serum concentrations of catecholamine than in patients with acute coronary syndrome [10]. It is also worth noting that methods of radionuclide scanning reflect the pathophysiological changes occurring in the body during certain pathological processes [11, 12] and with high sensitivity allow to assess the damage to cardiomyocytes *in vivo* [13].

The aim of this study was to assess the role of the autonomic nervous system in stress cardiomyopathy using an experimental model of Takotsubo syndrome.

MATERIALS AND METHODS

The study was approved by the Ethics Committee of the Cardiology Research Institute, Tomsk NRMС. The study was carried out in accordance with the Directive 2010/63/EU of the European Parliament and of the Council of September 22, 2010 on the protection of animals used for scientific purposes.

The study was carried out on 120 female Wistar rats. Stress was simulated by immobilizing the animals on their backs for 24 hours. The animals were randomly divided into groups of 12 animals each in accordance with the pharmacological agent used. To assess the effect of immobilization, a stress control group consisting of 12 animals was introduced. 16

hours before immobilization, the rats were deprived of food while maintaining access to water. The rats were fixed with standard plastic “clamps” manufactured by DKS JSC (Tver, Russia) at the upper and lower extremities, which constrained their movement, but did not cause pain. As our studies have shown, such an effect causes formation of Selye’s three phases of general adaptation (involution of the thymus and spleen, hypertrophy of the adrenal glands, and the appearance of stomach ulcers) [14]. As controls, we used 12 intact animals. The rats were decapitated after the termination of immobilization under general anesthesia with ether.

The quantitative assessment of SCM was carried out by accumulation of the radiopharmaceutical ^{99m}Tc -pyrophosphate (^{99m}Tc -PP) in the myocardium according to the method proposed by D.G. Miller and S. Mallov [13]. The radiopharmaceutical, obtained using a TEKCIS technetium-99m generator (France), was injected intravenously at a dose of 150 MBq / kg 30 minutes after the termination of immobilization. 100 minutes after the injection, the animals were decapitated under ether anesthesia. The incorporation of ^{99m}Tc -PP into the myocardial tissue was calculated as a percentage of the administered dose per 1 gram of tissue. After removal from the chest, the heart was washed from the blood by perfusing it with normal saline (10 ml) through the aorta. Radioactivity was recorded using a Philips Forte gamma camera (Philips, Netherlands).

The preparations were administered intraperitoneally two times: the first injection was carried out 30 minutes before immobilization and 12 hours after immobilization (except for guanethidine and hexamethonium). For pharmacological denervation, the ganglionic blocker hexamethonium chloride was used, which was injected five times at a dose of 20 mg / kg with an interval of 4 h 48 min [15]. Chemical sympathectomy was induced by administration of guanethidine. Guanethidine (50 mg / kg) was injected subcutaneously once a day for three days, the last injection was performed 24 hours before immobilization [16]. For the blockade of peripheral muscarinic receptors, atropine methyl nitrate was used, which was injected twice at a dose of 1 mg / kg [17]. Prazosin (2 mg / kg) was used to block α_1 -adrenergic receptors (AR) [18]. Yohimbine, an α_2 -AR antagonist, was administered at a dose of 2 mg / kg [19]. Nebivolol, a selective β_1 -AR antagonist, was used at a dose of 1.2 mg / kg [20]. The selective β_2 -AR antagonist ICI 118,551 was used at a dose of 0.3 mg / kg [21]. The selective β_3 -AR

antagonist L-748337 was administered at a dose of 0.1 mg / kg [22].

Statistical processing of the obtained data was carried out using the Statistica 13 software (StatSoft Inc., USA, AXA001J575030FAACD-K). To assess the statistical significance of differences between the groups, the Mann – Whitney test was used. The results were expressed as the mean and the standard error of the mean ($M \pm SD$). In all cases, $p \leq 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

As it can be seen from Figure 1, depletion of endogenous catecholamines after three-day administration of guanethidine reduced the ^{99m}Tc -PP accumulation in the rat myocardium by 35.9% compared with the stress control. The observed fall indicates a decrease in SCM after immobilization stress. This fact confirms that endogenous catecholamines are involved in the development of stress-induced damage to the myocardium. The introduction of hexamethonium had no effect on the degree of accumulation (Fig. 1). It was found that when administered intravenously, the effect from hexamethonium (10 mg/kg) lasted 60 minutes [23]. It is possible that the absence of the effect of hexamethonium in our study is determined by a short-termed effect of hexamethonium. In addition, it is possible that the lack of the effect of hexamethonium is associated with the fact that the medication blocks both sympathetic and parasympathetic ganglia. This assumption is based on our hypothesis that activation of the sympathoadrenal system contributes to cardiomyopathy, and stimulation of the parasympathetic link of the autonomic nervous system increases cardiac resistance to SCM.

Our hypothesis was also confirmed in a series of experiments with the blockade of muscarinic receptors using atropine methyl nitrate (Fig. 1). When using this pharmacological agent, we observed an increase in the accumulation of ^{99m}Tc -PP by 26.5% compared with the stress controls, which confirmed our hypothesis about the protective role of the activation of the parasympathetic nervous system. Our result is consistent with the data obtained by R.Q. Xue et al. [24]. Their study showed that stimulation of the vagus has a cardioprotective effect in myocardial damage after administration of isoproterenol. One of the mechanisms of adrenergic damage to the heart is calcium overload in cardiomyocytes, while acetylcholine attenuates calcium overload [25]. Therefore, activation of muscarinic receptors can inhibit SCM.

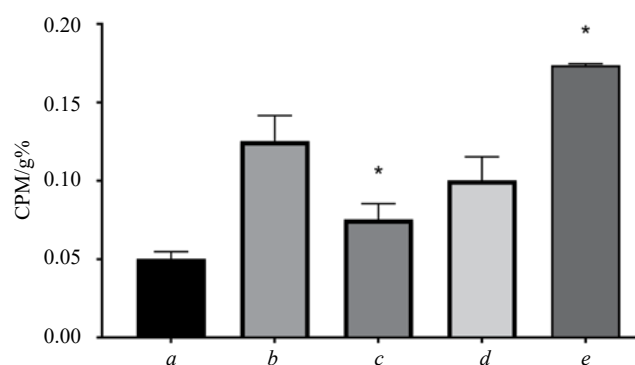


Fig.1. The degree of ^{99m}Tc -pyrophosphate accumulation during stress: after depletion of endogenous catecholamines with guanethidine; after pharmacological denervation with hexamethonium; with blockade of the muscarinic receptor with atropine methyl nitrate: *a* – intact animals, *b* – stress controls; *c* – stress + guanethidine; *d* – stress + hexamethonium; *e* – stress + atropine methyl nitrate; * $p < 0.05$ compared with the stress controls

During the experiments, data were obtained on the role of α -AR in SCM. As it can be seen from Figure 2, the blockade of α_1 -adrenergic receptors with prazosin did not affect the accumulation of ^{99m}Tc -PP during stress. Blockade of α_2 -adrenergic receptors with yohimbine (Fig. 2) caused an increase in ^{99m}Tc -PP by 220% compared with stressed animals. Yohimbine is known to block α_2 -ARs located on the sympathetic terminals, which leads to the release of norepinephrine [26]. Obviously, under stress, yohimbine causes an increase in the release of norepinephrine from the adrenergic nerve terminals that innervate the heart, which results in an increase in SCM.

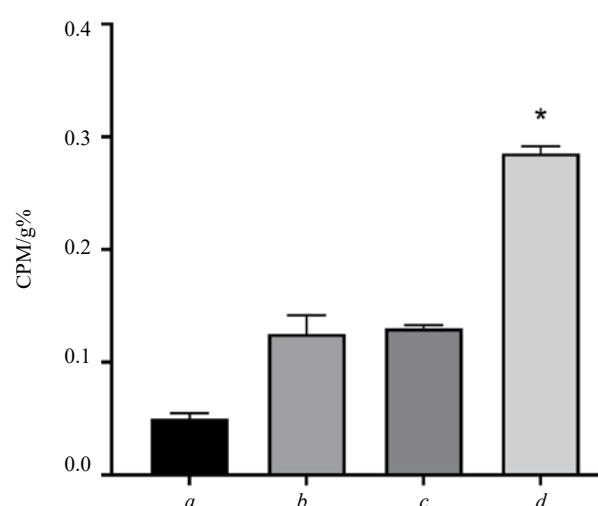


Fig. 2. The degree of accumulation of ^{99m}Tc -pyrophosphate during the blockade of α -adrenergic receptors under stress: *a* – intact animals, *b* – stress controls, *c* – stress + prazosin, *d* – stress + yohimbine; *E* – stress + atropine methyl nitrate; * $p < 0.05$ compared with the stress controls

During a series of experiments to determine the role of β -AR in SCM, data were obtained that the blockade of β_1 -AR reduced the accumulation of ^{99m}Tc -PP by 250% (Fig. 3). It is generally known that these receptors are associated with Gs proteins, activation of which leads to stimulation of adenylate cyclase and an increase in cAMP synthesis. The β_2 -AR antagonist ICI 118,551 has the opposite effect: after its administration, the accumulation of ^{99m}Tc -PP in the myocardium increased by 34.6%. It is known that β_2 -AR at high concentrations of catecholamines are able to switch from Gs-proteins to Gi/o-proteins [27]. It is also known that activation of these receptors along the pathway of Gi/o-protein stimulation causes inhibition of adenylate cyclase and a decrease in cAMP synthesis and has anti-apoptotic and cardioprotective effects [28, 29]. The activation of Gi/o-proteins most likely occurs in SCM. The increase in myocardial damage can be explained by the fact that the β_2 -AR blockade impairs the physiological mechanisms of reducing the pathological effects of excessive β_1 -AR activation. Inhibition of β_3 -AR had no effect on the degree of ^{99m}Tc -PP accumulation in the myocardium under stress (Fig. 3).

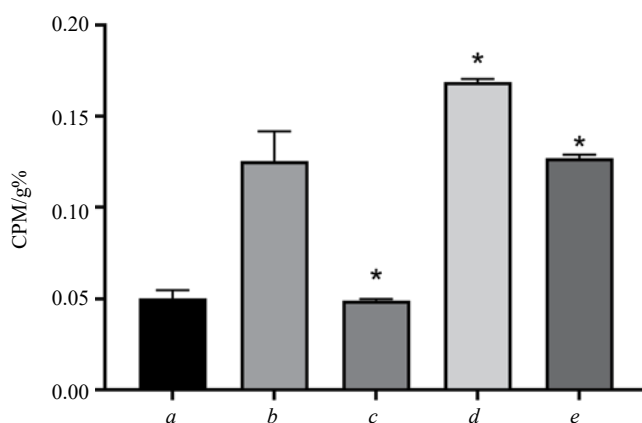


Fig. 3. The degree of ^{99m}Tc -pyrophosphate accumulation during the blockade of β -adrenergic receptors in stress: A – intact animals, B – stress controls; C – stress + nebivolol; D – stress + ICI 118.551; E – stress + L-748337; * $p < 0.05$ compared with the stress controls

CONCLUSION

Blockade of peripheral muscarinic receptors enhances SCM. α_1 -adrenergic receptors do not have a considerable impact on the pathogenesis of SCM. It was found that the blockade of α_2 -AR contributed to enhancement of SCM, most likely due to inhibition of pre-synaptic α_2 -AR. β_1 -adrenergic receptors play a crucial role in the pathogenesis of SCM. The activation of β_2 -adrenergic receptors by endogenous

catecholamines limits SCM. β_3 -adrenergic receptors do not significantly affect SCM. Consequently, the sympathoadrenal system and, in particular, β_1 -AR contribute to the development of mechanisms that are involved in the development of SCM. The parasympathetic nervous system ensures resistance of the heart to stress.

REFERENCES

- Johansson G., Jonsson L., Lannek N., Blomgren L., Lindberg P., Poupa O. Severe stress-cardiopathy in pigs. *American Heart Journal*. 1974; 87 (4): 451–457. DOI: 10.1016/0002-8703(74)90170-7.
- Sato H. Tako-tsubo-like left ventricular dysfunction due to multivessel coronary spasm. *Clinical Aspects of Myocardial Injury: From Ischemia to Heart Failure*. 1990: 56–64.
- Prokudina E.S., Kurbatov B.K., Zavadovsky K.V., Vrublevsky A.V., Naryzhnaya N. V., Lishmanov Y.B. et al. Takotsubo syndrome: clinical manifestations, etiology and pathogenesis. *Current Cardiology Reviews*. 2020; 16. DOI: 10.2174/1573403X16666200129114330.
- Stiermaier T., Moeller C., Oehler K., Desch S., Graf T., Eitel C. et al. Long-term excess mortality in takotsubo cardiomyopathy: predictors, causes and clinical consequences. *European Journal of Heart Failure*. 2016; 18 (6): 650–656. DOI: 10.1002/ehf.494.
- Aoki Y., Kodera S., Ooe K. Further patient information; Takotsubo cardiomyopathy related to autonomic nervous system. *International Journal of Cardiology*. 2016; 209: 341. DOI: 10.1016/j.ijcard.2016.02.056.
- Vaccaro A., Despas F., Delmas C., Lairez O., Lambert E., Lambert G. et al. Direct evidences for sympathetic hyperactivity and baroreflex impairment in Tako Tsubo cardiopathy. *PLoS ONE*. 2014; 9(3): e93278. DOI: 10.1371/journal.pone.0093278.
- Marfella R., Barbieri M., Sardù C., Rizzo M.R., Siniscalchi M., Paolisso P. et al. Effects of α -lipoic acid therapy on sympathetic heart innervation in patients with previous experience of transient takotsubo cardiomyopathy. *Journal of Cardiology*. 2016; 67(2): 153–161. DOI: 10.1016/j.jjcc.2015.07.012.
- Christensen T.E., Bang L.E., Holmvang L., Skovgaard D.C., Oturai D.B., Søholm H. et al. 123 I-MIBG scintigraphy in the subacute state of Takotsubo cardiomyopathy. *JACC: Cardiovascular Imaging*. 2016; 9(8): 982–990. DOI: 10.1016/j.jcmg.2016.01.028.
- Sobue Y., Watanabe E., Ichikawa T., Koshikawa M., Yamamoto M., Harada M. et al. Physically triggered Takotsubo cardiomyopathy has a higher in-hospital mortality rate. *International Journal of Cardiology*. 2017; 235: 87–93. DOI: 10.1016/j.ijcard.2017.02.090.
- Núñez-Gil I.J., Bernardo E., Feltes G., Escaned J., Mejía-Rentería H.D., De Agustín J.A. et al. Platelet function in Takotsubo cardiomyopathy. *Journal of Thrombosis and Thrombolysis*. 2015; 39 (4): 452–458. DOI: 10.1007/s11239-014-1109-y.
- Chernov V., Triss S., Skuridin V., Lishmanov Yu. Thallium-199: a new radiopharmaceutical for myocardial perfusion imaging. *Int. J. Cardiac Imaging* 1996; 12 (2): 119–126. DOI: 10.1007/BF01880743.

12. Chernov V.I., Medvedeva A.A., Sinilkin I.G., Zelchan R.V., Bragina O.D., Choyzonov E.L. Nuclear medicine as a tool for diagnosis and targeted cancer therapy. *Bulletin of Siberian Medicine*. 2018; 17 (1): 220–231 (in Russ.). DOI: 10.20538/1682-0363-2018-1-220-231.
13. Miller D.G., Mallov S. Quantitative determination of stress-induced myocardial damage in rats. *Pharmacology, Biochemistry and Behavior*. 1977; 7 (2): 139–145. DOI: 10.1016/0091-3057(77)90198-8.
14. Prokudina E., Maslov L., Naryzhnaya N., Tsibulnikov S., Lishmanov Y., Madias J. et al. Cardioprotective properties of opioid receptor agonists in rats with stress-induced cardiac injury. *Physiological Research*. 2019; 68 (3): 375–384. DOI: 10.33549/physiolres.933946.
15. Takeuchi K., Okabe S., Takagi K. A new model of stress ulcer in the rat with pylorus ligation and its pathogenesis. *The American Journal of Digestive Diseases*. 1976; 21 (9): 782–788. DOI: 10.1007/BF01073030.
16. Maslov L.N., Lishmanov Y.B., Oeltgen P.R., Barzakh E.I., Krylatov A. V., Govindaswami M. et al. Activation of peripheral $\delta 2$ opioid receptors increases cardiac tolerance to ischemia/reperfusion injury. Involvement of protein kinase C, NO-synthase, KATP channels and the autonomic nervous system. *Life Sciences*. 2009; 84 (19–20): 657–663. DOI: 10.1016/J.LFS.2009.02.016.
17. Ray A., Sullivan R.M., Henke P.G. Adrenergic modulation of gastric stress pathology in rats: a cholinergic link. *Journal of the Autonomic Nervous System*. 1987; 20 (3): 265–268. DOI: 10.1016/0165-1838(87)90155-X.
18. Bankwala Z., Hale S.L., Kloner R.A. Alpha-adrenoceptor stimulation with exogenous norepinephrine or release of endogenous catecholamines mimics ischemic preconditioning. *Circulation*. 1994; 90 (2): 1023–1028. DOI: 10.1161/01.cir.90.2.1023.
19. Martins J.L.R., Rodrigues O.R.L., Da Silva D.M., Galdino P.M., De Paula J.R., Romão W. et al. Mechanisms involved in the gastroprotective activity of *Celtis iguanaea* (Jacq.) Sargent on gastric lesions in mice. *Journal of Ethnopharmacology*. 2014; 155 (3): 1616–1624. DOI: 10.1016/j.jep.2014.08.006.
20. Loots W., De Clerck F. Differential effects of nebivolol on adrenoceptors in the heart and in resistance arterioles in the rat. Quantitative intravital microscopic analysis. *European Journal of Pharmacology*. 1990; 179 (1–2): 177–186. DOI: 10.1016/0014-2999(90)90416-4.
21. Kwolek G., Zakrzewska A., Schlicker E., Göthert M., Godlewski G., Malinowska B. Central and peripheral components of the pressor effect of anandamide in urethane-anaesthetized rats. *British Journal of Pharmacology*. 2005; 145 (5): 567–575. DOI: 10.1038/sj.bjp.0706195.
22. Mori A., Miwa T., Sakamoto K., Nakahara T., Ishii K. Pharmacological evidence for the presence of functional $\beta 3$ -adrenoceptors in rat retinal blood vessels. *Naunyn-Schmiedeberg's Archives of Pharmacology*. 2010; 382 (2): 119–126. DOI: 10.1007/s00210-010-0526-5.
23. Taheri P., Choe E., Lipton H., Hyman A., Flint L., Ferrara J. Autonomic control of the regional hemodynamic response to scald. *Life Sciences*. 1995; 56 (9): 701–707. DOI: 10.1016/0024-3205(94)00504-L.
24. Xue R.Q., Sun L., Yu X.J., Li D.L., Zang W.J. Vagal nerve stimulation improves mitochondrial dynamics via an M3 receptor/CaMKK β /AMPK pathway in isoproterenol-induced myocardial ischaemia. *Journal of Cellular and Molecular Medicine*. 2017; 21 (1): 58–71. DOI: 10.1111/jcmm.12938.
25. Zhao M., Jia H.H., Liu L.Z., Bi X. yuan, Xu M., Yu X.J. et al. Acetylcholine attenuated TNF- α -induced intracellular Ca²⁺ + overload by inhibiting the formation of the NCX1-TRPC3-IP3R1 complex in human umbilical vein endothelial cells. *Journal of Molecular and Cellular Cardiology*. 2017; 107: 1–12. DOI: 10.1016/j.yjmcc.2017.04.001.
26. Hoffman B.B. Drugs blocking adrenergic receptors, Ch. 10. In the book: B.G. Katzung. Basic and Clinical Pharmacology; vol. 1. M.: Binom, 1998 (in Russ.).
27. Daaka Y., Luttrell L.M., Lefkowitz R.J. Switching of the coupling of the $\beta 2$ -adrenergic receptor to different G proteins by protein kinase A. *Nature*. 1997; 390 (6655): 88–91. DOI: 10.1038/36362.
28. Chesley A., Lundberg M.S., Asai T., Xiao R.P., Ohtani S., Lakatta E.G. et al. The $\beta 2$ -adrenergic receptor delivers an antiapoptotic signal to cardiac myocytes through G(i)-dependent coupling to phosphatidylinositol 3'-kinase. *Circulation Research*. 2000; 87 (12): 1172–1179. DOI: 10.1161/01.RES.87.12.1172.
29. Communal C., Singh K., Sawyer D.B., Colucci W.S. Opposing effects of $\beta 1$ - and $\beta 2$ -adrenergic receptors on cardiac myocyte apoptosis: Role of a pertussis toxin-sensitive G protein. *Circulation*. 1999; 100 (22): 2210–2212. DOI: 10.1161/01.CIR.100.22.2210.

Authors contribution

All authors contributed to conception and design, analysis and interpretation of data, substantiation of the manuscript, and critical revision of the manuscript for important intellectual content.

Authors information

Prokudina Ekaterina S., Cand. Sci. (Med.), Researcher, Laboratory of Experimental Cardiology, Cardiology Research Institute, Tomsk NRMC, Tomsk, Russian Federation. ORCID 0000-0002-1991-6516.

Maslov Leonid N., Dr. Sci. (Med.), Professor, Laboratory of Experimental Cardiology, Cardiology Research Institute, Tomsk NRMC, Tomsk, Russian Federation. ORCID 0000-0002-6020-1598.

Naryzhnaya Natalya V., Dr. Sci. (Med.), Leading Researcher, Laboratory of Experimental Cardiology, Cardiology Research Institute, Tomsk NRMC, Tomsk, Russian Federation. ORCID 0000-0003-2264-1928.

Kurbatov Boris K., Junior Researcher, Laboratory of Experimental Cardiology, Cardiology Research Institute, Tomsk NRMC, Tomsk, Russian Federation. ORCID 0000-0001-9603-822X.

Mukhomedzyanov Alexander V., Junior Researcher, Laboratory of Experimental Cardiology, Cardiology Research Institute, Tomsk NRMC, Tomsk, Russian Federation. ORCID 0000-0003-2264-1928.

Shipulin Vladimir V., Laboratory Assistant, Laboratory of Radionuclide Research Methods, Cardiology Research Institute, Tomsk NRMC, Tomsk, Russian Federation. ORCID 0000-0001-9887-8214.

Krylatov Andrey V., Cand. Sci. (Med.), Researcher, Laboratory of Experimental Cardiology, Cardiology Research Institute, Tomsk NRMC, Tomsk, Russian Federation. ORCID 0000-0003-4341-3844.

Tsibulnikov Sergey Yu., Cand. Sci. (Med.), Senior Researcher, Laboratory of Experimental Cardiology, Cardiology Research Institute, Tomsk NRMC, Tomsk, Russian Federation. ORCID 0000-0003-1534-2516.

Voronkov Nikita S., Junior Researcher, Laboratory of Experimental Cardiology, Cardiology Research Institute, Tomsk NRMC, Tomsk, Russian Federation. ORCID 0000-0001-5261-8062.

(✉) **Kurbatov Boris K.**, e-mail: bobersanker@gmail.com

Received 26.03.2020

Accepted 28.12.2020

MRI assessment of the abdominal adipose tissue and the state of the abdominal aorta in patients with coronary artery disease: association with metabolic disorders

Ryumshina N.I.¹, Koshelskaya O.A.¹, Kologrivova I.V.¹, Kharitonova O.A.¹, Nasekina K.A.², Ussov V.Yu.¹

¹ Cardiology Research Institute, Tomsk National Research Medical Center (NRMC), Russian Academy of Sciences 111a, Kievskaya Str., Tomsk, 634012, Russian Federation

² Siberian State Medical University (SSMU) 2, Moscow Trakt, Tomsk, 634050, Russian Federation

ABSTRACT

Aim. To evaluate potential associations between quantitative features of visceral and subcutaneous adipose tissue (AT) and anthropometric characteristics of obesity, metabolic disorders, and the state of the abdominal aorta in patients with chronic coronary artery disease (CAD).

Materials and methods. The study included 55 patients (average age 61.2 ± 7.2 years) with chronic CAD. Magnetic resonance imaging (MRI) was performed on a 1.5 T MRI scanner using T2-weighted spin-echo modes. The area and volume of abdominal subcutaneous (SAT) and visceral adipose tissue (VAT) were calculated at the L4–L5 level; the total volumes of abdominal SAT and VAT were determined. Parameters of lipid and carbohydrate metabolism, as well as adipokine profile were studied in the blood serum.

Results. In the course of a multiple linear regression analysis, we detected the independent determinants, which described 95% of the total VAT volume variability and were represented by waist circumference (WC) and serum levels of high-density lipoprotein (HDL) cholesterol and adiponectin. The model was characterized by the significance level $p < 0.000001$, the residuals of the model were normal. We calculated the coefficients in the model: 1.39 for WC, -0.26 for HDL cholesterol, and -0.19 for adiponectin. We detected a positive correlation between the abdominal aorta (AA) diameter and SAT area at the L4–L5 level ($r_s = 0.48$; $p = 0.0014$), which does not depend on gender, and reverse correlations between the aorta diameter and glycated hemoglobin (HbA1c) level ($r_s = -0.40$; $p = 0.0359$) and postprandial glycemia ($r_s = -0.40$; $p = 0.0273$). The patients with a dilated aorta (group 2), when compared with the patients with a normal aorta diameter (group 1), did not differ in the AT accumulation, but demonstrated decreased levels of HbA1c and postprandial glycemia, which resulted in a smaller number of patients with type 2 diabetes mellitus.

Conclusion. We identified independent determinants of an increase in the total volume of abdominal visceral AT, such as an increase in WC and a decrease in serum adiponectin and HDL cholesterol levels. Results of the study indicate the presence of a link between the AA remodeling, accumulation of subcutaneous AT, and impaired glucose metabolism.

Key words: magnetic resonance imaging, abdominal adipose tissue, abdominal aorta, lipid and glucose metabolism disturbances, adiponectin.

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

Source of financing. The study was carried out within the state assignment of Cardiology Research Institute, Tomsk NRMC, on the topic of fundamental research No. AAAA-A15-115123110026-3 “Fundamental aspects of the emergence and development of socially sensitive cardiovascular diseases; identification of targets for diagnosis,

✉ Koshelskaya Olga A., e-mail: koshel@live.ru

treatment, and prognosis improvement; protection mechanisms”.

Conformity with the principles of ethics. All patients signed an informed consent to participate in the study and process the examination results. The study was approved by the Ethics Committee at Cardiology Research Institute, Tomsk NRMС (Protocol No. 146 of 16.06.2016).

For citation: Ryumshina N.I., Koshelskaya O.A., Kologrivova I.V., Kharitonova O.A., Nasekina K.A., Ussov V.Yu. MRI assessment of the abdominal adipose tissue and the state of the abdominal aorta in patients with coronary artery disease: association with metabolic disorders. *Bulletin of Siberian Medicine*. 2021; 20 (3): 95–104. <https://doi.org/10.20538/1682-0363-2021-2-95-104>.

Магнитно-резонансная томографическая оценка абдоминальной жировой ткани и состояние брюшной аорты у пациентов с ишемической болезнью сердца: связь с нарушениями метаболизма

Рюмшина Н.И.¹, Кошельская О.А.¹, Кологривова И.В.¹, Харитонов О.А.¹,
Насекина К.А.², Усов В.Ю.¹

¹ Научно-исследовательский институт (НИИ) кардиологии, Томский национальный исследовательский медицинский центр (НИМЦ) Российской академии наук
Россия, 634012, г. Томск, ул. Киевская, 111а

² Сибирский государственный медицинский университет (СибГМУ)
Россия, 634050, г. Томск, Московский тракт, 2

РЕЗЮМЕ

Цель. Изучение потенциальных взаимосвязей количественных характеристик висцеральной и подкожной жировых тканей (ЖТ) с антропометрическими показателями ожирения, нарушениями метаболизма и состоянием брюшного отдела аорты у пациентов с хронической ишемической болезнью сердца (ИБС).

Материалы и методы. В исследовании приняли участие 55 пациентов (средний возраст $61,2 \pm 7,2$ лет) с хронической ИБС. Магнитно-резонансную томографию выполняли на 1,5 Т магнитно-резонансном томографе в T2-взвешенных спин-эхо режимах. Расчет площади и объема абдоминальных подкожной (ПЖТ) и висцеральной ЖТ (ВЖТ) проводился на уровне L4–L5, а также измерялся общий объем абдоминальных ПЖТ и ВЖТ. В сыворотке крови исследовали показатели липидного и углеводного метаболизма и состояние адипокинового профиля.

Результаты. В ходе множественного линейного регрессионного анализа установлены независимые детерминанты вариабельности общего объема абдоминальной ВЖТ, которые описывают 95% вариабельности этого показателя: окружность талии (ОТ), содержание в крови холестерина липопротеинов высокой плотности (ХС-ЛВП) и адипонектина. Уровень значимости модели составил $p < 0,000001$, остатки модели нормальны. Оценки коэффициентов в модели для: ОТ – 1,39; ХС-ЛВП – 0,26 и адипонектина – 0,19. Документированы корреляционные взаимосвязи диаметра абдоминальной аорты (АА): прямая – с площадью ПЖТ L4–L5 ($r_s = 0,48$; $p = 0,0014$), что не зависит от пола, и обратные – с уровнем гликированного гемоглобина (HbA1c) ($r_s = -0,40$; $p = 0,0359$) и постпрандиальной гликемией ($r_s = -0,40$; $p = 0,0273$). В сравнении с пациентами, имеющими нормальные значения диаметра АА (группа 1), у пациентов с расширенной АА (группа 2) не было значимых различий накопления ЖТ, но имел место более низкий уровень HbA1c и постпрандиальной гликемии, что определялось меньшим числом пациентов с сахарным диабетом типа 2.

Заключение. Установлены независимые детерминанты возрастания общего объема абдоминальной висцеральной ЖТ, которыми являются: увеличение окружности талии и сниженные значения содержания в крови адипонектина и ХС-ЛВП. Результаты исследования свидетельствуют о наличии взаимосвязи между процессами ремоделирования АА, накоплением подкожной абдоминальной ЖТ и нарушениями метаболизма глюкозы.

Ключевые слова: магнитно-резонансная томография, абдоминальная жировая ткань, брюшная аорта, нарушения метаболизма липидов и глюкозы, адипонектин.

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

Источник финансирования. Статья подготовлена в рамках госзадания НИИ кардиологии Томского НИМЦ по теме фундаментальных исследований № АААА-А15-115123110026-3 «Фундаментальные аспекты возникновения и развития социально значимых сердечно-сосудистых заболеваний; выявление мишеней для диагностики, лечения и улучшения прогноза; механизмы защиты».

Соответствие принципам этики. Все пациенты дали информированное согласие на участие и обработку результатов обследования. Исследование одобрено независимым этическим комитетом НИИ кардиологии, Томский НИМЦ (протокол № 146 от 16.06.2016).

Для цитирования: Рюмшина Н.И., Кошельская О.А., Кологривова И.В., Харитонов О.А., Насекина К.А., Усов В.Ю. Магнитно-резонансная томографическая оценка абдоминальной жировой ткани и состояние брюшной аорты у пациентов с ишемической болезнью сердца: связь с нарушениями метаболизма. *Бюллетень сибирской медицины*. 2021; 20 (3): 95–104. <https://doi.org/10.20538/1682-0363-2021-3-95-104>.

INTRODUCTION

A progressive increase in the prevalence of metabolic disorders and obesity is one of the most important features of modern society. According to the World Health Organization, more than 650 million people are obese and more than 1.9 billion are overweight. It is known that adipose tissue (AT) is the largest and the most active endocrine organ, participating in the regulation of energy balance, glucose and lipid homeostasis, as well as the functioning of the immune system in response to fluctuations in nutritional status, environment, lifestyle, and aging [1]. Numerous studies have shown a strong association between excessive accumulation of visceral adipose tissue (VAT) and the development of metabolic syndrome [2], type 2 diabetes mellitus (T2DM) [3], obstructive coronary artery disease, and myocardial infarction [4]. Adipose tissue is able to produce a wide spectrum of cytokines and adipokines, whereas the metabolic and functional properties of subcutaneous and visceral fat differ significantly [5], and the available data on the secretory potential of subcutaneous adipose tissue (SAT) are very contradictory.

Instrumental methods for assessing the degree of visceral obesity are important in the diagnosis and risk stratification of cardiovascular diseases [6]. Tomographic techniques, such as computed tomography (CT) and magnetic resonance imaging (MRI), are considered to be the golden standard [7]. According to some data, MRI may outperform CT in measurement accuracy [8]. MRI has high tissue differentiation, which makes it possible to quantify various fat depots while simultaneously evaluating adjacent organs and tissues. Approaches to quantitative analysis of AT are still an unanswered question, since there is no single protocol for measuring AT.

Only a few studies investigated a potential relationship between the quantitative characteristics of

abdominal AT and AA aneurysm, and the results of these studies are very controversial [9]. Since the majority of patients with abdominal aortic dilatation and aneurysm have pronounced atherosclerosis and CAD of various severity, it cannot be ruled out that this pathological relationship may be determined by common risk factors, including visceral obesity, which is confirmed by the results of some studies [10].

Since aortic dilatation is a complication in a number of pathologies with a high risk of adverse events, early detection of AA expansion signs will reduce mortality from conditions, such as aortic dissection and aneurysm ruptures. MRI has a class of evidence IC for diagnosing the aortic condition according to modern recommendations [11]. Tomographic methods are preferred for AA visualization, as this area is difficult to access for ultrasound examination. To date, no studies have been conducted on quantitative parameters of abdominal AT accumulation and parameters reflecting the nature of AA remodeling compared with the assessment of clinical characteristics of patients and the state of their metabolic and adipokine profiles. It is still unclear whether the presence of visceral abdominal obesity can modulate a relationship between abdominal fat accumulation and the degree of AA expansion, and what factors may be involved in this process.

The aim of the study was to evaluate potential associations between the quantitative features of visceral and subcutaneous adipose tissue and anthropometric characteristics of obesity, metabolic disorders, and the state of the abdominal aorta in patients with chronic coronary artery disease (CAD).

MATERIALS AND METHODS

The study included patients aged 48–78 years (60% of them were men) with verified CAD. All patients gave an informed consent to participate in the study and process the examination results. A single-stage

retrospective study was conducted in accordance with the standards of Good Clinical Practice and the principles of the Declaration of Helsinki. The study was approved by the local Ethics Committee at Cardiology Research Institute (Protocol No. 146 of 16.06.16).

The exclusion criteria included an acute complication of atherosclerosis less than 6 months ago; any inflammatory disease; T2DM with poor glycemic control and HbA1c > 10% or a daily glycemic level > 11 mmol / l; chronic kidney disease above C3b; left ventricular ejection fraction < 40%; and oncological, hematological, and immune diseases.

Table 1 presents the clinical characteristics of the recruited patients. The study included 55 men and women with verified chronic CAD at the age of 61.68 ± 6.7 years.

Table 1

Clinical characteristics of the patients recruited in the study (n = 55)	
Parameter	Value
Gender (male / female)	33 / 22
Age, years, $M \pm SM$	61.68 ± 6.7
Patients with myocardial infarction, n (%)	36 (65.5)
Duration of CAD, years*	3 (1; 8)
Patients with hypertension, n (%)	48 (87.3)
Duration of hypertension, years*	15 (10; 20)
Type 2 diabetes mellitus, n (%)	23 (41.8)
Duration of T2DM, years*	10 (1; 15)
Systolic blood pressure, mm Hg., $M \pm SM$	131.8 ± 15.2
Diastolic blood pressure, mm Hg., $M \pm SM$	75.9 ± 8.4
Smokers, n (%)	23 (41.8)
Patients with obesity, n (%):	45 (81.8)
– class I	23 (51.1)
– class II	14 (31.1)
– class III	8 (17.8)
Therapy with statins, n (%)	53 (96)

Metabolic syndrome was revealed in 78% of patients [12]. About half of the included patients had T2DM and were smokers. Anatomical stenosis in more than 70% of at least one of the main coronary arteries were established in 42 patients (76.4%), 2 (3.6%) patients had microvascular pathology, while changes in the coronary arteries were insignificant in other cases.

The MRI study was carried out on a high-field MRI scanner with 1.5 T magnetic field strength (Titan Vantage, Toshiba Medical, 2010, Japan) in the supine position of the patient using built-in and external 4- and 8-channel quadrature coils for the whole body. The scanned area included the distance from the dome of the diaphragm to the entrance to the small pelvis. Measurements of the thickness of the anterior and posterior abdominal wall and the area of the ab-

dominal SAT and VAT were carried out at the level of the intervertebral L4–L5 discs. The AA diameter and wall thickness were measured at the L2–L3 level, 1–2 slices above the aortic bifurcation. The measurement of the total volume of the abdominal SAT and VAT was carried out taking into account all sections of the scanned area according to the method proposed by Fang et al. (2018).

Post-processing image analysis and measurements were performed using the eFilm 3.4 software package (MergeHealth, 2010) and 3D Slicer 4.9.0. The AT was automatically circled according to the specified intensity range of the MR signal corresponding to the AT, and, if necessary, adjustments were made. After that, the volume (cm³) and surface area (cm²) of all the selected voxels were calculated. Since no convincing data showing the compliance of single-slice measurements with the total amount of AT were published, it was decided to carry out the calculation as extensively as possible.

Samples of venous blood were obtained from patients in the morning in the fasted state. The serum levels of leptin (Mediagnost, Germany), adiponectin (Assaypro, USA), their ratio, and insulin (Accu-Bind, USA) were determined by solid-phase enzyme immunoassay. The content of total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) was determined by the enzymatic colorimetric method (Diakon-DS kits, Russia). The glucose content in the blood serum was evaluated by the hexokinase method. The glucose and insulin levels were also evaluated 2 hours after the standard food intake (postprandial content).

Statistical analysis was conducted using the STATISTICA 10.0 software package for Windows. The normality of data distribution was defined by the Shapiro – Wilk test. Continuous variables with normal distribution were presented as $M \pm SD$, where M is the mean and SD is the standard deviation; with non-normal distribution – as the median and the interquartile range $Me (Q_{25\%}; Q_{75\%})$. Categorical variables were represented by the absolute and relative (%) frequencies. The nonparametric Mann – Whitney test was used to assess the statistical significance of differences, when the distribution of data was different from normal. The Spearman's rank correlation coefficient (r_s) was used to assess the relationships between the variables. A multiple linear regression model was constructed to assess the effect of biometric and laboratory parameters on the total volume of visceral fat. The differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

The average values of the abdominal AT and AA, calculated using MRI and 3D Slicer, are presented in Table 2.

Table 2

Average values of the abdominal AT depot, the diameter of the AA and its wall thickness in the overall sample		
Parameter	<i>M</i>	<i>SD</i>
Cross-sectional area of the vertebral body of L4–L5, cm ²	823.8	208.7
VAT area at L4–L5, cm ²	186.0	78.6
SAT area at L4–L5, cm ²	327.8	120.1
Total AT area at L4–L5, cm ²	513.8	161.7
SAT volume at L4–L5, cm ³	260.8	111.3
VAT volume at L4–L5, cm ³	140.4	53.8
Total AT volume at L4–L5, cm ³	404.3	141.5
Total VAT volume, cm ³	5,595.4	3,371.4
Total SAT volume, cm ³	4,217.7	1,363.9
Total volume of abdominal AT, cm ³	9,871.1	4,143.1
External diameter of AA, mm	20.5	4.4
Abdominal aorta wall thickness, mm	2.7	0.8

In cases of an increased diameter and, in particular, aneurysmal dilatation of the aorta, hyperintensive inclusions were visualized in the aortic wall on T2-weighted spin-echo images, indirectly reflecting hemorrhagic impregnation (pathological angiogenesis) in the wall thickness. The presence of hyperintensive foci in the arterial wall is a characteristic symptom of the pathological aortic dilatation, which, in response to LDL accumulation and local hypoxia, is accompanied by thickening of the intimal membrane and an increase in the amounts of extracellular matrix with development of an extensive network of microvessels (*vasa vasorum*). It is the small newly formed vessels that have a hyperintensive signal in the wall thickness in T2-weighted images. It is believed that neoangiogenesis can develop in the early stages of the atherosclerotic process and maintain local subclinical inflammation, so it is a good and fairly early marker of a high risk of aneurysm development in this area of the vessel wall [13].

Statistical processing of the data revealed that the existing gender characteristics of the accumulation and distribution of AT have a significant impact on a number of the studied parameters. Therefore, to eliminate this factor, the data were adjusted for gender, height, and body weight of patients.

In the overall sample, after correction of statistically significant differences in the parameters, the correlations of the area and volume of the abdominal VAT and SAT with anthropometric parameters of

obesity and key biomarkers of lipid and carbohydrate metabolism were studied. The values of the total VAT showed significant positive correlations with WC ($r_s = 0.62$, $p = 0.00000$) and blood triglyceride levels ($r_s = 0.34$, $p = 0.0459$), leptin levels ($r_s = 0.64$, $p = 0.0020$), as well as negative correlations with HDL-C ($r_s = -0.49$, $p = 0.0028$) and adiponectin levels ($r_s = -0.37$, $p = 0.0486$). None of the quantitative parameters of SAT accumulation had associations with blood levels of adiponectin, triglycerides, and HDL-C, while the total volume of SAT showed positive correlations with WC ($r_s = 0.51$, $p = 0.0003$) and leptin levels ($r_s = 0.50$, $p = 0.0126$). There were no correlations between the values of the total SAT and VAT volumes and the content of glucose, HbA1c, and insulin.

It should be noted that the relationships between the quantitative characteristics of abdominal AT and the state of metabolism and adipokine profile have been analyzed in a very limited number of studies. [14]. Although adiponectin is known to be secreted by both subcutaneous and visceral AT [15], the results of studies on the contribution of various fat depots to determination of systemic adiponectin levels are contradictory and gender specific. Thus, a number of studies have shown that the development of local AT inflammation and insulin resistance negatively affects visceral production of adiponectin, while its synthesis in SAT remains unchanged, especially in women [16].

At the same time, there is evidence of a relationship between SAT area and the plasma level of adiponectin in men [17]. Leptin is an adiponectin antagonist which reflects the degree of AT hormonal activity and also a key marker of chronic subclinical inflammation in AT [18]. In the article by I.J. Neeland et al. (2013), the leptin content in the blood plasma was shown to be mainly associated with the SAT volume [14].

Our results on the presence of correlations between the content of serum leptin not only with the total volume of abdominal VAT, but also with the total volume of abdominal SAT confirm these data and question the idea of SAT as a metabolically inert fat depot.

Indeed, the existing data on the contribution of SAT to the formation of cardiovascular risk and metabolic imbalance, in comparison with VAT, are inconsistent [17]. The vast majority of studies present data on the VAT effect on the processes occurring in the aortic wall. It was shown that if VAT prevails, the aortic diameter [19] and calcification of the aortic wall decrease [20], its elastic properties change [21; 22], and intima-media thickness (IMT) increases [23]. In addition, a positive correlation between visceral obesity and the

development of atherosclerosis was proven [24–26]. At the same time, the role of SAT in changes in the aortic wall was not demonstrated. However, it is believed that SAT has a protective function in calcium deposition, thereby reducing the risk of atherosclerosis [20].

In the course of a multiple step-by-step linear regression analysis with an exception, conducted in the overall sample, we established the following independent determinants of the variability of the total VAT volume: waist circumference and blood levels of HDL-C and adiponectin. The significance level of the model was $p < 0.000001$, the coefficient of determination $R^2 = 0.949$, and the model residuals were normal. Estimates of the coefficients in the model: for WC –1.39, for HDL-C –0.26, and for adiponectin –0.19. It is known that obesity is associated not only with a decrease in the HDL-C content, but also with a change in their qualitative composition and a decrease in the ability to evacuate excess cholesterol from fat depots [27], and adiponectin has a direct effect on the HDL-C synthesis [28].

Next, we analyzed potential correlations of vascular remodeling parameters (values of AA diameter and wall thickness) with the content of serum biomarkers

and with all quantitative parameters of the abdominal SAT and VAT accumulation, including the total volume of abdominal AT. As it turned out, the only parameter of AT accumulation that had a correlation with AA diameter was the area of abdominal SAT at L4–L5 ($r_s = 0.48$, $p = 0.0014$) (Fig. 1). AA diameter also showed a negative correlation with the level of HbA1c ($r_s = -0.40$, $p = 0.0359$) and postprandial glycemia ($r_s = -0.40$, $p = 0.0273$).

To compare the clinical and anthropometric characteristics, the amount of abdominal AT and the levels of biomarkers depending on the AA diameter values, the overall sample of patients was divided into two groups – a group with normal values of the AA diameter ($n = 32$, group 1) and a group with its dilatation (outer diameter of more than 20 mm) ($n = 23$, group 2) [11]. Examples of MR images of the normal and dilated abdominal aorta with the corresponding typical variants of the SAT and VAT distribution are shown in Figures 2 and 3.

Table 3 shows the results of comparing the two groups after correction for gender differences of the studied parameters.

Table 3

Clinical characteristics, anthropometric parameters of obesity, quantitative parameters of abdominal AT, and laboratory data of patients depending on the AA diameter				
Parameter	Overall sample ($n = 55$)	Group 1 ($n = 32$)	Group 2 ($n = 23$)	p
Age, years, $M \pm SM$	61.7 $\pm$ 6.7	60.4 $\pm$ 7	61.1 $\pm$ 6.9	
Patients with T2DM, n (%)	23 (41.8)	17 (30.9)	6 (10.9)	0.04
Diameter of AA, mm, $Me (Q_{25\%}; Q_{75\%})$	20 (18; 21.5)	19 (18; 20)	22.5 (21.5; 24)	< 0.001
AA wall thickness, mm, $M \pm SM$	2.7 $\pm$ 0.8	2.4 $\pm$ 0.6	3.2 $\pm$ 0.9	
Body mass index, kg / m ²	29.6 (26.5; 34.5)	29.7 (26.5; 34.1)	31.8 (29.1; 37.7)	
Waist circumference, cm, $M \pm SM$	106.6 $\pm$ 13.9	103.2 $\pm$ 13.1	109.2 $\pm$ 14.1	
Hip circumference, cm, $M \pm SM$	108.8 $\pm$ 9.9	108.2 $\pm$ 9.1	109.2 $\pm$ 10.1	
Waist circumference / hip circumference ratio, $M \pm SM$	0.98 $\pm$ 0.08	0.95 $\pm$ 0.08	0.99 $\pm$ 0.07	0.02
VAT area, cm ² , $Me (Q_{25\%}; Q_{75\%})$	293 (240; 380)	140 (121; 251)	168 (133; 220)	
SAT area, cm ² , $Me (Q_{25\%}; Q_{75\%})$	164 (128; 220)	271 (237; 347)	309 (281; 419)	
Total AT area, cm ² , $Me (Q_{25\%}; Q_{75\%})$	487 (399.5; 599.5)	481 (411; 545)	503.3 (388; 623)	
Total VAT volume, cm ³ , $Me (Q_{25\%}; Q_{75\%})$	4,288 (3,146; 5,496)	4,220.5 (2,824; 5,181.5)	4,424 (3,516.5; 5,957.5)	
Total SAT area, cm ³ , $Me (Q_{25\%}; Q_{75\%})$	4,938 (3,634; 6,841)	4,766.5 (3,543; 6,667.5)	4,888 (3,653.5; 7,842)	
Total abdominal AT volume, cm ³ , $Me (Q_{25\%}; Q_{75\%})$	9,186.5 (7,233; 12,177.5)	9,344.5 (7,401; 11,816)	8,982.5 (7,065; 13,344)	
Glycemia, mmol / l, $Me (Q_{25\%}; Q_{75\%})$	5.7 (4.4; 10.6)	6.0 (5.1; 10.4)	5.6 (4.4; 8.8)	0.038
Postprandial glycemia, mmol / l, $Me (Q_{25\%}; Q_{75\%})$	7.5 (4.7; 14.96)	7.7 (5.6; 14.96)	7.1 (4.7; 12.7)	
HbA1c, %, $Me (Q_{25\%}; Q_{75\%})$	6.0 (5.5; 6.9)	6.5 (5.6; 7.2)	5.5 (5.5; 6.1)	0.003
Total cholesterol, mmol / l, $Me (Q_{25\%}; Q_{75\%})$	3.9 (3.3; 4.7)	4.1 (3.4; 4.7)	3.7 (3.3; 5.1)	
LDL-C, mmol / l, $Me (Q_{25\%}; Q_{75\%})$	2.1 (1.4; 2.9)	2.1 (1.6; 2.8)	1.9 (1.4; 3.3)	
HDL-C, mmol / l, $Me (Q_{25\%}; Q_{75\%})$	1.1 (0.9; 1.4)	1.1 (0.9; 1.4)	1.07 (0.9; 1.3)	
Triglycerides, mmol / l, $Me (Q_{25\%}; Q_{75\%})$	1.5 (1.1; 1.9)	1.3 (0.9; 1.7)	1.49 (1.4; 1.9)	
Adiponectin, μ g / ml, $Me (Q_{25\%}; Q_{75\%})$	7.3 (2.5; 19.3)	7.6 (4.7; 11.9)	6.9 (6.2; 9.9)	
Leptin, ng / ml, $Me (Q_{25\%}; Q_{75\%})$	19.1 (9.3; 34.3)	14.1 (8.6; 34.1)	23.3 (11.2; 34.3)	

Note: the p values are given only for parameters for which statistically significant inter-group differences were revealed.

* in the overall sample, the parameters are given after correction for gender differences.

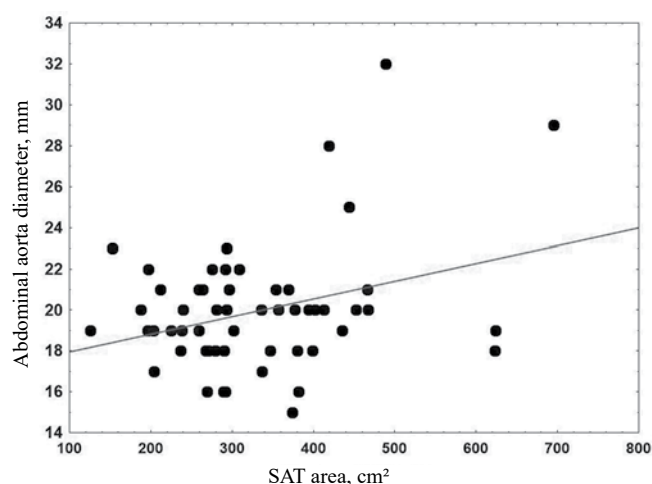


Fig. 1. Correlation between the SAT area at the L4–L5 level and the abdominal aorta diameter, $r_s = 0.48$, $p = 0.0014$

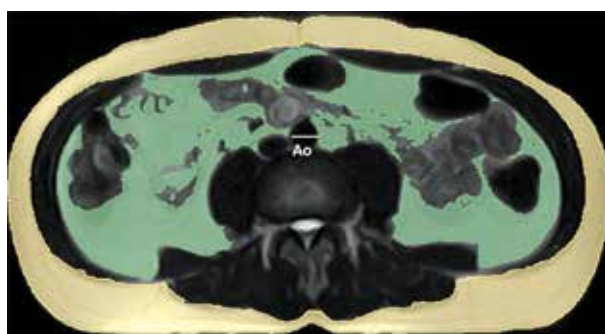


Fig. 2. MRI of the abdominal AT and aorta: male, 55 years old, BMI – 26, WC – 98 cm, postprandial glycaemia – 7.7 mmol / l, HbA1c – 5.7%; axial T2-weighted spin-echo slice; volume of abdominal subcutaneous adipose tissue (yellow) – 2,125 cm³, abdominal visceral adipose tissue (green) – 2,405 cm³, aorta diameter – 19 mm, the wall is smooth, without changes in the MR signal

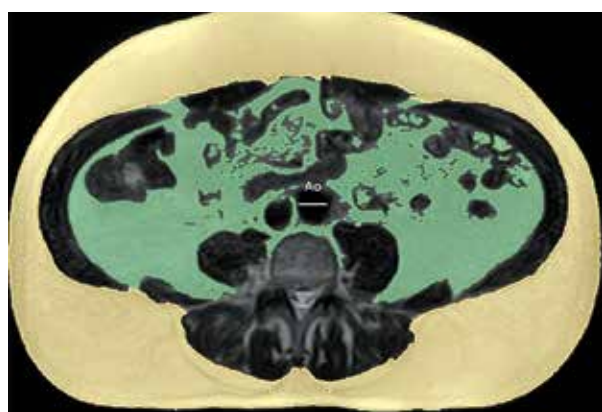


Fig. 3. MRI of the abdominal AT and aorta: male, 63 years old, BMI – 31, WC – 114 cm, postprandial glycaemia – 7.7 mmol / l, HbA1c – 6.2%; axial T2-weighted spin-echo slice; volume of abdominal subcutaneous adipose tissue (yellow) – 5,284 cm³, abdominal visceral adipose tissue (green) – 4,127 cm³, aorta diameter – 25 mm, the wall is smooth with small foci of the amplified MR signal

After the gender adjustment, the patients in group 2 showed higher values of the waist-to-hip circumference ratio, lower levels of HbA1c and basal glucose, as well as a tendency to lower values of postprandial glycemia, which was determined by a significantly lower number of patients with T2DM. In addition, in the patients from group 2, a tendency to a reduced level of adiponectin was observed, while there were no inter-group differences in body mass index and the area and volume of abdominal SAT and VAT.

Our results are consistent with the data of other research groups, which showed that patients with manifest disorders of carbohydrate metabolism tend to have a decrease in the aortic diameter, which may be mediated by a decline in the extracellular matrix degradation in hyperglycemia and an increase in the vascular wall stiffness [29].

Our data show a different nature of the relationships between subcutaneous and visceral abdominal fat depots and metabolic parameters. It is the volume of abdominal VAT that is closely related to the parameters of lipid metabolism and the level of adiponectin, while the lipid and adipokine profiles do not have an association with abdominal SAT accumulation. At the same time, the results of our study demonstrated for the first time that the abdominal SAT area was the only parameter associated with the AA diameter, and, apparently, the level of glycaemia can be considered a potential metabolic factor involved in the modulation of AA remodeling. The correlation of AA diameter with the SAT area at L4–L5 and the absence of an association between parameters of AA wall remodeling and the values of the abdominal AT volume require further studies. It is possible to assume that the total volume of abdominal AT, most fully reflecting its accumulation, is capable of correlating with the state of systemic metabolism. On the other hand, it is quite difficult to directly link the metabolic activity of such a large endocrine organ as abdominal AT only with the state of the abdominal aortic wall. In addition, it is known that SAT regresses more badly and is a source of a significant number of cellular mediators of inflammation and damage to the vascular wall. For these reasons, the demonstrated dependence of the abdominal aortic diameter on the SAT area at L4–L5 seems to most accurately reflect the negative impact of the abdominal AT on the processes of vascular remodeling.

In the future, it is of particular interest to study the potential relationships between abdominal aortic wall remodeling and quantitative parameters of perivascular aortic fat, as well as to conduct prospective studies

on the mechanisms linking quantitative characteristics of subcutaneous and visceral abdominal obesity with glucose (insulin) metabolism disorders.

CONCLUSION

According to the results of the study, independent determinants of an increase in the total volume of abdominal VAT were established, such as an increase in WC and a decrease in the adiponectin and HDL-C levels. Our data demonstrate the existence of a relationship between AA remodeling, abdominal AT accumulation, and impaired glucose metabolism. New data have been obtained suggesting a relationship between AA expansion and an increase in the abdominal SAT area, regardless of gender. There are negative correlations between the AA diameter values, on the one hand, and the HbA1c levels and postprandial glycaemia, on the other hand.

The limitations of the study are its single-stage design, small sample size, and a lack of a separate study of the quantitative characteristics of abdominal AT and its relationships in men, women, and patients with T2DM and obesity. Due to a lack of reference values for the total volume of abdominal VAT, it was not possible to create a predictive model for this parameter.

REFERENCES

1. Liu Z., Wu K.K.L., Jiang X., Xu A., Cheng K.K.Y. The role of adipose tissue senescence in obesity- and ageing-related metabolic disorders. *Clin. Sci. (Lond.)*. 2020; 134 (2): 315–330. DOI: 10.1042/CS20190966.
2. Wajchenberg B.L. Subcutaneous and visceral adipose tissue: their relation to the metabolic syndrome. *Endocr. Rev.* 2000; 21 (6):697–738. DOI: 10.1210/edrv.21.6.0415.
3. Freemantle N., Holmes J., Hockey A., Kumar S. How strong is the association between abdominal obesity and the incidence of type 2 diabetes? *Int. J. Clin. Pract.* 2008; 62 (9): 1391–1396. DOI: 10.1111/j.1742-1241.2008.01805.x.
4. Mandviwala T., Khalid U., Deswal A. Obesity and cardiovascular disease: a risk factor or a risk marker? *Curr. Atheroscler. Rep.* 2016;18 (5): 21. DOI: 10.1007/s11883-016-0575-4.
5. Lind L., Strand R., Kullberg J., Ahlström H. Cardiovascular-related proteins and the abdominal visceral to subcutaneous adipose tissue ratio. *Nutrition, Metabolism & Cardiovascular Diseases*. 2021; 31 (2): 532–539. DOI: 10.1016/j.numecd.2020.09.010.
6. Marzetti M., Brunton T., McCreight L., Pearson E., Docherty S., Gandy S.J. Quantitative MRI evaluation of whole abdomen adipose tissue volumes in healthy volunteers – validation of technique and implications for clinical studies. *BJR*. 2018; 91 (1087): 108720180025. DOI: 10.1259/bjr.20180025.
7. Klingensmith J.D., Elliott A.L., Givan A.H., Faszold Z.D., Mahan C.L., Doedtman A.M., Fernandez-Del-Valle M. Development and evaluation of a method for segmentation of cardiac, subcutaneous, and visceral adipose tissue from Dixon magnetic resonance images. *J. Med. Imaging (Bellingham)*. 2019; 6 (1):014004. DOI: 10.1117/1.JMI.6.1.014004.
8. Pescatori L.C., Savarino E., Mauri G., Silvestri E., Cariati M., Sardanelli F., Sconfienza L.M. Quantification of visceral adipose tissue by computed tomography and magnetic resonance imaging: reproducibility and accuracy. *Radiol. Bras.* 2019; 52 (1): 1–6. DOI: 10.1590/0100-3984.2017.0211.
9. Cronin O., Liu D., Bradshaw B., Iyer V., Buttner P., Cunningham M., Walker P.J., Golledge J. Visceral adiposity is not associated with abdominal aortic aneurysm presence and growth. *Vascular Medicine* 2014; 19 (4): 272–280. DOI: 10.1177/1358863X14537883.
10. Golledge J., Clancy P., Jamrozik K., Norman P.E. Obesity, adipokines, and abdominal aortic aneurysm: Health in Men study. *Circulation*. 2007; 116 (20): 2275–2279. DOI: 10.1161/CIRCULATIONAHA.107.717926.
11. Erbel R., Aboyans V., Boileau C., Bossone E., Bartolomeo R.D., Eggebrecht H., Evangelista A., Falk V., Frank H., Gaemperli O., Grabenwöger M., Haverich A., Jung B., Manolis A.J., Meijboom F., Nienaber C.A., Roffi M., Rousseau H., Sechtem U., Sirnes P.A., Allmen R.S., Vrints C.J.; ESC Committee for Practice Guidelines. 2014 ESC Guidelines on the diagnosis and treatment of aortic diseases: Document covering acute and chronic aortic diseases of the thoracic and abdominal aorta of the adult. The Task Force for the Diagnosis and Treatment of Aortic Diseases of the European Society of Cardiology (ESC). *Eur. Heart J.* 2014; 35 (41): 2873–2926. DOI: 10.1093/eurheartj/ehu281.
12. Ahmedzhanov N.M., Butrova S.A., Dedov I.I., Zvenigorodskaya L.A., Kislyak O.A., Koshel'skaya O.A., Kuznetsova I.V., Kuharchuk V.V., Litvin A.YU., Medvedeva I.V., Mkrtumyan A.M., Mychka V.B., Nebieridze D.V., Nedogoda S.V., Oganov V.G., Ogarkov M.YU., Perepech N.B., Podzolkov V.I., Smetnik V.P., Susekov A.V., Titov V.N., Tyurina T.V., Fursov A.N., Hirmanov V.N., Chazova I.E., Chukaeva I.I., SHeStakova M.V., Shubina A.T. Russian experts' consensus on metabolic syndrome problem in the Russian Federation: definition, diagnostic criteria, primary prevention, and treatment. *Preventive medicine*. 2010; 13 (5): 27–32 (in Russ.).
13. Coolen B.F., Calcagno C., van Ooij P., Fayad Z.A., Strijkers G.J., Nederveen A.J. Vessel wall characterization using quantitative MRI: what's in a number? *MAGMA*. 2018; 31 (1): 201–222. DOI: 10.1007/s10334-017-0644-x.
14. Neeland I.J., Ayers C.R., Rohatgi A.K., Turer A.T., Berry J.D., Das S.R., Vega G.L., Khera A., McGuire D.K., Grundy S.M., de Lemos J.A. Associations of visceral and abdominal subcutaneous adipose tissue with markers of cardiac and metabolic risk in obese adults. *Obesity*. 2013; 21 (9): E439–E447. DOI: 10.1002/oby.20135.
15. Gruzdeva O.V., Borodkina D.A., Dyleva Y.A., Kuzmina A.A., Belik E.V., Brel N.K., Karetnikova V.N., Kashtalap V.V., Bychkova E.E., Barbarash O.L. The relationship of the epicardial fat and adipokines in myocardial infarction. *Russian Clinical Laboratory Diagnostics*. 2020; 65 (9): 533–540 (in Russ.).

16. Reneau J., Goldblatt M., Gould J., Kindel T., Kastenmeier A., Higgins R., Rengel L.R., Schoyer K., James R., Obi B., Moosreiner A., Nicholson K., Sahoo D., Kidambi S. Effect of adiposity on tissue-specific adiponectin secretion. *PLoS One*. 2018; 13 (6): e0198889. DOI: 10.1371/journal.pone.0198889.
17. Frederiksen L., Nielsen T.L., Wraae K., Hagen C., Frystyk J., Flyvbjerg A., Brixen K., Andersen M. Subcutaneous rather than visceral adipose tissue is associated with adiponectin levels and insulin resistance in young men. *J. Clin. Endocrinol. Metab.* 2009; 94 (10): 4010-5. DOI: 10.1210/jc.2009-0980.
18. Beshpalova I.D., Ryazantseva N.V., Kalyuzhin V.V., Dzyuman A.N., Osikhov I.A., Medyantsev Y.A., Klinovitskiy I.Y., Murashev B.Y., Afanasyeva D.S., Bychkov V.A. Clinicomorphological parallels in abdominal obesity. *Bulletin of the Siberian Branch of the Russian Academy of Medical Sciences*. 2014; 34 (4): 51–58 (in Russ.).
19. Apoloni R.C., Zerati A.E., Wolosker N., Saes G.F., Wolosker M., Curado T., Puech-Leão P., De Luccia N. Analysis of the Correlation Between Central Obesity and Abdominal Aortic Diseases. *Ann. Vasc. Surg.* 2019; 54: 176–184. DOI: 10.1016/j.avsg.2018.06.016.
20. Goldenberg L., Saliba W., Hayeq H., Hasadia R., Zeina A.R. The impact of abdominal fat on abdominal aorta calcification measured on non-enhanced CT. *Medicine (Baltimore)*. 2018; 97 (49): e13233. DOI: 10.1097/MD.00000000000013233.
21. Muenkaew M., Boonyasirinant T., Krittayaphong R. Correlation between aortic stiffness and visceral fat determined by magnetic resonance imaging. *J. Med. Assoc. Thai.* 2012; 95 (2): S117–S126.
22. Sutton-Tyrrell K., Newman A., Simonsick E.M., Havlik R., Pahor M., Lakatta E., Spurgeon H., Vaitkevicius P. Aortic stiffness is associated with visceral adiposity in older adults enrolled in the study of health, aging, and body composition. *Hypertension*. 2001; 38 (3): 429–433. DOI: 10.1161/01.hyp.38.3.429.
23. Mzayek F., Wang L.E., Relyea G., Yu X., Terry J.G., Carr J., Hundley G.W., Hall M.E., Correa A. Impact of abdominal Obesity on Proximal and Distal Aorta Wall Thickness in African Americans: The Jackson Heart Study. *Obesity (Silver Spring)*. 2019; 27 (9): 1527–1532. DOI: 10.1002/oby.22563.
24. Polat T.B., Urganci N., Caliskan K.C., Akyildiz B. Correlation of abdominal fat accumulation and stiffness of the abdominal aorta in obese children. *J. Pediatr. Endocrinol. Metab.* 2008; 21 (11): 1031–1040. DOI: 10.1515/jpem.2008.21.11.1031.
25. Efe D., Aygün F., Acar T., Yildiz M., Gemici K. Investigation of relation between visceral and subcutaneous abdominal fat volumes and calcified aortic plaques via multislice computed tomography. *Vascular*. 2015; 23 (4): 396–402. DOI: 10.1177/1708538114552012.
26. Reijrink M., de Boer S.A., Spoor D.S., Lefrandt J.D., Lambers Heerspink H.J., Boellaard R., Greuter M.J., Borra R.J.H., Hillebrands J.L., Slart R.H.J.A., Mulder D.J. Visceral adipose tissue volume is associated with premature atherosclerosis in early type 2 diabetes mellitus independent of traditional risk factors. *Atherosclerosis*. 2019; 290: 87–93. DOI: 10.1016/j.atherosclerosis.2019.09.016.
27. Wang H., Peng D.Q. New insights into the mechanism of low high-density lipoprotein cholesterol in obesity. *Lipids Health Dis.* 2011; 10: 176. DOI: 10.1186/1476-511X-10-176.
28. Yanai H., Yoshida H. Beneficial effects of adiponectin on glucose and lipid metabolism and atherosclerotic progression: mechanisms and perspectives. *International Journal of Molecular Sciences*. 2019; 20 (5): 1190. DOI: 10.3390/ijms20051190.
29. Dereziński T., Zozulińska-Ziółkiewicz D., Uruska A., Dąbrowski M. Abdominal aorta diameter as a novel marker of diabetes incidence risk in elderly women. *Sci. Rep.* 2020; 10 (1): 13734. DOI: 10.1038/s41598-020-70736-1.

Acknowledgements

The authors express their gratitude to Natalia Y. Margolis for her assistance in statistical processing of the research results.

Authors contribution

Ryumshina N.I. – analysis and interpretation of data, critical revision of the manuscript for important intellectual content, drafting of the manuscript. Koshelskaya O.A. – conception and design, critical revision of the manuscript for important intellectual content, final approval of the manuscript for publication. Kologrivova I.V. – analysis and interpretation of data, critical revision of the manuscript for important intellectual content. Kharitonova O.A. – analysis and interpretation of data. Nasekina K.A. – analysis and interpretation of data. Ussov V.Yu. – conception and design, final approval of the manuscript for publication.

Authors information

Ryumshina Nadezhda I., Cand. Sci. (Med.), Researcher, Department of Radiology and Tomography, Cardiology Research Institute, Tomsk NRC, Tomsk, Russian Federation. ORCID 0000-0002-6158-026X.

Koshelskaya Olga A., Dr. Sci. (Med.), Professor, Principal Researcher, Department of Atherosclerosis and Coronary Artery Disease, Cardiology Research Institute, Tomsk NRMC, Tomsk, Russian Federation. ORCID 0000-0002-6679-1269.

Kologrivova Irina V., Cand. Sci. (Med.), Researcher, Department of Clinical Laboratory Diagnostics, Cardiology Research Institute, Tomsk NRMC, Tomsk, Russian Federation. ORCID 0000-0003-4537-0008.

Kharitonova Olga A., Junior Researcher, Department of Atherosclerosis and Coronary Artery Disease, Cardiology Research Institute, Tomsk NRMC, Tomsk, Russian Federation. ORCID 0000-0002-2818-5882.

Nasekina Kristina A., Student, Siberian State Medical University, Tomsk, Russian Federation. ORCID 0000-0003-0530-5703.

Ussov Vladimir Yu., Dr. Sci. (Med.), Professor, Head of the Department of Radiology and Tomography, Cardiology Research Institute, Tomsk NRMC, Tomsk, Russian Federation. ORCID 0000-0001-7978-5514.

(✉) **Koshelskaya Olga A.**, e-mail: koshel@live.ru

Received 14.04.2021

Accepted 27.05.2021

The role of cytokines in the pathogenesis of infectious complications in surgical treatment of obstructive jaundice of gallstone origin

Smirnova O.V., Elmanova N.G.

*Research Institute of Medical Problems of the North (RI MPN), Federal Research Center "Krasnoyarsk Scientific Center of the Siberian Branch of the Russian Academy of Sciences" (FRC KSC SB RAS)
3G, Partizana Zheleznaya Str., Krasnoyarsk, 660022, Russian Federation*

ABSTRACT

Aim. To study the content of cytokines in the blood serum of patients with obstructive jaundice of gallstone origin before and after surgical treatment, depending on the development of postoperative complications.

Materials and methods. The treatment group consisted of 70 patients with the diagnosis of obstructive jaundice of gallstone origin, verified following a comprehensive clinical and instrumental examination. In 54 patients, the postoperative period was uncomplicated, and in 16 patients, various infectious complications in the postoperative period were revealed. The control group consisted of 125 healthy volunteers. The concentration of six cytokines (interleukin (IL)-2, IL-4, IL-18, IL-10, tumor necrosis factor alpha (TNF α), and interferon gamma (INF γ)) was determined using reagent kits manufactured by Vector-Best LLC (Novosibirsk, Russian Federation) by enzyme-linked immunosorbent assay on the Thermo Scientific analyzer (BioMerieux, France).

Results. We identified significantly high concentrations of proinflammatory cytokines in the blood serum of patients with obstructive jaundice of gallstone origin upon admission, compared with the data obtained in the study of blood serum in the control group and in patients with obstructive jaundice after surgery. In the postoperative period in patients with obstructive jaundice without complications, the proinflammatory cytokines are significantly reduced and IL-4 is increased, whereas with the development of infectious complications, the level of proinflammatory cytokines is significantly elevated.

Conclusion. In the pathogenesis of obstructive jaundice, a local inflammatory process plays an essential role. This is confirmed by statistically significant changes in the studied cytokines. The established increase in the concentration of IL-4, which has anti-inflammatory activity, indicates its importance in the mechanisms underlying the absence of infectious complications in the postoperative period of obstructive jaundice of gallstone origin. The revealed increase in the levels of IL-18, TNF α , and INF γ in the blood serum of patients suggests their role in the pathogenesis of infectious complications in the postoperative period of obstructive jaundice of gallstone origin.

Key words: cytokines, pathogenesis, postoperative complications, inflammation, obstructive jaundice.

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

Source of financing. The study was supported by the funding within the state assignment of RI MPN, FRC KSC SB RAS.

Conformity with the principles of ethics. All participants signed an informed consent to take part in the study. The study was approved by the local Ethics Committee at Research Institute of Medical Problems of the North (Protocol No. 7 or 16.11.2012).

For citation: Smirnova O.V., Elmanova N.G. The role of cytokines in the pathogenesis of infectious complications in surgical treatment of obstructive jaundice of gallstone origin. *Bulletin of Siberian Medicine*. 2021; 20 (3): 105–111. <https://doi.org/10.20538/1682-0363-2021-3-105-111>.

✉ Smirnova Olga V., e-mail: ovsmirnova71@mail.ru

Роль цитокинов в патогенезе инфекционных осложнений при хирургическом лечении механической желтухи желчнокаменного генеза

Смирнова О.В., Елманова Н.Г.

Научно-исследовательский институт медицинских проблем Севера (НИИ МПС), Федеральный исследовательский центр «Красноярский научный центр Сибирского отделения Российской академии наук» (ФИЦ КНЦ СО РАН)

660022, г. Красноярск, ул. Партизана Железняка, 3г

РЕЗЮМЕ

Цель. Изучить содержание цитокинов в сыворотке крови пациентов с механической желтухой желчнокаменного генеза до и после хирургического лечения в зависимости от развития послеоперационных осложнений.

Материалы и методы. Основная группа состояла из 70 пациентов с верифицированным на основании комплексного клинично-инструментального обследования диагнозом «механическая желтуха желчнокаменного генеза». У 54 больных послеоперационный период был без осложнений и у 16 больных выявлялись различные инфекционные осложнения в послеоперационном периоде. Контрольную группу составили 125 практически здоровых добровольцев. Концентрацию шести цитокинов (интерлейкина (IL) 2, IL-4, IL-18, IL-10, фактора некроза опухоли α (TNF α), интерферона γ (INF γ)) определяли с использованием набора реагентов производства ЗАО «Вектор-Бест» (г. Новосибирск) методом иммуноферментного анализа на иммуноферментном анализаторе Thermo Scientific (BioMerieux, Франция).

Результаты. Установлено наличие статистически значимо высоких концентраций провоспалительных цитокинов в сыворотке крови пациентов с механической желтухой желчнокаменного генеза при поступлении относительно данных, полученных при исследовании сыворотки крови контрольной группы и больных с механической желтухой после операции. В послеоперационном периоде у пациентов с механической желтухой без осложнений статистически значимо снижаются показатели провоспалительных цитокинов и увеличивается IL-4, а при развитии инфекционных осложнений – статистически значимо увеличивается уровень провоспалительных цитокинов.

Заключение. В патогенезе механической желтухи важную роль играет локальный воспалительный процесс. Это подтверждается статистически значимыми изменениями изучаемых цитокинов. Установленное нарастание концентрации IL-4, обладающего противовоспалительной активностью, свидетельствует о его значимости в механизмах отсутствия развития инфекционных осложнений в послеоперационном периоде механической желтухи желчнокаменного генеза. Выявленное повышение в сыворотке крови уровня IL-18, TNF α , INF γ позволяет предположить их участие в патогенезе инфекционных осложнений в послеоперационном периоде у пациентов с механической желтухой желчнокаменного генеза.

Ключевые слова: цитокины, патогенез, послеоперационные осложнения, воспаление, механическая желтуха.

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

Источник финансирования. Исследование проводилось за счет средств государственного задания НИИ МПС ФИЦ КНЦ СО РАН.

Соответствие принципам этики. Все участники подписали информированное согласие на участие в исследовании. Исследование одобрено НИИ МПС (протокол № 7 от 16.11.2012).

Для цитирования: Смирнова О.В., Елманова Н.Г. Роль цитокинов в патогенезе инфекционных осложнений при хирургическом лечении механической желтухи желчнокаменного генеза. *Бюллетень сибирской медицины*. 2021; 20 (3): 105–111. <https://doi.org/10.20538/1682-0363-2021-3-105-111>.

INTRODUCTION

Obstructive jaundice (OJ) is an acute clinical condition caused by the appearance of an obstacle to the outflow of bile in the duodenum [1]. OJ of benign genesis is commonly caused by gallstone disease. Currently, many researchers point to the increasing number of patients with OJ in many countries of the world, therefore, OJ is a medical and social problem of modern society [2, 3]. The pathogenesis of OJ is complex; the development of a local and systemic inflammatory response and endotoxemia contributes to destruction and disorganization of the gastrointestinal tract and requires urgent surgical aid to restore patency of the bile ducts and eliminate all complications [4–7]. Considering the data of numerous studies on the leading role of the destructive and inflammatory process in the pathogenesis of OJ, which directly involves cytokines, it can be assumed that signs of imbalance in cytokine production affect the course of the postoperative period in patients with OJ. The data of such studies are ambiguous and few in number [8–10].

Following tissue infection in the postoperative period, a patient with OJ develops a complex, multicomponent sequence of reactions aimed at isolating and destroying the pathogen, preventing tissue destruction, triggering repair processes, and restoring homeostasis. The initiation and development of the inflammatory response is controlled by proinflammatory cytokines produced by T cells, neutrophils, and macrophages in response to a bacterial pathogen. Cells of the immune system produce IL-2, TNF- α , INF- γ , and IL-8. Proinflammatory cytokines play a protective role by stimulating the phagocytic and bactericidal activity of neutrophils and macrophages and triggering antigen-specific immunity aimed at eliminating the pathogen. The protective role of proinflammatory cytokines is carried out when mediators work locally in the focus of inflammation, while systemic overproduction of these cytokines contributes to the appearance of toxic shock syndrome and organ dysfunctions. To overcome excessive manifestations of systemic inflammation in a patient with OJ, negative regulation mechanisms are activated due to the production of anti-inflammatory cytokines (IL-4 and IL-10) and soluble inhibitors of proinflammatory cytokines. The basis of the pathogenesis of infectious complications in the postoperative period in OJ is the development of cytokine storm, which includes pro-inflammatory and anti-inflammatory mediators.

The balance between the two opposite groups largely determines the course and outcome of infectious complications in OJ in the postoperative period [11, 12].

Therefore, the above-stated data allow to make an important conclusion about the relevance of a comprehensive study of the content and balance of cytokines before and after surgical treatment of patients with OJ of gallstone origin in order to understand the molecular mechanisms of the development of postoperative complications in OJ and formulate the aim of this study.

The aim of the study was to determine the content of cytokines (IL-2, TNF- α , INF- γ , IL-18, IL-4, and IL-10) in patients with OJ caused by gallstones before and after surgical treatment, depending on the development of postoperative complications.

MATERIALS AND METHODS

To accomplish this aim, 70 patients were examined (the average age was (44.02 ± 14.88) years) with the diagnosis of OJ of gallstone origin, verified following a comprehensive clinical and instrumental examination. All the patients were admitted to the surgical department of the clinic of the Research Institute of Medical Problems of the North of FRC of the KSC SB RAS. All the patients were diagnosed on the basis of a standard examination, including history taking, clinical presentation, detailed blood count, biochemical parameters (bilirubin level, alanine aminotransferase (ALT), alkaline phosphatase, total protein, etc.), and an instrumental examination (ultrasound, MRI, etc.).

In the postoperative period, 16 out of 70 patients (22.8%) developed inflammatory infectious complications that required additional drug correction. As control values of the cytokines in the blood serum determined in the present study, we used the data obtained from 125 practically healthy volunteers, comparable in sex and age with the group of the examined patients, who were selected during regular health checkups in Krasnoyarsk medical facilities. The examination of the participants was approved by the Ethics Committee of the Research Institute of Medical Problems of the North (Protocol No. 7 of November 16, 2012). Each participant signed an informed consent for the examination. When working with the examined patients, we followed the ethical principles set out in Art. 24 of the Constitution of the Russian Federation and the Declaration of Helsinki developed by the World Medical Association.

The exclusion criteria were: persons with HIV, tuberculosis, and drug addiction; refusal to participate in the study; age over 80 years. The material for the study was venous blood which was drawn from the ulnar vein of the patients twice: before the start of etiotropic therapy and 7 days after surgical treatment. Serum samples were frozen and stored in the refrigerator at -70°C prior to testing. Once frozen, the blood serum was thawed to room temperature before testing.

The concentration of six cytokines (interleukin (IL)-2, IL-4, IL-18, IL-10, tumor necrosis factor alpha (TNF α), and interferon gamma (INF γ)) was determined using reagent kits manufactured by Vector-Best LLC (Novosibirsk, Russian Federation) by enzyme-linked immunosorbent assay on the Thermo Scientific analyzer (BioMerieux, France).

The concentration of the cytokines was determined in pg / ml [13, 14]. Statistical data were analyzed using the Statistica 10 software package (StatSoft Inc., USA). Non-normal distribution of parameters in all the obtained samples was found using the Kolmogorov – Smirnov test with Lilliefors correction. The statistical significance of the differences was assessed using the nonparametric Wilcoxon test for dependent samples and using the Mann – Whitney test with Bonferroni's correction for independent samples. All data were presented as the median and the interquartile range (*Me*) [Q_1 – Q_3]. The differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

In the present study, in the blood serum of the patients with uncomplicated OJ of gallstone origin, statistically significantly high concentrations of the proinflammatory cytokines were detected, compared with the data obtained from practically healthy volunteers (Table 1). An increase in the concentration of TNF α by more than 60 times was revealed in the patients with OJ before the surgery, compared with the control group ($p = 0.0006$); after surgery, the value decreased. This indicates an increase in the natural cytotoxicity of monocytes, macrophages, and natural killer (NK) cells [15, 16]. It plays an important role in inflammation, promotes adhesion of granulocytes to the vascular endothelium, accelerates transendothelial migration of leukocytes to the inflammation focus, and increases the phagocytic activity of neutrophils and monocytes, implementing development of complete phagocytosis [17].

The concentration of INF γ was elevated in all OJ patients with an uncomplicated postoperative period

($p = 0.001$), whereas statistically significant differences were detected in OJ patients before the surgery compared with the control group. Its formation and secretion occur after a repeated response of previously sensitized lymphocytes to the antigen. INF γ stimulates cells of the immune system to develop an immune response.

Overproduction of the cytokine IL-18 was found in the OJ patients before and after surgical treatment ($p = 0.001$); the most pronounced increase in the parameter value was detected in the patients before the pathogen-specific treatment of OJ of gallstone origin. Under the influence of IL-18, antimicrobial resistance of the whole body increased [18].

Overproduction of proinflammatory cytokines in OJ proves the presence of local and systemic inflammation, which aggravate the clinical course of the disease in patients. After surgical treatment, the concentration of proinflammatory cytokines decreases, but does not reach normal control values, proving that the surgery itself is a surgical injury that maintains the inflammatory process in the body [19–21].

The increased content of IL-2, a regulator of cellular antigen-specific immune response, was identified. The median IL-2 concentration was 7 times higher in the OJ patients before the surgery and 5 times higher after the surgery, compared with the control group. By activating various signaling pathways, it causes proliferation and activation of T cells for implementing the immune response.

When assessing the content of cytokines, the increase in which is associated with the activation of type 2 inflammation, a statistically significant decrease in the median concentrations of IL-4 and IL-10 in the patients with OJ before etiotropic treatment was found, compared with the control group. Therefore, in the patients with OJ of gallstone origin before surgical treatment, type 1 inflammation prevailed. At runtime, in the patients with OJ, the concentration of IL-4 increased after surgical treatment, compared with the control group. It is likely that the revealed changes in the content of IL-4 do not exclude its ability to participate in development of connective tissue and fibrosis at the site of surgical injury. The role of anti-inflammatory cytokines in limiting local inflammation and preventing the development of systemic phenomena is not excluded [22, 23].

In 16 patients with OJ of gallstone origin, infectious complications developed in the postoperative period (Table 2). Evaluation of the content of pro- and anti-inflammatory cytokines revealed statistically

significantly higher concentrations of cytokines with pro-inflammatory properties (TNF α , INF γ , IL-18) at baseline and in the postoperative period, while the median concentrations of anti-inflammatory cytokines remained low. Acute overproduction of proinflammatory cytokines in the bloodstream contributes to development of a systemic inflammatory response with corresponding clinical manifestations (cytokine storm), to correct which involvement of all organs and systems of the body is required in case of severe impairment in hemostasis. Prolonged pathologically high concentrations of proinflammatory cytokines cause septic shock and death of the patient [17, 24, 25]. Thus, in conditions when the concentration of cytokines exceeds physiological values for a long time, their protective function changes to a pathological one [15, 26]. Due to inadequate overproduction, cytokines themselves have a histodestructive effect, causing multiple organ failure.

CONCLUSION

The study made it possible to establish that statistically significantly higher concentrations of proinflammatory cytokines (IL-2, TNF α , INF γ , IL-18) are detected in the blood serum of patients with OJ of gallstone origin before the surgery, which indicates the significance of type 1 inflammation in the pathogenesis of OJ of gallstone origin. It was found that in the blood serum of the patients with OJ with an uncomplicated postoperative period, statistically significantly higher concentrations of IL-4, which action is aimed at compensating the consequences of the inflammatory process and restoring the damaged tissue of the hepatobiliary system, were revealed along with a combination of type 1 and type 2 inflammation. The identified high concentrations of TNF α , INF γ , and IL-18 in the postoperative period of OJ suggest their participation in the development of infectious complications.

Table 1

The content of cytokines in the blood serum of patients with OJ with an uncomplicated postoperative period at runtime, compared with the control group, pg / ml						
Parameters	Control group, n = 125 (1)		OJ patients before the surgery, n = 54 (2)		OJ patients after the surgery, n = 54 (3)	
	Me	Q_1-Q_3	Me	Q_1-Q_3	Me	Q_1-Q_3
IL-2	1.1	0.5–3.05	7.25	4.72–9	5.5	3–7.3
	–		$p_{1-2} = 0.001$		$p_{1-3} = 0.001$	
TNF α	0.54	0.38–0.88	35.7	4.82–86.5	8.1	3.1–51
	–		$p_{1-2} = 0.0006$		$p_{1-3} = 0.001$	
INF γ	0.6	0.22–4.0	9.2	2.8–21.83	7.3	2.7–8.1
	–		$p_{1-2} = 0.001$		–	
IL-18	1.6	0.1–2.1	90	56.9–2452.	61.7	53.88–99.3
	–		$p_{1-2} = 0.001$		$p_{1-3} = 0.001$	
IL-4	2.15	0.6–4.8	0.01	0.009–2.2	38.4	4.3–377.6
	–		$p_{1-2} = 0.003$		$p_{1-3} < 0.001$ $p_{2-3} < 0.001$	
IL-10	14.8	8.65–26.85	2.6	1.1–5.4	2.1	1.05–2.75
	–		$p_{1-2} = 0.001$		$p_{1-3} = 0.04$	

Note: p_{1-2} – statistically significant differences between the control group and the group of patients with OJ before the surgery; p_{1-3} – statistically significant differences between the control group and the group of patients with OJ after the surgery; p_{2-3} – statistically significant differences between the group of OJ patients after the surgery and the group of patients with OJ before the surgery (here and in Table 2).

Table 2

The concentration of cytokines in the blood serum of patients with OJ with a complicated postoperative period at runtime, compared with the control group, pg / ml						
Parameters	Control group, n = 125 (1)		OJ patients before the surgery, n = 16 (2)		OJ patients after the surgery, n = 16 (3)	
	Me	Q_1-Q_3	Me	Q_1-Q_3	Me	Q_1-Q_3
IL-2	1.1	0.5–3.05	9.43	6.12–11.7	12.26	7.96–15.21
	–		$p_{1-2} = 0.001$		$p_{1-3} = 0.001$	
TNF α	0.54	0.38–0.88	46.41	6.27–112.5	60.33	8.15–146.19
	–		$p_{1-2} = 0.001$		$p_{1-3} = 0.001$	

Table 2 (continued)

	Control group, <i>n</i> = 125 (1)		OJ patients before the surgery, <i>n</i> = 16 (2)		OJ patients after the surgery, <i>n</i> = 16 (3)	
	<i>Me</i>	<i>Q</i> ₁ – <i>Q</i> ₃	<i>Me</i>	<i>Q</i> ₁ – <i>Q</i> ₃	<i>Me</i>	<i>Q</i> ₁ – <i>Q</i> ₃
INF γ	0.6	0.22–4.0	12.0	3.64–28.4	15.6	4.73–36.9
	–		<i>p</i> ₁₋₂ = 0.001		–	
IL-18	1.6	0.1–2.1	117	73.97–318.8	80.21	70.04–129.1
	–		<i>p</i> ₁₋₂ = 0.001		<i>p</i> ₁₋₃ = 0.0006	
IL-4	2.15	0.6–4.8	0.013	0.012–2.86	0.017	0.015–2.9
	–		<i>p</i> ₁₋₂ = 0.001		<i>p</i> ₁₋₃ = 0.00001	
IL-10	14.8	8.65–26.85	2.2	0.19–4.4	0.9	0.85–2.75
	–		<i>p</i> ₁₋₂ = 0.001		<i>p</i> ₁₋₃ = 0.001	

REFERENCES

- Galperin E.I. Classification of the severity of obstructive jaundice. *Annals of Surgical Hepatology*. 2012; 2 (17): 26–33 (in Russ.).
- Voronova E.A., Pakhomova R.A. Modern ideas about the classification of obstructive jaundice. *Modern Issues in Science and Education*. 2015; 6: 298 (in Russ.).
- Kalakhanova B.Kh., Chechenin G.M. Modern approach to the choice of the timing of endobiliary prosthesis in patients with obstructive jaundice of tumor genesis. *Clinical Medicine*. 2014; 6 (4): 97–101 (in Russ.).
- Datsenko B.M., Borisenko V.B. Obstructive jaundice, acute cholangitis, biliary sepsis: their pathogenetic relationship and principles of differential diagnosis. *News of Surgery*. 2013; 21 (5): 31–39 (in Russ.).
- Koshevsky P.P., Alekseev S.A., Bovtyuk N.Ya., Kartun L.V. Correction of endotoxemia and imbalance of anti-inflammatory cytokines in the early postoperative period in patients with obstructive jaundice and biliary hypertension of non-tumor etiology. *Russian Medical Journal*. 2014; 74(1): 74–78 (in Russ.).
- Rutenberg G.M., Romyantsev G.M., Protasov A.V. The effectiveness of minimally invasive surgical approaches in the surgical treatment of choledocholithiasis. *Endoscopic Surgery*. 2008; 14 (1): 3–8 (in Russ.).
- Kint J.F., van den Bergh J.E., van Gelder R.E. Percutaneous treatment of common bile duct stones: results and complications in 110 consecutive patients. *Dig. Surg*. 2015; 32 (1): 9–15. DOI: 10.1159/000370129.
- Mostyuk E.M., Dergach V.Ya., Kriventsov M.A., Shelepa E.D., Segal D.F. Prognosis and prevention of postoperative hepatic failure in obstructive jaundice. *Crimean Journal of Experimental and Clinical Medicine*. 2015; 2 (18): 48–51 (in Russ.).
- Hajiyev J.N., Tagiyev E.G., Hajiyev N.J. The state of the cytokine status in patients with obstructive jaundice of benign genesis. *Surgery*. 2015; 5: 56–58 (in Russ.).
- Voronova A.A., Vinnik Yu.S., Pakhomova R.A. Surgical tactics of benign surgical jaundice, depending on its severity. *Basic Research*. 2015; 1(8): 1554–1557 (in Russ.).
- Simbirtsev A.S. Cytokines – classification and biological function. *Cytokines and Inflammation*. 2004; 3 (2): 16–22 (in Russ.).
- Khaitov R.M. Immunology. M.: GEOTAR-Media, 2016: 716 (in Russ.).
- Smirnova O.V., Titova N.M., Kasparov E.V., Elmanova N.G. Characteristics of cytokine regulation in patients with obstructive jaundice syndrome of benign, benign tumor, and malignant genesis. *In the World of Scientific Discoveries*. 2016; 11 (83): 27–41 (in Russ.).
- Smirnova O.V., Titova N.M., Manchuk V.T., Elmanova N.G. Features of cytokine regulation in patients with obstructive jaundice of various origins. *Modern Issues in Science and Education*. 2015; 4: 425 (in Russ.).
- Bebezov Kh.S., Osmonov T.A., Bebezov B.Kh., Raimkulov A.E., Ermekov T.A. Results of percutaneous transhepatic endobiliary interventions in biliary tract surgery. *Annals of Surgical Hepatology*. 2006; 11 (4): 44–49 (in Russ.).
- Xu F., Dai C., Peng S., Zhao Y., Jia C.-J., Xu Y.-Q. Preconditioning with glutamine protects against ischemia/reperfusion-induced hepatic injury in rats with obstructive jaundice. *Pharmacology*. 2014; 93 (3-4): 155–165. DOI: 10.1159/000360181.
- Chen J., Wu F., Long Y., Yu W. Glutathione supplementation attenuates oxidative stress and improves vascular hyporesponsiveness in experimental obstructive jaundice. *Oxid. Med. Cell Longev*. 2015; 486148. DOI: 10.1155/2015/486148.
- Smirnova O.V., Tsukanov V.V., Titova N.M., Gubanov B.G. Features of cytokine regulation in patients with obstructive jaundice of malignant genesis. *Medical Immunology*. 2018; 20 (1): 135–140 (in Russ.). DOI: 10.15789/1563-0625-2018-1-135-144
- Tarasenko S.V., Natalsky A.A., Nikiforov A.A. Polymorphism of genes for biotransformation of xenobiotics, TNF α , and IL-4 in patients with obstructive jaundice. *Cytokines and Inflammation*. 2014; 13 (1): 51–59 (in Russ.).
- Jones C., Badger S., Black J. The use of antiendotoxin peptides in obstructive jaundice endotoxemia. *Eur. J. Gastroenterol. Hepatol*. 2012; 24 (3): 248–254. DOI: 10.1097/MEG.0b013e32834dfb8c.
- Galperin E.I., Akhaladze G.G., Vetshev P.S., Dyuzheva T.G. Differential approach to the use of minimally invasive methods for treating obstructive jaundice of tumor genesis. *Annals of Surgical Hepatology*. 2019; 24(2): 10–24 (in Russ.). DOI: 10.16931/1995-5464.2019210-24.
- Belyaev A.N., Khvostunov S.I., Belyaev S.A., Kostin S.V. Metabolic correction of cerebral disorders in obstructive

- cholestasis (experimental study). *Annals of Surgical Hepatology*. 2016; 21 (2): 86–97 (in Russ.).
24. Vetshev P.S., Stoyko Yu.M., Levchuk A.L., Bardakov V.G. Possibilities of modern diagnostic methods and substantiation of therapeutic tactics in obstructive jaundice. *Bulletin of Surgical Gastroenterology*. 2008; 2: 24–32 (in Russ.).
 25. Artemov A. V., Rozikov Yu.Sh., Bulanov N.N., Cheminaeva N.R. The effectiveness of new methods of diagnosis and treatment in diseases of the extrahepatic bile ducts. *Medical Imaging*. 2006; 6: 60–64 (in Russ.).
 26. Badger S.A., Jones C. Cytokine response to portal endotoxaemia and neutrophil stimulation in obstructive jaundice. *Eur. J. Gastroenterol. Hepatol.* 2012; 24 (1): 25–32. DOI: 10.1097/MEG.0b013e32834b0dd3.
 27. Kryshen V.P., Ryazanov D.Yu., Polyudov A.A., Zadorozhny V.V., Ruban A.G. Therapeutic and diagnostic tactics in patients with obstructive jaundice syndrome. *Kharkiv Surgical School*. 2013; 5 (62): 19–25 (in Russ.).

Authors information

Smirnova Olga V., Dr. Sci. (Med.), Associate Professor, Head of the Laboratory of Clinical Pathophysiology, RI MPN of FIC KSC SB RAS, Krasnoyarsk, Russian Federation. ORCID 0000-0003-3992-9207.

Elmanova Nina G., Junior Researcher, Laboratory of Clinical Pathophysiology, RI MPN of FIC KSC SB RAS, Krasnoyarsk, Russian Federation.

(✉) **Smirnova Olga V.**, e-mail: ovsmirnova71@mail.ru

Received 17.04.2020

Accepted 29.09.2020

Incidence rate and clinical characteristics of seasonal affective disorders in senior medical students

Ukraintsev I.I.¹, Schastnyy E.D.², Bokhan N.A.^{1,2}

¹ Siberian State Medical University (SSMU)
2, Moscow Trakt, Tomsk, 634055, Russian Federation

² Mental Health Research Institute, Tomsk National Research Medical Center of the Russian Academy of Sciences (Tomsk NRMC RAS)
4, Aleutskaya Str., Tomsk, 634014, Russian Federation

ABSTRACT

Aim. To study the incidence rate, clinical features, and prognosis of seasonal affective disorder (SAD) in senior (6th-year) medical students.

Materials and methods. SAD screening using the Seasonal Pattern Assessment Questionnaire (SPAQ, 1987) included 119 undergraduate medical students. 78 students were females (65.5%) and 41 – males (34.5%) ($p = 0.001$). The average age of women was 23 (22; 23) years, the average age of men – 23 (22; 24) years. Statistical processing was performed using the Mann – Whitney U-test, Pearson's χ^2 test, and Spearman's rank correlation coefficient (r_s).

Results. The data on the prevalence of affective disorders with a seasonal pattern in medical students were obtained: SAD – 9.2%, sub-SAD – 13.5%, psychological undulation of season perception (PUSP) – 16.8%. The number of students who did not exhibit seasonal undulation of the six main characteristics recorded by the SPAQ was 72 (60.5%) ($p = 0.001$). There were statistically significant differences in the higher median Global Seasonality Score of the SPAQ for SAD compared with PUSP, both with and without account of the gender factor ($p = 0.001$). The use of a binary logistic regression model made it possible to identify groups of students with or without SAD according to the SPAQ. The data obtained determined the contribution of the following factors: gender, seasonality, body weight, and the number of sleep hours per day in spring.

Conclusion. The study made it possible to obtain a logistic regression model that allowed to predict the greatest likelihood of developing SAD.

Key words: seasonal affective disorder, medical students, Seasonal Pattern Assessment Questionnaire (SPAQ).

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

Source of financing. The study was carried out within the state assignment (budgetary funding within the comprehensive research topic AAAA-A19-119020690013-2 “A comprehensive study of clinical and psychopathological patterns and pathobiological mechanisms of the formation and progression of socially sensitive mental and behavioral disorders with the development of innovative methods for early diagnosis, personalized treatment, and prevention strategies”).

Conformity with the principles of ethics. All participants of the study signed an informed consent. The study was approved by the local Ethics Committee at Mental Health Research Institute, Tomsk NRMC RAS (Protocol No. 115 of 26.11.2018).

For citation: Ukraintsev I.I., Schastnyy E.D., Bokhan N.A. Incidence rate and clinical characteristics of seasonal affective disorders in senior medical students. *Bulletin of Siberian Medicine*. 2021; 20 (3): 112–119. <https://doi.org/10.20538/1682-0363-2021-3-112-119>.

Частота встречаемости и клинические характеристики сезонного аффективного расстройства у студентов-медиков старшего курса обучения

Украинцев И.И.¹, Счастный Е.Д.², Бохан Н.А.^{1,2}

¹ Сибирский государственный медицинский университет (СибГМУ)
Россия, 634055, г. Томск, Московский тракт, 2

² Научно-исследовательский институт (НИИ) психического здоровья, Томский национальный исследовательский медицинский центр (ТНИМЦ) Российской академии наук
Россия, 634014, г. Томск, ул. Алеутская, 4

РЕЗЮМЕ

Цель – изучение частоты встречаемости, клинических особенностей и прогнозирования развития сезонного аффективного расстройства (САР) у студентов-медиков 6-го курса обучения.

Материалы и методы. В скрининге на обнаружение САР с применением опросника для оценки сезонного паттерна (SPAQ, 1987) участвовали 119 студентов последнего курса медицинского университета, в том числе 78 представителей женского пола (65,5%) и 41 – мужского (34,5%) ($p = 0,001$). У женщин возраст составил 23 (22; 23) года, у мужчин – 23 (22; 24). Статистическую обработку проводили с использованием критерия χ^2 Пирсона и метода ранговой корреляции Спирмена (r_s).

Результаты. Получены данные о распространенности аффективных расстройств с сезонным паттерном у студентов-медиков: САР – 9,2%, суб-САР – 13,5%, психологическая ундуляция восприятия времен года (ПУВВГ) – 16,8%. Количество студентов, не проявляющих сезонной ундуляции шести основных характеристик, фиксируемых опросником SPAQ, составляло 72 (60,5%) ($p = 0,001$). Выявлены статистически значимые различия более высокого уровня медианы балла по общей сезонной шкале опросника SPAQ в случае САР по сравнению с ПУВВГ обследуемых как с учетом фактора пола, так и с его отсутствием ($p = 0,001$). Применение бинарной логистической регрессии позволило определить группы студентов с наличием или отсутствием САР по шкале SPAQ. Полученные данные определили величину вклада следующих факторов: пол, сезонность, масса тела и количества часов сна в сутки весной.

Заключение. Проведенное исследование позволило получить логистическую регрессионную модель, позволяющую прогнозировать наибольшую вероятность развития САР.

Ключевые слова: сезонное аффективное расстройство, студенты медицинского университета, опросник SPAQ.

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

Источник финансирования. Исследование выполнено в рамках государственного задания (бюджетное финансирование в рамках комплексной темы НИР АААА-А19-119020690013-2 «Комплексное исследование клинико-психопатологических закономерностей и патобиологических механизмов формирования и прогрессивности социально значимых психических и поведенческих расстройств с разработкой инновационных методов ранней диагностики, персонализированных стратегий терапии и профилактики»).

Соответствие принципам этики. Все участники исследования подписали информированное согласие. Исследование одобрено локальным этическим комитетом НИИ психического здоровья, Томский НИМЦ (протокол № 115 от 26.11.2018).

Для цитирования: Украинцев И.И., Счастный Е.Д., Бохан Н.А. Частота встречаемости и клинические характеристики сезонного аффективного расстройства у студентов-медиков старшего курса обучения. *Бюллетень сибирской медицины*. 2021; 20 (3): 112–119. <https://doi.org/10.20538/1682-0363-2021-3-112-119>.

INTRODUCTION

The etiology and pathophysiology of seasonal affective disorder (SAD) are associated with a particular season and light intensity. Long-term clinical and biological studies of SAD make it possible to identify its typical features, including recurrent affective episodes, a rapid response to non-pharmacological treatment, specific neurovegetative symptoms, and nutritional patterns [1]. Recent years have witnessed new results explaining the main biological hypotheses of SAD, with an emphasis on circadian rhythms, neurotransmitters, and molecular genetics. Integrative issues in the study of SAD are being discussed, including the research value of a dual vulnerability hypothesis, which conceptualizes seasonality as a dimensional spatial construct and the importance of studying endophenotypes. Chronotypes are defined as individual preferences for activity and sleep during the day. Morning and evening individuals differ in sleep-wake cycle time, peak performance times, and sensation upon awakening.

Circadian rhythm dysregulation is important in the development of affective disorders and widespread among people with mood disorders [2]. Sleep changes are the most significant diagnostic criterion for these disorders. Patients with bipolar disorder (BD) usually have more irregular sleep and corresponding social patterns, as well as abnormal secretion of melatonin and cortisol. Numerous studies on the effect of a chronotype on mood symptoms have conflicting results. The evening chronotype is often associated with an affective pathology. A large cohort study showed that depressive and anxiety disorders are associated with evening circadian rhythms, taking into account relevant socio-demographic, clinical, physical health, and sleep-related factors [3]. Evening activity is more common in adults with bipolar disorder. Research by M.C. Melo et al. (2020) showed that the evening chronotype has a poor prognosis for BD, which confirms the need for treatment of circadian rhythm disorders even in euthymia to improve the patient's quality of life and prevent the development of affective episodes [2].

In young respondents, seasonal variability in mild depression symptoms was shown in the Canadian population [4]. Higher levels of depressive symptoms were reported during the winter months compared to the summer months.

A study by A.V. Zhedik et al. [5] that included 81 medical university students of all years of study (av-

erage age 18.8 ± 0.8 years) revealed SAD signs in 40 individuals (37 women, 3 men). The authors associated this disorder with high mental and psychological strain. Another study showed higher prevalence of SAD in female students, and an important role in the development of the disorder in question was attributed to students' character and temperament characteristics [6]. The gender factor influence was also revealed in the study that showed the predominance of anxiety disorders in female students [7].

The aim of this study was to investigate the incidence rate, clinical features, and prognosis of seasonal affective disorder in senior (6th-year) medical students.

MATERIALS AND METHODS

The study included senior medical students. The inclusion criteria for the study encompassed age of 18–30 years and the ability to give a written informed consent. The exclusion criteria were the presence of organic, neurological, and severe somatic disorders leading to organ failure and refusal to participate in the study. The study was carried out in compliance with the ethical standards developed in accordance with the Declaration of Helsinki of the World Medical Association, "Ethical Principles for Medical Research Involving Human Subjects" as amended in 2000 and "Rules of Clinical Practice in the Russian Federation" approved by the Order of the Ministry of Health of the Russian Federation No. 266 of 19.06.2003.

SAD screening using the Seasonal Pattern Assessment Questionnaire (SPAQ, 1987) involved 119 undergraduate medical students. 78 students were females (65.5%) and 41 – males (34.5%) ($p = 0.001$). The average age of females was 23 (22; 23) years, and the average age of males was 23 (22; 24) years.

When studying the prevalence of sub-SAD and SAD, the SPAQ [8] was used. This study used the modified version of the questionnaire, which was developed in our country [9]. The diagnosis of SAD required a Global Seasonality Score (GSS) greater than 11, with a problem being marked or severe. Subsyndromal forms of SAD were diagnosed by GSS 11 or more in the absence of the problem or by GSS 9–10 in case the problem was marked or severe. The absence of SAD was determined in the following cases: the absence of a problem; the problem was assessed as moderate and had GSS less than 9. The entire surveyed group was assessed in terms of values in 4 main categories: 1 – the presence of SAD; 2 – the absence of SAD; 3 – psychological undulation

of the season perception (PUSP); 4 – subsyndromal SAD.

Assignment of the surveyed to any category due to the screening findings was based on the cumulative scoring and categorical assessment. As presented in the SPAQ text, the questionnaire includes six questions about seasonal changes in sleep, activity, mood, body weight, appetite, and energy, which makes up a Global Seasonality Score (GSS), where each question corresponds to the answer, estimated in the range from 0 to 4 points. Thus, the maximum possible result may be 24 points. The SPAQ also includes a question about the difficulties arising from these changes (seasonality as a problem), implying one of the following answers: absence of the problem, moderate, marked or severe problem.

Statistical data processing was carried out using the standard software package IBM SPSS Statistics 26. Quantitative data were presented as the median and the interquartile range $Me [Q_1; Q_3]$. Qualitative data were presented by frequency parameters (n (%)). Statistical processing was performed using the Spearman's rank correlation coefficient (r_s). Qualitative variables were analyzed through the study of their frequencies by means of contingency tables using the Pearson's χ^2 test.

To construct a binary logistic model, a step-by-step inclusion method was used. The value of the weighting factors (W), standard error (SE), and the level of significance (p) were calculated. To assess the quality of the predictive model built on the basis of the logistic regression algorithm, ROC analysis was used, with calculation of the sensitivity (Se), specificity (Sp), and the area under the curve (AUC). Assessment of the specificity and sensitivity values made it possible to test the adequacy of the model.

RESULTS

Students often underestimate the problem that is caused by seasonal mood changes. A prerequisite for diagnosing SAD was assessment of seasonal fluctuations as at least a marked problem and GSS of 11 or more. Considering this categorization, the prevalence of SAD among senior medical students was studied and the following results were obtained (Fig. 1).

Figure 1 shows that the prevalence of SAD was 9.2% (11 persons), sub-SAD – 13.5% (16 persons), and PUSP – 16.8% (20 persons). The number of students who did not show seasonal undulation of the six main characteristics recorded by the SPAQ questionnaire was 60.5% (72 persons).

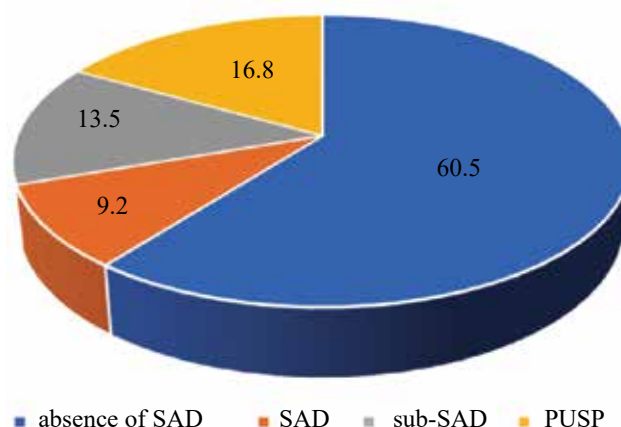


Fig. 1. Incidence rate of SAD, sub-SAD, and PUSP

Figure 2 shows that when assessing the gender-related differences in the selected groups of the disorder in women, the values of SAD (11.5%), sub-SAD (18.0%), and PUSP (18.0%) differed from the corresponding parameters (4.9; 4.9, and 14.6%) in men ($p = 0.034$, Pearson's χ^2 test).

Figure 2 illustrates the gender-related incidence rate of SAD, sub-SAD, and PUSP.

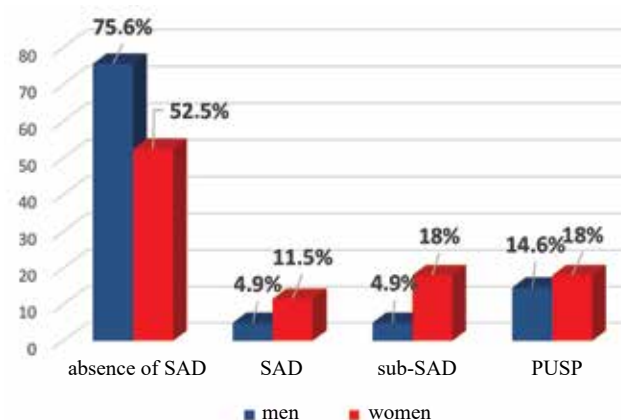


Fig. 2. The gender-related incidence rate of SAD, sub-SAD, and PUSP

A statistical analysis of the correlations between different variables was carried out to determine their most significant interrelations. When considering the ratios of seasonal undulation of the six main characteristics in the patients with SAD, determined by the SPAQ, the closest significant positive correlation ($p = 0.03$) was found between the energy level and social activity ($r_s = 0.74$). In the patients with SAD and sub-SAD, a correlation was found between increased appetite and sleep length ($r_s = 0.52$), as well as between increased appetite ($r_s = 0.52$) (preference for

carbohydrate foods) and weight in the fall and winter months (September and January). The obtained data show the practical significance of the seasonal undulation of the above-mentioned six main characteristics on the Global Seasonality Score for the diagnosis of SAD and sub-SAD at the screening stage.

Statistically significant relationships ($p < 0.05$) were revealed between the final GSS and the two parameters assessed on this scale: sleep length ($r_s = 0.74$) and body weight ($r_s = 0.82$).

Additionally, an association between seasonal variations in the six main characteristics (sleep length, social activity, mood (overall wellbeing), body weight, appetite, and energy level), the severity of the problem (absence of the problem, moderate, marked or severe problem), and gender was analyzed (Fig. 3). The differences in the selected groups were assessed. A moderate problem in women was statistically significantly more common than in men (37.2% and 22.0%, respectively; $p = 0.046$, Pearson's χ^2 test). In contrast, men showed the absence of the problem statistically significantly more often than women (73.2 and 48.7%, respectively; $p = 0.01$, Pearson's χ^2 test).

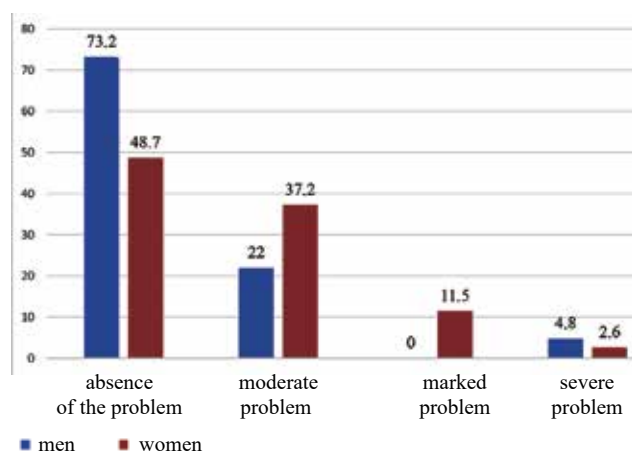


Fig. 3. Influence of the gender factor on the assessment of mood and behavior seasonality

When assigning the individuals to sub-SAD without considering the gender factor and assessing seasonality as a problem, a correlation with the mood parameter measured on the SPAQ Global Seasonality Score was revealed ($r_s = -0.51$). When assigning the individuals to the PUSP category without considering the gender factor and assessing seasonality as a problem, a correlation with sleep length ($r_s = 0.60$) and decreased appetite ($r_s = 0.46$) was identified.

A binary logistic regression analysis was used to select a group of factors that determine the possibility

of assigning students to a group with or without SAD according to the SPAQ score. A logistic regression model was constructed to predict the highest likelihood of developing SAD. Interpretation of the results was determined by the values of the regression coefficients and their levels of significance. From the data obtained using logistic regression, the contribution of the following factors was determined. Gender parameters: $W = 1.615$, $SE = 0.760$, $Wald = 4.511$ (Wald statistics), $Sig = 0.034$ (significance level), $Exp(B) = 5.029$. Parameters of seasonality as a problem: $W = 2.345$, $SE = 0.625$, $Wald = 14.070$, $Sig = 0.001$, $Exp(B) = 10.434$. Parameters of body weight: $W = 0.622$, $SE = 0.316$, $Wald = 3.879$, $Sig = 0.049$, $Exp(B) = 1.863$. Parameters of sleep length per day in spring: $W = 0.542$, $SE = 0.195$, $Wald = 7.686$, $Sig = 0.006$, $Exp(B) = 1.719$.

At the first stage, the value of the function $Z(x)$ was determined using the formula:

$$Z = -10,378 + 1,615 \cdot X_1 + 2,345 \cdot X_2 + 0,622 \cdot X_3 + 0,542 \cdot X_4$$

where Z – SAD; $W = -10.378$; X_1, X_2, X_3, X_4 – values of the factors. X_1 – gender; X_2 – seasonality assessment scale as a problem according to the SPAQ; X_3 – parameters of sleep length per day in spring.

The main results of binary logistic regression among medical students were characterized by the following parameters: $W = -10.378$; $SE = 2.496$; $Wald = 17.295$; $Sig = 0.001$; $Exp(B) = 0.001$.

Then the likelihood of developing a seasonal affective disorder in students was assessed using the formula: $P = e^z / (1 + e^z)$, where P is the likelihood of students' belonging to a group with / without SAD; e is the base of the natural logarithm ($e = 2.7183$).

According to the analysis carried out with the use of the logistic regression algorithm, parameters and weighting factors were identified that made it possible to construct a separating function with sufficient operational characteristics. When the number of input parameters decreased, the recognition quality of this model deteriorated.

ROC analysis was used to assess the quality of the model. For the variable "Scale for assessing seasonality as a problem" in the SPAQ, $AUC = 0.792$; $SE = 0.050$; asymptotic significance was 0.001; the lower limit was 0.693 and the upper limit was 0.891 of the asymptotic 95% confidence interval; $Se = 89.0\%$; $Sp = 63.0\%$. If this model is used, then in 83.1% of cases it will give a correct result, which is a fairly good indicator for biomedical systems.

DISCUSSION

This study contributes to the field of circadian and chronobiological research, showing a close relationship between SAD development and young age [10, 11]. From a diagnostic and preventive viewpoint, it is important to distinguish the three main categories of SAD according to cumulative scoring and categorical assessment. The PUSP category is close to sub-SAD in its clinical characteristics, which made it possible to create a continuous series of seasonal patterns of mood and behavior changes from normal values to a clinically most marked seasonal affective disorder. In terms of chronobiological parameters, subsyndromal SAD forms, respectively, are closer to SAD than to healthy individuals.

Analysis and systematization of epidemiological studies by searching in the electronic databases Medline, Excerpta Medica, and PsychLIT made it possible to estimate the prevalence of SAD using 20 retrospective studies [12]. SAD prevalence varied from 0% to 9.7%, which was confirmed by our study (9.2%).

In adolescence, depression is mostly characterized by atypical symptoms, including social withdrawal, increased sleepiness, overeating, and a desire for carbohydrates. The presence of atypical depressive symptoms in the structure of an episode of depression in progress often correlates with bipolar mood disorder. Sleep disorders are associated with a decreased quality of life and a risk of relapse in BD. At the same time, sleep disorders very frequently persist in BD, despite adequate pharmacological treatment [13]. In the modern literature, there is a small amount of studies devoted to sleep disorders in SAD. The issue of sleep disorders, in which circadian or behavioral abnormalities play a leading role in SAD patients, remains unclear. This is supported by the discrepancy between self-reported and actigraphic (polysomnographic) sleep length in SAD. Some studies largely ignore the period of summer remission in SAD, which could provide valuable information on the role of sleep disorders in the onset and recurrence of winter depressive episodes. Therefore, further studies should comprise a comprehensive assessment of sleep and circadian rhythms, including all seasons of the year, to fully characterize the corresponding phenotypes [14].

The obtained data on SAD predominance in women were consistent with the data of other researchers, who showed a positive correlation between age and the frequency of seasonal affective disorders. For

instance, this parameter was higher in post-pubertal girls, which was probably associated with sexual neurohormonal characteristics [15, 16].

A study of a large sample of young people with depression demonstrated high incidence of anhedonia and anergy [17]. At the same time, the study by N.A. Majrashi et al. [18] found a negative correlation between the length of the light period and bad mood and anhedonia in women. No mediated effects were observed in men. This study expands the understanding of the neurobiological factors that influence the pathophysiology of SAD.

The use of methods for prompt diagnosis of mood disorders in practical healthcare (intelligent systems for express diagnosis) is of practical importance. It allows for timely identification of persons belonging to an increased risk group to carry out interventions aimed at improving their quality of life in the future [19]. Timely diagnosis of all SAD categories in students will make it possible to effectively use the existing preventive and therapeutic potential [20–22].

CONCLUSION

The data obtained show that in seasonal affective disorders, vegetative symptoms, such as seasonal fluctuations in sleep length ($r_s = 0.74$) and body weight ($r_s = 0.82$), were most significant for the subjective assessment of seasonal fluctuations as a problem of varying severity. The revealed patterns confirmed the previously obtained data on the relationship between the typical rhythms of seasonal mood fluctuations (vegetative and behavioral characteristics, school performance) and SAD [23]. Additionally, the results obtained in this study were comparable with the research confirming fairly high prevalence of SAD and sub-SAD in different age groups [24].

These data were of practical importance for the timeliest primary detection of sub-SAD and SAD with a focus on the diagnosis of seasonal fluctuations in vegetative manifestations of subdepressive or depressive disorders, not only circadian changes in mood.

Timely diagnosis and further use of therapeutic and preventive approaches determine a set of actions aimed at regulating biopsychosocial functioning of medical students [19]. To achieve this, relevant treatment methods are used along with rhythm-stabilizing measures (correct daily regimen, planning the rhythm of study load in student curricula, light regime).

REFERENCES

1. Riccobono G., Iannitelli A., Pompili A., Iorio C., Stratta P., Rossi R., Bersani G., Pacitti F. Night Eating Syndrome, circadian rhythms and seasonality: a study in a population of Italian university students. *Rivista di Psichiatria*. 2020; 55 (1): 47–52. DOI: 10.1708/3301.32719.
2. Melo M.C., Garcia R.F., Araújo C.F., Luz J.H., Bruin P.F., Bruin V.M. Chronotype in bipolar disorder: an 18-month prospective study. *Brazilian Journal of Psychiatry*. 2020; 42 (1): 68–71. DOI: 10.1590/1516-4446-2019-0489.
3. Antypa N., Vogelzangs N., Meesters Y., Schoevers R., Penninx B.W. Chronotype associations with depression and anxiety disorders in a large cohort study. *Depression and Anxiety*. 2016; 33 (1): 75–83. DOI: 10.1002/da.22422.
4. Takaes Y. Circadian rhythm in bipolar disorder: A review of the literature. *Psychiatry and clinical neurosciences*. 2018; 72 (9): 673–682. DOI: 10.1111/pcn.12688.5.
5. Zhedik A.V., Korotkaya V.A. Prevalence of seasonal affective disorder among students of BSMU [Electronic resource]. Actual problems of modern medicine and pharmacy 2018 : Collection of Materials of the LXXII International Scientific and Practical Conference for Students and Young Scientists, Minsk, April 18–20, 2018; ed. A.V. Sikorsky, O.K. Doronina. Minsk : BSMU, 2018: 1504–1507 (in Russ.).
6. Korotkaya V.A. Prevalence of seasonal affective disorder among students of BSMU [Electronic resource]. Actual problems of modern medicine and pharmacy 2018 : Collection of Abstracts of the LXXII International Scientific and Practical Conference for Students and Young Scientists, Minsk, April 18–20, 2018; ed. A.V. Sikorsky, O.K. Doronina. Minsk : BSMU, 2018: 1148 (in Russ.).
7. Ukraintsev I.I., Schastnyy E.D., Bokhan N.A. Incidence rate of anxiety and personality disorders and their interrelationship in senior-year students of the medical university. *Bulletin of Siberian Medicine*. 2019; 18 (4): 143–149 (in Russ.). DOI: 10.20538/1682-0363-2019-4-143–149.
8. Rosenthal N.E., Bradt G.H., Wehr T.A. Seasonal Pattern Assessment Questionnaire (SPAQ). National Institute of Mental Health, Bethesda, USA, 1984.
9. Putilov A.A. Chronophysiological mechanisms mediating the effect of bright light on human activity and mood: *Dr. Sci. (Biol.)... dissertation*. Tomsk, 1999: 383.
10. Lukmanji A., Williams J.V.A., Bulloch A.G.M., Patten S.B. Seasonal variation in specific depressive symptoms: A population based study. *Journal of Affective Disorders*. 2020; 261: 153–159. DOI:10.1016/j.jad.2019.10.003.
11. Wescott D.L., Soehner A.M., Roecklein K.A. Sleep in seasonal affective disorder. *Current Opinion in Psychology*. 2019; 34: 7–11. DOI: 10.1016/j.copsyc.2019.08.023.
12. Magnusson A. An overview of epidemiological studies on seasonal affective disorder. *Acta Psychiatr Scand*. 2000; 101 (3): 176–184.
13. Harvey A.G., Kaplan K.A., Soehner A. Interventions for sleep disturbance in bipolar disorder. *Sleep Med. Clin*. 2015; 10 (1): 101–105. DOI: 10.1016/j.jsmc.2014.11.005.
14. Wescott D.L., Soehner A.M., Roecklein K.A. Sleep in seasonal affective disorder. *Curr. Opin. Psychol*. 2020; 34: 7–11. DOI: 10.1016/j.copsyc.2019.08.023.
15. Low K.G., Feissner J.M. Seasonal affective disorder in college students: prevalence and latitude. *J. Am. Coll. Health*. 1998; 47 (3): 135–137. DOI: 10.1080/07448489809595634.
16. Swedo S.E., Pleeter J.D., Richter D.M., Hoffman C.L., Allen A.J., Hamburger S.D., Turner E.H., Yamada E.M., Rosenthal N.E. Rates of seasonal affective disorder in children and adolescents. *Am. J. Psychiatry*. 1995; 152 (7): 1016–1019. DOI: 10.1176/ajp.152.7.1016.
17. Kornetov A.N. Ontogenetic aspects of depressive disorders. *S.S. Korsakov Journal of Neurology and Psychiatry*. 2003; 103 (8): 80–81 (in Russ.).
18. Majrashi N.A., Ahearn T.S., Waiter G.D. Brainstem volume mediates seasonal variation in depressive symptoms: A cross sectional study in the UK Biobank cohort. *Sci Rep*. 2020; 10 (1): 3592. DOI: 10.1038/s41598-020-60620-3.
19. Yankovskaya A.E., Kornetov A.N., Il'inskikh N.N., Obukhovskaya V.B. An expansion of intelligent systems complex for express-diagnostics and prevention of organizational stress, depression, and deviant behavior on the basis of the biopsychosocial approach. *Pattern Recognition and Image Analysis (Advances in Mathematical Theory and Applications)*. 2017, 27 (4): 783–788. DOI: 10.1134/S1054661817040204.
20. Pjrek E., Friedrich M.E., Cambioli L., Dold M., Jäger F., Komorowski A., Lanzenberger R., Kasper S., Winkler D. The Efficacy of Light Therapy in the Treatment of Seasonal Affective Disorder: A Meta-Analysis of Randomized Controlled Trials. *Psychotherapy and psychosomatics*. 2020; 89 (1): 17–24. DOI: 10.1159/000502891.
21. Lam R.W., Teng M.Y., Jung Y.E., Evans V.C., Gottlieb J.F., Chakrabarty T., Michalak E.E., Murphy J.K., Yatham L.N., Sit D.K. Light Therapy for Patients With Bipolar Depression: Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Canadian Journal of Psychiatry*. 2020 ;65 (5): 290–300. DOI: 10.1177/0706743719892471.
22. May I.C. Light therapy for preventing seasonal affective disorder: Summary of a Cochrane Review. *Explore: The Journal of Science and Healing*. 2020; 16 (2): 133–134. DOI: 10.1016/j.explore.2019.12.004.
23. Simutkin G.G., Popova N.M. Seasonal affective disorder in schoolchildren. *Siberian Herald of Psychiatry and Addiction Psychiatry*. 2005; 2 (36): 65–68 (in Russ.).
24. Simutkin G.G. Clinical-constitutional and chronobiological patterns in seasonal affective disorders: *author's abstract of ... Dr. Sci. (Med.) dissertation*. Tomsk, 2002: 50 (in Russ.).

Authors information

Ukrainitsev Igor I., Cand. Sci. (Med.), Assistant of the Psychiatry, Narcology, and Psychotherapy Division, SibMed, SSMU, Tomsk, Russian Federation. ORCID 0000-0001-5112-2188.

Schastnyy Evgeny D., Dr. Sci. (Med.), Professor, Head of the Affective States Department, Mental Health Research Institute, Tomsk NRMC RAS, Tomsk, Russian Federation. ORCID 0000-0003-2148-297X.

Bokhan Nikolay A., Dr. Sci. (Med.), Professor, Academician of RAS, Director of Mental Health Research Institute, Tomsk NRMC RAS; Head of the Psychiatry, Narcology, and Psychotherapy Division, SibMed, SSMU, Tomsk, Russian Federation. ORCID 0000-0002-1052-855X.

(✉) **Schastnyy Evgeny D.**, e-mail: evgeny.schastnyy@gmail.com

Received 05.05.2020

Accepted 28.12.2020

Adherence to treatment in patients with inflammatory bowel disease

**Andreev K.A., Gorbenko A.V., Skirdenko Yu.P., Nikolaev N.A., Livzan M.A.,
Bikbavova G.R., Fedorin M.M.**

*Omsk State Medical University
12, Lenina Str., Omsk, 644099, Russian Federation*

ABSTRACT

Inflammatory bowel disease (IBD) is a common pathology that reduces the quality and duration of a patient's life. The cornerstone of treatment of IBD patients is polypharmacotherapy based on the use of salicylates, antibiotics, immunomodulatory and biological drugs, and topical dosage forms. Multicomponent treatment has shown to reduce the quality of life and negatively affect adherence to drug therapy in IBD patients.

One of the leading causes of treatment failure is low treatment adherence, which leads to disease progression, disability, and increased financial costs. Currently, there are many factors that affect adherence to therapy, some of them are modifiable, which creates opportunities to improve the effectiveness of existing medical interventions. However, the available data on the level of adherence in IBD patients are not numerous and homogeneous, so a low level of adherence to drug therapy in IBD patients is registered in 7–72% of cases.

An important issue in understanding adherence in IBD patients is a lack of research on the level of adherence to counselling and lifestyle modification. However, the course of IBD, treatment features related to the duration of therapy and necessary lifestyle modifications (nutrition), as well as regular monitoring of laboratory and instrumental parameters determine the need to assess adherence to lifestyle modification and counselling along with adherence to drug therapy.

Key words: inflammatory bowel disease, ulcerative colitis, Crohn's disease, treatment adherence, quality of life.

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

Source of financing. The research was carried out with the state support for the leading scientific schools of the Russian Federation (Grant No. SS-2558.2020.7, Agreement No. 075-15-2020- 036 of 17.03.2020 "Development of Health-Preserving Technology for a Comorbid Gastroenterological Patient Based on Adherence Control").

For citation: Andreev K.A., Gorbenko A.V., Skirdenko Yu.P., Nikolaev N.A., Livzan M.A., Bikbavova G.R., Fedorin M.M. Adherence to treatment in patients with inflammatory bowel disease. *Bulletin of Siberian Medicine*. 2021; 20 (3): 120–128. <https://doi.org/10.20538/1682-0363-2021-3-120-128>.

Приверженность лечению больных воспалительными заболеваниями кишечника

**Андреев К.А., Горбенко А.В., Скирденко Ю.П., Николаев Н.А., Ливзан М.А.,
Бикбавова Г.Р., Федорин М.М.**

*Омский государственный медицинский университет (ОмГМУ)
Россия, 644099, г. Омск, ул. Ленина, 12*

✉ Gorbenko Aleksander V., e-mail: prime.dr.alex@gmail.com

РЕЗЮМЕ

Воспалительные заболевания кишечника (ВЗК) – это распространенная патология, снижающая качество и продолжительность жизни пациента. Краеугольным камнем лечения больных ВЗК является полифармакотерапия, основанная на применении салицилатов, антибиотиков, иммуномодулирующих и биологических препаратов, использовании местных лекарственных форм. Показано, что сложные схемы лечения снижают качество жизни и отрицательно сказываются на приверженности лекарственной терапии больных ВЗК.

Одной из ведущих причин неэффективности лечения является низкий уровень приверженности, что приводит к прогрессированию заболевания, инвалидизации и увеличению финансовых затрат. В настоящий момент известно много факторов, влияющих на приверженность терапии, часть из них модифицируема, что создает возможности для повышения эффективности существующих медицинских вмешательств. Однако имеющиеся данные об уровне приверженности больных ВЗК не отличаются многочисленностью и однородностью. Так, низкий уровень приверженности лекарственной терапии больных ВЗК регистрируется в 7–72% случаев.

Важной проблемой понимания приверженности больных ВЗК является отсутствие исследований об уровне приверженности медицинскому сопровождению и модификации образа жизни. Тогда как течение ВЗК и особенности лечения, связанные с длительностью терапии, необходимой модификацией образа жизни (питания), а также регулярный контроль лабораторных и инструментальных параметров диктуют необходимость оценки приверженности модификации образа жизни и медицинскому сопровождению наряду с приверженностью лекарственной терапии.

Ключевые слова: воспалительные заболевания кишечника, язвенный колит, болезнь Крона, приверженность, качество жизни.

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

Источник финансирования. Публикация подготовлена при государственной поддержке ведущих научных школ Российской Федерации (грант НШ-2558.2020.7, соглашение № 075-15-2020-036 от 17.03.2020 «Разработка технологии здоровьесбережения коморбидного больного гастроэнтерологического профиля на основе контроля приверженности»).

Для цитирования: Андреев К.А., Горбенко А.В., Скирденко Ю.П., Николаев Н.А., Ливзан М.А., Бикбазова Г.Р., Федорин М.М. Приверженность лечению больных воспалительными заболеваниями кишечника. *Бюллетень сибирской медицины*. 2021; 20 (3): 120–128. <https://doi.org/10.20538/1682-0363-2021-3-120-128>.

RATIONALE

The evolution of mankind has long gone beyond biology. Socialization, urbanization, and technological progress have brought benefits and made life comfortable. The development of science and technology has made it possible to improve methods of diagnosis, treatment, and disease prevention. However, incidence of diseases in general is not reducing, but their spectrum is changing. Thus, in developed countries, lifestyle-associated diseases are increasingly common [1]. Inflammatory bowel disease (IBD) is a striking consequence of modern lifestyle.

To date, evidence of the influence of environmental factors on the state of the intestinal mucosa, microbiota composition and its interaction with the intestinal wall has been accumulated, which triggers a progressive immune response in genetically predisposed individuals [2]. The pathogenesis of in-

flammatory bowel diseases is not fully understood. It is known that in IBD under the influence of a complex of factors (genetic, microbial, dietary, and psychological), dysfunction of the intestinal barrier develops, which contributes to translocation of microorganisms and products of microbial origin and leads to activation of the immune response. The immune-mediated reaction to the intestinal microflora is considered the basis of pathogenesis [3].

According to some data, improvement of sanitary and hygienic living conditions in childhood leads to a decrease in the antigenic effect on the body, which can cause the development of hyperreactivity upon contact with microorganisms later in adulthood [4]. Diet was shown to be an important environmental factor in the course of IBD [2]. Lack of time, stress, and hypodynamia have significantly changed our diet over the past 30 years [5], with more ready-to-

eat foods, flavor enhancers, sugar and sugar substitutes, high-calorie foods, animal proteins, and less dietary fiber [6].

Few diseases affect human life as dramatically as IBD [7]. The debut of the disease occurs at a young age (20–30 years), when a person lives an active social life [3]. The disease proceeds for a long period of time and causes severe pain, with constantly alternating periods of remission and relapse [8]. The main complaint of patients is chronic diarrhea, with stool frequency up to 20 times a day during day and night [3]. Ulcerative colitis (UC) and Crohn's disease (CD) adversely impact a patient's quality of life, increase the risk of colorectal cancer, the incidence of which is 15% after 30 years of the disease, and often lead to disability [9].

MAIN PART

Multicomponent pharmacological therapy, based on the long-term use of salicylates, antibiotics, and immunomodulatory and biological agents, is pivotal in treatment of IBD [10]. However, these groups of drugs are characterized by a number of side effects, such as nausea, vomiting, fever, aggravation of diarrhea, elevated blood pressure, seizures, joint and muscle pain, disruption of growth processes in children, and development of infectious complications [11]. In addition, researchers from Italy found that the use of topical drugs in the form of suppositories and rectal foams is associated with low adherence compared with oral forms, with 68% and 40% of non-compliant patients in the respective groups [12]. Multifaceted clinical presentation, complex treatment regimen, and its undesirable effects pose difficulties for an active social life of the patient [3].

All of these factors significantly reduce the quality of life and negatively affect adherence to drug therapy [7]. Patients' adherence to treatment in all chronic diseases decreases over time, which is also caused by a need for chronic drug administration [13]. In the outpatient setting, non-compliance in patients with chronic diseases is 30–60% [14]. Low adherence to treatment is one of the main reasons for low efficiency of chronic disease therapy and has a number of negative economic consequences for both the patient and the healthcare system [15].

Therefore, public healthcare systems spend vast financial resources on treating patients with IBD. For example, six-month treatment of one IBD patient costs € 2,500 in Belgium, while the cost of a year-

long treatment of one IBD patient exceeds £ 2,000 in the United Kingdom [16]. In the United States, treatment of one IBD patient costs about \$35,000, which totals about \$2 billion for all the patients in the country [17]. In Russia, there is no official information on the estimated cost of treatment for patients with IBD [18]. However, in 2019, researchers from Tatarstan calculated that treatment of one IBD patient cost from 50,000 to 200,000 rubles per year, depending on the severity of the disease, type of care, and disease duration, with calculations carried out on the basis of tariff agreements with the compulsory medical insurance fund [19].

A survey of 100 patients with IBD, carried out by M.F. Vladimirovsky Moscow Regional Clinical Research Institute, revealed that 40% of the respondents violated the prescribed treatment regimen, and the patients mentioned high treatment costs as one of the main reasons [20]. The patient takes up a considerable part of the treatment cost, which, given difficulties associated with the inability to work full-time, results in a significant financial loss and affects the patient's willingness to undergo treatment [1]. Patients with IBD have to receive life-long pharmacotherapy, which is the cornerstone of therapy [21, 22].

Currently, data on therapy adherence in patients with gastrointestinal pathology remain scarce and heterogeneous. Researchers from London analyzed 17 studies involving 4,322 patients with IBD; lack of adherence to treatment was detected in 7–72% of cases [23]. Treatment of IBD patients includes not only the use of medications, but also dieting, which over time reduces the patients' adherence to therapy in general [24]. At the same time, very few studies have investigated adherence to a new lifestyle (in case of IBD, adherence to a diet).

Adherence is pivotal in treatment of patients with IBD. In a study of a cohort of 99 UC patients who had been in remission for more than six months and were receiving mesalazine maintenance therapy, researchers from the University of Michigan and the University of Chicago concluded that non-compliant patients had a five times higher risk of relapse [25]. Later, researchers from Louisiana conducted a large retrospective study involving 13,062 patients with UC over a 10-year period. It revealed that patients with low adherence had a 60% higher risk of relapse [26]. Researchers from Salford Royal Hospital carried out a similar retrospective study, but

in one group of patients, they periodically changed the form of mesalazine administration. As a result, it was found that the risk of relapse was 3.5 times higher in this group of patients [27].

In addition to a significant impact on therapy effectiveness and reduction of the risk of complications and disability, high adherence to treatment considerably impacts the costs [28]. For example, regular use of salicylates in patients with IBD reduces hospitalization costs by 62%, with a decrease in outpatient care by 13%, in emergency care by 45%, and in total costs of the healthcare system for treating adherent patients by 50% [14]. Thus, increasing patient adherence to treatment is important, according to some data, it is even more important than improving specific treatment methods [24].

In a study conducted in Chelyabinsk, based on the results of a survey of 133 patients with IBD, 58.4% of the respondents were found to have a low level of adherence. We used our own questionnaire to assess the level of adherence. The questionnaire included questions about the reasons for non-compliance with doctor's recommendations. The following social and economic reasons were identified: insufficient financial resources (31.5%), irregular supply of subsidized drugs (26%), and lack of drugs in the pharmacy (14.6%).

As for reasons related to the patient, 49.7% of the respondents mentioned lack of awareness about their disease, 42.2% of the individuals did not believe in the treatment success, 24.5% of the patients expressed fear of "getting addicted" to medications, 12.6% of the patients had doubts about the necessity of therapy, and only 13% of the respondents mentioned forgetfulness. Poor drug tolerance, allergic reactions, and development of adverse effects were noted by 3.8%, 2.7%, and 6.3% of the respondents, respectively [29]. Analyzing the reasons for low adherence, it should be noted that most of them are related to the financial, logistical, and informational aspects of the issue, i.e. they can be changed.

The adherence of UC patients is affected by many aspects, including intensity and duration of the disease, treatment cost, adverse events of therapy, individual psychosocial characteristics, and the level of patient-doctor cooperation [30]. Low adherence to treatment can be particularly noted in patients in remission, due to the absence of symptoms. A large number of pills and complicated treatment regimens are believed to be among the main factors determi-

ning low adherence [31]. This is confirmed by the results of a survey conducted among 1,595 patients with IBD in the United States. Thus, 65% (944) of the respondents showed low adherence, while the reported causes included inconvenience of taking medications frequently during the day and the use of rectal forms [32].

At Ryzhikh National Medical Research Center of Coloproctology, a study was conducted with 380 patients with active UC. It revealed that 79.1% of the patients who took a daily dose of mesalazine once a day and 75.7% of the patients who took a daily dose of mesalazine three times a day went into clinical remission. On this basis, researchers recommended to give preference to a single daily dose of oral mesalazine, as this has a positive effect on the adherence level and will ultimately increase the effectiveness of the entire treatment [33].

In terms of the impact on adherence, the total number of medications taken per day is also important. Patients taking four or more medications every day have a higher rate of poor adherence than those taking fewer medications per day (60% and 40%, respectively) [34]. Currently, this becomes particularly relevant given the prevalence of comorbidity among patients.

Scholars from the Research Institute of Physiology and Fundamental Medicine found that there are manifestations of nonalcoholic fatty liver disease in half of IBD patients. Abdominal obesity, metabolic syndrome, age over 40 years, and arterial hypertension were the main factors associated with the presence of nonalcoholic steatohepatitis in IBD patients. CD with comorbid nonalcoholic steatohepatitis was characterized by a need for a higher dose of azathioprine [35]. Assessment of the comorbid background in patients with IBD is a serious task in the joint work of a doctor and a patient, when selecting an individualized treatment regimen.

According to C. Selinger et al., in Australia, non-compliance with maintenance therapy was detected in 30% of IBD patients. At the same time, among patients receiving biological therapy, low adherence was detected only in 5.3% of cases [36]. Researchers from the University Hospital of Nancy Brabois conducted a systematic review to analyze the level of adherence to therapy with tumor necrosis factor inhibitors (anti-TNF). According to 13 studies involving 93,998 patients with IBD, the overall adherence was 82.6% [37].

Therefore, the use of biological therapy in treatment of inflammatory bowel diseases can be considered as a factor associated with high adherence to treatment. The revealed fact needs to be studied more thoroughly. A lack of adherence to anti-TNF therapy leads to subsequent immunogenicity and a lack of a response to the ongoing biological therapy [14].

The study of socio-psychological factors as modifiers of adherence levels showed that among non-compliant patients, there are considerably more employed people (85.9%, $p < 0.05$) at the age of 40–49 years (25.6%, $p < 0.05$), with completed secondary education (56.4%, $p < 0.05$), with an identified non-constructive model of child–parent relationships (85.9%, $p < 0.05$), and in an unfavorable, conflict family environment (18.2%, $p < 0.05$) [38]. Previously, researchers in Italy found that insufficient adherence was significantly more common in the group of patients under 40 years (43% as opposed to 34%). Disease duration of less than 5 years was also characterized by lower adherence (24% as opposed to 15%). It should be noted that with these two factors combined the non-compliance level reached 75% [39]. As for gender differences, there is no single opinion. In some studies, male gender was found to be a predictor of low adherence, while other studies revealed female gender to be the one, and some studies found no significant differences between men and women at all [34, 40, 41].

Anxiety disorders in gastroenterological patients undergoing treatment for a long time reduce adherence to therapy. Changing the patient's mental status and a proactive attitude of the attending doctor were found to have a positive effect on the patient's compliance [42]. Complete understanding of the disease and clear statement of therapy goals and objectives have a positive effect on the patient's readiness for counselling and lifestyle modifications, ultimately increasing the level of adherence to treatment [43].

Researchers at Cincinnati Children's Hospital Medical Center examined the quality of life and adherence in 36 adult patients with IBD. As a result, a correlation was found between insufficient adherence to azathioprine and a poor quality of life [44]. In addition, a research team from Hungary carried out a study involving 592 patients with IBD and did not reveal a relationship between the level of adherence and the quality of life, but found a higher incidence of disability in low adherence [45]. Australian

researchers found that low adherence to drug therapy is one of the most significant risk factors for disability in patients with IBD ($p < 0.0001$) [46].

In issues of adherence to treatment, a well-established partnership between a patient and a doctor and their effective communication are often crucial in determining the level of adherence to therapy [12]. The doctor – patient interaction has an enormous impact on the efficacy of therapy and reduces the treatment cost. It was found that failure to schedule a follow-up visit by the attending doctor is accompanied by greater development of depression in patients, reduces the level of trust in the physician, and worsens adherence, eventually leading to refusal from the recommended treatment [47, 48].

The Selecting Therapeutic Targets in Inflammatory Bowel Disease committee (STRIDE) identified achievement of a high quality of life as the main goal of treating IBD patients. A decisive approach to achieving remission and tertiary prevention of IBD patients is the treat-to-target concept (T2T), based on patient's subjective needs [49]. Individual therapy regimens are designed, taking into account the extent of the lesion, the severity of the disease, tolerability of pharmacotherapy, lifestyle, and diet therapy. Patients with IBD are required to control semiotics, and doctor's work is focused on biochemical, morphological, and endoscopic criteria of disease intensity [50].

There are two groups of methods for assessing adherence. Direct assessment methods include blood biochemistry for detecting the content of drugs and their metabolites. Indirect methods include pill counts, prescription dispensing logs and patient medication logs, questionnaires, and the use of electronic devices. All methods are far from perfect, but adherence questionnaires are most commonly used. The use of customized questionnaires to assess adherence makes it difficult to assess the results and makes it almost impossible to compare them with others.

Uniform questionnaires facilitate analysis and comparison of obtained data. The first scale used in patients with IBD was the modified Morisky – Green – Levine Medication Adherence Scale [51]. However, it shows only the level of adherence to drug therapy, without considering other components of compliance, such as adherence to counselling and lifestyle modifications, the assessment of which is critical for understanding adherence to IBD therapy

[52]. The Quantitative Assessment of Treatment Adherence Questionnaire (QAT-25) recommended by the Russian Scientific Medical Society of Therapists allows for quantitative (i.e., as a percentage) calculation of adherence to drug therapy, lifestyle modification, and counselling separately and has high rates of specificity (78%), sensitivity (94%), and reliability (94%), according to an independent assessment [52].

Currently, there are no generally accepted methods to increase the level of adherence. Methodological systems for teaching patients, digital reminders for taking medications, and the most effective logistics of drug supply for the convenience of patients are actively used abroad [53, 54]. Australian researchers found that an individual consultation of non-adherent IBD patients with a clinical pharmacologist regarding drug therapy increased their adherence to the level of the initially adherent group and had maintained for at least 15 months [55].

According to a meta-analysis conducted by scientists from North Carolina, regarding reminder systems (text and voice reminders on the phone, pill boxes, etc.), a significant increase in adherence was observed compared with controls, regardless of the reminder method used [56]. In Russia, the most widespread method of influencing adherence has been "Health-Promoting Schools", including patients with IBD [53]. However, there are no data showing long-lasting results in IBD patients.

CONCLUSION

The literature review revealed that high adherence to treatment in IBD patients increases therapy effectiveness, reduces the risk of relapses and complications, and significantly affects financial savings for both public healthcare systems and patients. According to the combined research results, adherence to drug therapy in IBD patients is low, whereas adherence to lifestyle modification and counselling in patients with IBD has not been studied at all. The importance of studying adherence to lifestyle modification in patients with IBD is determined by a great contribution of diet therapy, but patients' readiness for it is currently unknown.

The study of adherence to counselling will make it possible to understand readiness for regular visits to the doctor and control of clinical, laboratory, and instrumental criteria of the disease course. The overwhelming majority of the revealed reasons for low adherence are potentially modifiable. Possibilities of

modifying adherence in IBD patients, both in short and long term, require further study to develop effective algorithms to increase it.

An important factor associated with high adherence is joint work of the doctor and the patient during selection of an individual treatment regimen based on personal needs of the IBD patient and assessment of the comorbid background in order to improve the patient's quality of life. A comprehensive approach to the study of adherence to treatment in IBD patients will help to unify methods for assessing and analyzing the level of compliance and design methods to impact it.

REFERENCES

1. Knyazev V.O., Shkurko V.T., Fadeeva A.N., Bakulin G.I., Bordin S.D. Epidemiology of chronic inflammatory bowel disease. Yesterday, today, tomorrow. *Experimental and Clinical Gastroenterology*. 2017; 139 (3): 4–12 (in Russ.).
2. Cuiv P.O., Begun J., Keely S. Towards an integrated understanding of the therapeutic utility of exclusive enteral nutrition in the treatment of Crohn's disease. *Food Funct*. 2016; 7 (4): 1741–1751. DOI: 10.1039/C5FO01196E.
3. Ivashkin V.T., Shelygin Yu.A., Belousova E.A., Abdulganieva D.I., Alekseeva O.A., Achkasov S.I., Valuisikh E.Yu., Vardanyan A.V., Veselov A.V., Veselov V.V., Golovenko O.V., Gubonina I.V., Zhigalova T.N., Kashnikov V.N., Knyazev O.V., Makarchuk P.A., Moskalev A.I. Project: Clinical guidelines for the diagnostics and treatment of ulcerative colitis. *Koloproktologia*. 2019; 18 (4): 7–36 (in Russ.). DOI: 10.33878/2073-7556-2019-18-4-7-36.
4. Fiocchi C. Etiopathogenesis of inflammatory bowel diseases. *Koloproktologia*. 2015; 51 (1): 5–20 (in Russ.).
5. Livzan M.A., Nikolaev N.A., Skirdenko Yu.P., Fedorin M.M., Gorbenko A.V., Andreev K.A., Andreeva E.K., Ivanov A.I., Ivakhnenko A.T. Nutritional behavior in the student environment. *Kremlin Medicine Journal*. 2019; 2: 13–16 (in Russ.). DOI: 10.26269/tvs8-by34.
6. Bikbavova G.R., Livzan M.A., Sovalkin V.I., Turchaninov D.V., Lopatina O.E., Tretyakova T.V., Akhramovich A.P., Gordienko T.S. Modern lifestyle and its impact on the ulcerative colitis incidence. *The Russian Archives of Internal Medicine*. 2019; 9 (3): 188–193 (in Russ.). DOI: 10.20514/2226-6704-2019-9-3-188-193.
7. Paulino S., Dias J. The role of nutritional approach in the management of Crohn's disease. *Pediatrics. Suppl*. 2018; 10–16 (in Russ.). DOI: 10.26442/2413-8460_2018.2.10-16.
8. Sovalkin V.I., Akhmedov V.A., Bikbavova G.R., Emel'yanova Yu.A. Modern views on prevalence, risk factors, and causes of ulcerative colitis. *Experimental and Clinical Gastroenterology*. 2017; 10 (146): 110–115 (in Russ.).

9. Guseinova Z.K., Taizhanova D.Zh., Tauesheva Z.B., Mandybaeva M.K., Holoshenko A.O. Prevalence and screening diagnosis of colorectal cancer. *Medical sciences*. 2015; 3 (2): 190–197 (in Russ.).
10. Rubin D.T., Ananthakrishnan A.N., Siegel C.A., Sauer B.G., Long M.D. ACG Clinical guideline: ulcerative colitis in adults. *Am. J. Gastroenterol.* 2019; 114 (3): 384–413. DOI: 10.14309/AJG.000000000000152.
11. Ahmed I., Roy B., Khan S., Septer S., Umar S. Microbiome, metabolome and inflammatory bowel disease. *Microorganisms*. 2016; 4 (2): 20. DOI: 10.3390/microorganisms4020020.
12. D'Inca R., Bertomoro P., Mazzocco K., Vettorato MG., Rumiati R., Sturniolo GC. Risk factors for non-adherence to medication in inflammatory bowel disease patients. *Aliment. Pharmacol. Ther.* 2008; 27 (2): 166–172. DOI: 10.1111/j.1365-2036.2007.03555.x.
13. Nalyotov S.V., Alesinsky M.M., Nalyotova E.N. Compliance of hypertensive patients in the blockade Donbass. Solving the problem in collaboration of a doctor and a pharmacist. 2017: 55 (in Russ.).
14. Kane S., Shaya F. Medication non-adherence is associated with increased medical health care costs. *Dig. Dis. Sci.* 2008; 53 (4): 1020–1024. DOI: 10.1007/s10620-007-9968-0.
15. Mikirtichan G.L., Kaurova T.V., Ochkur O.K. Compliance as a medico-social and ethical problem in pediatrics. *Current Pediatrics*. 2012; 11 (6): 5–10 (in Russ.). DOI: 10.15690/vsp.v11i6.485.
16. Muller-Lissner S.A., Bollani S., Brummer R.J. et al. Epidemiological aspects of irritable bowel syndrome in Europe and North America. *Digestion*. 2001; 64 (3): 200–204. DOI: 10.1159/000048862.
17. Ward F.M., Bodger K., Laly M.G., Heatley R.V. Clinical economic review: medical management of inflammatory bowel disease. *Pharmacol. Ther.* 1999; 13 (1): 15–25. DOI: 10.1046/j.1365-2036.1999.00437.x.
18. Kuposova K.A., Khlynova O.V. Project routing patients with inflammatory bowel disease in the Perm region. *Kazan Medical Journal*. 2016; 2: 32–35 (in Russ.).
19. Bezdezhnykh T.P., Fedyaev D.V., Khachatryan G.R., Arutyunov G.G., Gerasimova K.V. Economic consequences of healthcare optimization for IBD patients in the Republic of Tatarstan. *Modern pharmacoeconomics and pharmacoepidemiology*. 2019; 12 (1): 14–26 (in Russ.). DOI: 10.17749/2070-4909.2019.12.1.14-26.
20. Buzunova Yu.M., Belousova E.A. Adherence to the treatment of patients with inflammatory bowel disease. *Gastroenterologiya Sankt-Peterburga*. 2015; 1–2: M5–M5b (in Russ.).
21. Kuvshinova N.Yu. The problem of compliance therapy in different fields of medicine. *Izvestia of Samara Scientific Center of the Russian Academy of Sciences*. 2015; 17 (5–3): 1014–1020. 22 (in Russ.).
22. Parfenov A.I., Kagramanova A.V., Babayan A.F., Knyazev O.V. Adherence to therapy in patients with inflammatory bowel diseases. *Therapeutic archive*. 2018; 90 (12): 4–11 (in Russ.). DOI: 10.26442/00403660.2018.12.000001.
23. Jackson C.A., Clatworthy J., Robinson A., Horne R. Factors associated with non-adherence to oral medication for inflammatory bowel disease: a systematic review. *Am. J. Gastroenterol.* 2010; 105 (3): 525–539. DOI: 10.1038/ajg.2009.685.
24. Maev I.V., Kucheryavyy Yu.A., Andreev D.N. Modern approaches to antihelicobacter therapy optimization. *Lechaschi Vrach Journal*. 2014; 4: 73–79.
25. Kane S., Huo D., Aikens J., Hanauer S. Medication non-adherence and the outcomes of patients with quiescent ulcerative colitis. *Am. J. Med.* 2003; 114 (1): 39–43. DOI: 10.1016/s0002-9343(02)01383-9.
26. Khan N., Abbas A.M., Bazzano L.A., Koleva Y.N., Krousel-Wood M. Long-term oral mesalazine adherence and the risk of disease flare in ulcerative colitis: nationwide 10-year retrospective cohort from the veterans affairs healthcare system. *Aliment. Pharmacol. Ther.* 2012; 36 (8): 755–764. DOI: 10.1111/apt.12013.
27. Robinson A., Hankins M., Wiseman G., Jones M. Maintaining stable symptom control in inflammatory bowel disease: a retrospective analysis of adherence, medication switches and the risk of relapse. *Aliment. Pharmacol. Ther.* 2013; 38 (5): 531–538. DOI: 10.1111/apt.12396.
28. Nelidova A.V., Usacheva E.V., Zamakhina O.V., Suprun E.V. Influencing factors for adherence to treatment of patients with coronary atherosclerosis in the long term vascular events. *Modern Problems of Science and Education. Surgery*. 2015; 4: 354 (in Russ.).
29. Vasilenko A.G., Vasilenko Z.G., Nedomukova E.S., Smagina N.V., Popp V.Ya., Romza L.B., Efimov A.I., Levin M.Z., Efimov I.A. Analysis of causes of low adherence to the treatment of patients with inflammatory bowel diseases. *Bulletin of the Chelyabinsk Regional Clinical Hospital*. 2014; 3: 31–33 (in Russ.).
30. Kane S., Holderman W., Jacques P., Miodek T. Once daily versus conventional dosing of pH- dependent mesalamine long-term to maintain quiescent ulcerative colitis: Preliminary results from a randomized trial. *Patient Prefer Adherence*. 2008; 2: 253–258. DOI: 10.2147/PPA.S3698.
31. Ediger J.P., Walker J.R., Graff L., Lix L., Clara I., Rawsthorne P., Rogala L., Miller N., McPhail C., Deering K., Bernstein C.N. Predictors of medication adherence in inflammatory bowel disease. *Am. J. Gastroenterol.* 2007; 102 (7): 1417–1426. DOI: 10.1111/j.1572-0241.2007.01212.x.
32. Lachaine J. Medication adherence and persistence in the treatment of Canadian ulcerative colitis patients: analyses with the RAMQ database. *BMC Gastroenterol.* 2013; 13: 23. DOI: 10.1186/1471-230X-13-23.
33. Shapina M.V., Khalif I.L. Use of 5-aminosalicylic acid for treatment of ulcerative colitis in different dosage

- modes. *Med. Counc. Remedum, Ltd.* 2017; 15: 44–50. DOI: 10.21518/2079-701x-2017-15-44-50.
34. Kane S.V., Cohen R.D., Aikens J.E., Hanauer S.B. Prevalence of nonadherence with maintenance mesalazine in quiescent ulcerative colitis. *Am. J. Gastroenterol.* 2001; 96 (10): 2929–2933. DOI: 10.1111/J.1572-0241.2001.04683.X.
 35. Krasner Ya.A., Osipenko M.F., Valuyskikh E.Yu., Rezakova M.V., Babich K.A. Frequency and features of non-alcoholic fatty liver disease steatohepatitis in patients with inflammatory bowel disease. *J. Sib. Med. Sci.* 2019; 3: 63–73. DOI: 10.31549/2542-1174-2019-3-63-73.
 36. Selinger C., Robinson A., Leong R. Non-adherence to inflammatory bowel disease maintenance medication: extent and predictors. *J. Gastroenterol. Hepatol.* 2011; 26: 56–56.
 37. Lopez A., Billioud V., Peyrin-Biroulet C., Peyrin-Biroulet L. Adherence to anti-TNF therapy in inflammatory bowel diseases: a systematic review. *Inflamm. Bowel Dis.* 2013; 19 (7): 1528–1533. DOI: 10.1097/MIB.0B013E31828132CB.
 38. Alekseyeva O.P., Kasimova L.N., Goryacheva Y.Y. Treatment compliance of patients with gastro-intestinal diseases. *Russian Journal of Gastroenterology, Hepatology, Coloproctology.* 2016; 26 (1): 20–28 (in Russ.). DOI: 10.22416/1382-4376-2016-26-1-20-28.
 39. Shale M.J., Riley S.A. Studies of compliance with delayed-release mesalazine therapy in patients with inflammatory bowel disease. *Aliment. Pharmacol. Ther.* 2003; 18 (2): 191–198. DOI: 10.1046/J.1365-2036.2003.01648.X.
 40. Cervený P., Bortlík M., Kubena A., Vlcek J., Lakatos P.L., Lukás M. Nonadherence in inflammatory bowel disease: results of factor analysis. *Inflamm. Bowel Dis.* 2007; 13 (10): 1244–1249. DOI: 10.1002/IBD.20189.
 41. Mackner L.M., Crandall W.V. Oral medication adherence in pediatric inflammatory bowel disease. *Inflamm. Bowel Dis.* 2005; 11 (11): 1006–1112. DOI: 10.1097/01.MIB.0000186409.15392.54.
 42. Gorjacheva E.E., Kitaeva I.I. Structure of anxiety disorders and treatment compliance of patients with functional gastrointestinal pathologies. *Neurological Bulletin.* 2015; 44 (2): 72–76 (in Russ.). DOI: 10.17816/NB13932.
 43. Nalyotov A.V., Nalyotov S.V., Barinova A.S., Vunichenko Yu.S. The problem of compliance in current gastroenterology. *University clinic.* 2017; 13 (2): 213–218 (in Russ.).
 44. Hommel K.A., Davis C.M., Baldassano R.N. Medication adherence and quality of life in pediatric inflammatory bowel disease. *J. Pediatr. Psychol.* 2008; 33 (8): 867–874. DOI: 10.1093/jpepsy/jsn022.
 45. Horváth G., Farkas K., Hollósi R. Is there any association between impaired health-related quality of life and non-adherence to medical therapy in inflammatory bowel disease? *Scand. J. Gastroenterol.* 2012; 47 (11): 1298–303. DOI: 10.3109/00365521.2012.703233.
 46. Perry J., Chen A., Kariyawasam V., Choong C., Teh W.L., Mitrev N., Kohler F., Leong R.W.L. Medication non-adherence in inflammatory bowel diseases is associated with disability. *Intest. Res.* 2018; 16 (4): 571–578. DOI: 10.5217/IR.2018.00033.
 47. Sewitch M.J., Abrahamowicz M., Barkun A., Bitton A., Wild G.E., Cohen A., Dobkin PL. Patient nonadherence to medication in inflammatory bowel disease. *Am. J. Gastroenterol.* 2003; 98 (7): 1535–1544. DOI: 10.1111/J.1572-0241.2003.07522.X.
 48. López San Román A., Bermejo F., Carrera E., Pérez-Abad M., Boixeda D. Adherence to treatment in inflammatory bowel disease. *Rev. Esp. Enferm. Dig.* 2005; 97 (4): 249–257. DOI: 10.4321/S1130-01082005000400005.
 49. Ungaro R., Colombel J.-F., Lissos T., Peyrin-Biroulet L. A treat-to-target update in ulcerative colitis: a systematic review. *Am. J. Gastroenterol.* 2019; 114 (6): 874–883. DOI: 10.14309/ajg.0000000000000183.
 50. Agrawal M., Colombel J.-F. Treat-to-target in inflammatory bowel diseases, what is the target and how do we treat? *Gastrointest. Endosc. Clin. N. Am.* 2019; 29 (3): 421–436. DOI: 10.1016/j.giec.2019.02.004.
 51. Knyazev O.V., Babayan A.F., Parfyonov A.I., Kagramanova A.V. Commitment to treatment of inflammatory bowel disease. *Medical alphabet.* 2019; 1 (6): 13–19 (in Russ.). DOI: 10.33667/2078-5631-2019-1-6(381)-13-18.
 52. Drapkina O.M., Livzan M.A., Martynov A.I., Moiseev S.V., Nikolaev N.A., Skirdenko Yu.P. The first The Russian consensus on the quantitative assessment of the adherence to treatment. *Medical News of the North Caucasus.* 2018; 13 (1-2): 259–271 (in Russ.). DOI: 10.14300/mnnc.2018.13039.
 53. Shchukina B.O., Grigoryan V.V. Organization of specialized units – centers of IBD as a way of optimizing medical care for patients with ulcerative colitis and Crohn's disease. *Experimental and Clinical Gastroenterology.* 2017; 146 (10): 116–123 (in Russ.).
 54. Choudhry N.K., Krumme A.A., Ercole P.M., Girdish C., Tong A.Y., Khan N.F., Brennan T.A., Matlin O.S., Shrank W.H., Franklin J.M. Effect of reminder devices on medication adherence: The REMIND randomized clinical trial. *JAMA Intern Med. American Medical Association.* 2017; 177 (5): 624–631. DOI: 10.1001/jamainternmed.2016.9627.
 55. Tiao D.K., Jeganathan J., Chen A. Impact of targeted pharmacist counselling intervention on IBD medication adherence. *J. Gastroenterol. Hepatol.* 2014; 29: 106.
 56. Fenerty S.D., West C., Davis S.A., Kaplan S.G., Feldman S.R. The effect of reminder systems on patients' adherence to treatment. *Patient Prefer Adherence.* 2012; 6: 127–35. DOI: 10.2147/PPA.S26314.

Authors contribution

Skirdenko Yu.P., Nikolaev N.A. – conception and design, final approval of the manuscript for publication. Gorbenko A.V., Andreev K.A., Fedorin M.M. – analysis and interpretation of data, final approval of the manuscript for publication. Livzan M.A., Bikbavova G.R. – critical revision of the manuscript for important intellectual content, final approval of the manuscript for publication.

Authors information

Andreev Kirill A., Inspector, Department for the Development of Regional Healthcare and Medical Activity, Omsk State Medical University, Omsk, Russian Federation. ORCID 0000-0001-9976-573X.

Gorbenko Aleksandr V., Inspector, Department for the Development of Regional Healthcare and Medical Activity, Omsk State Medical University, Omsk, Russian Federation. ORCID 0000-0001-9703-9371.

Skirdenko Yulia P., Cand. Sci. (Med.), Assistant, Department of Internal Medicine and Gastroenterology, Omsk State Medical University, Omsk, Russian Federation. ORCID 0000-0002-6225-2444.

Nikolaev Nikolai A., Dr. Sci. (Med.), Associate Professor, Professor, Department of Internal Medicine and Gastroenterology, Vice-Rector for Medical Activity and Regional Healthcare, Omsk State Medical University, Omsk, Russian Federation. ORCID 0000-0002-3758-4930.

Livzan Maria A., Dr. Sci. (Med.), Professor, Head of the Department of Internal Medicine and Gastroenterology, Rector of Omsk State Medical University, Omsk, Russian Federation. ORCID 0000-0002-6581-7017.

Bikbavova Galiya R., Cand. Sci. (Med.), Associate Professor, Department of Internal Medicine and Endocrinology, Omsk State Medical University, Omsk, Russian Federation. ORCID 0000-0001-9252-9152.

Fedorin Maxim M., Inspector, Department for the Development of Regional Healthcare and Medical Activity, Omsk State Medical University, Omsk, Russian Federation. ORCID 0000-0002-0238-4664.

(✉) **Gorbenko Aleksander V.**, e-mail: prime.dr.alex@gmail.com

Received 14.08.2020

Accepted 28.12.2020

The potential use of miRNAs in forensic science

Gareev I.F.¹, Beylerli O.A.¹, Izmailov A.A.²

¹Bashkir State Medical University (BSMU)
3, Lenina Str., Ufa, Republic of Bashkortostan, 450008, Russian Federation

²Republican Clinical Oncological Dispensary
73/1, Oktyabrya Av., Ufa, Republic of Bashkortostan, 450054, Russian Federation

ABSTRACT

The use of molecular genetic approaches to identification of tissues and biological fluids of the body, which often provide important information for reconstruction of a potential crime, is relevant for forensic studies. MicroRNAs (miRNAs) are short, single-stranded noncoding RNAs (containing on average 18–22 nucleotides) that regulate gene expression at the post-transcriptional level by binding to the 3'-untranslated region (3'-UTR) of specific mRNA targets, which results in a decrease in protein expression by blocking translation and / or promotes degradation of target mRNAs. MiRNAs are involved in virtually all biological processes, including cell proliferation, apoptosis, and differentiation. By acting on target genes, miRNAs are involved in regulation of many pathological processes. In addition, numerous miRNAs called circulating miRNAs were found in many biological fluids of the human body, for example, in blood. Molecular genetic approaches undoubtedly outperform histological and immunological tests in tissue characterization, and miRNAs, due to their characteristic tissue specificity and stability in biological fluids, have potential for application in forensic practice and are of great interest for experts.

Key words: microRNA, biological fluids, forensic science, expression, circulating.

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

Source of financing. The study was supported by Russian Foundation for Basic Research and National Natural Science Foundation (Project No. 21-51-53001).

For citation: Gareev I.F., Beylerli O.A., Izmailov A.A. The potential use of miRNAs in forensic science. *Bulletin of Siberian Medicine*. 2021; 20 (3): 129–140. <https://doi.org/10.20538/1682-0363-2021-3-129-140>.

Потенциал использования микроРНК в судебно-медицинской экспертизе

Гареев И.Ф.¹, Бейлерли О.А.¹, Измайлов А.А.²

¹ Башкирский государственный медицинский университет (БГМУ)
Россия, 450008, Республика Башкортостан, г. Уфа, ул. Ленина, 3

² Республиканский клинический онкологический диспансер
Россия, 450054, Республика Башкортостан, г. Уфа, пр. Октября, 73/1

РЕЗЮМЕ

Применение молекулярно-генетических подходов для идентификации тканей и биологических жидкостей организма, которые часто дают важную информацию для реконструкции потенциального преступления.

ния, является актуальной темой судебно-медицинских исследований. МикроРНК – это короткие, в среднем 18–22 нуклеотида, одноцепочечные некодирующие РНК, которые регулируют экспрессию генов на посттранскрипционном уровне путем связывания с 3'-нетранслируемой областью (3'-НТО) специфических мРНК-мишеней, что приводит к уменьшению экспрессии белка посредством блокады трансляции и (или) способствуя деградации мРНК-мишеней. МикроРНК участвуют практически во всех биологических процессах, включая клеточную пролиферацию, апоптоз и дифференцировку клеток. Через воздействие на гены-мишени микроРНК участвуют в регуляции многих патологических процессов. Кроме того, во многих биологических жидкостях организма человека, таких как кровь, были обнаружены многочисленные микроРНК, называемые циркулирующими микроРНК. Молекулярно-генетические подходы, несомненно, превосходят гистологические и иммунологические анализы в характеристике тканей, и микроРНК, благодаря характерной для них тканеспецифичности и стабильности в биологических жидкостях, несомненно, имеют потенциал в применении в судебно-медицинской практике и заслуживают внимания экспертов.

Ключевые слова: микроРНК, биологические жидкости, судебно-медицинская экспертиза, экспрессия, циркулирующие.

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

Источник финансирования. Исследование выполнено при финансовой поддержке РФФИ и ГФЕН (проект № 21-51-53001).

Для цитирования: Гареев И.Ф., Бейлерли О.А., Измаилов А.А. Потенциал использования микроРНК в судебно-медицинской экспертизе. *Бюллетень сибирской медицины*. 2021; 20 (3): 129–140. <https://doi.org/10.20538/1682-0363-2021-3-129-140>.

INTRODUCTION

MicroRNAs (miRNAs) are short (containing on average 18–22 nucleotides), single-stranded non-coding RNAs that regulate gene expression at the post-transcriptional level by binding to the 3'-untranslated region (3'-UTR) of specific messenger RNA (mRNA) targets, which results in a decrease in protein expression by blocking translation and / or promotes degradation of target mRNA [1]. There are many studies proving the importance of miRNA in the regulation of many biological processes, such as neurogenesis, cell proliferation and differentiation, apoptosis, regulation of immune processes, fat metabolism, glucose homeostasis, etc. [1, 2].

It was shown that dysregulation or aberrant expression of miRNAs associated with these processes leads to development of various human diseases, such as cancer, cardiovascular, autoimmune, and neurodegenerative diseases [3]. In addition, in many biological fluids of the human body, such as blood, urine, cerebrospinal fluid, saliva, etc., numerous miRNAs, called circulating miRNAs, were found [4]. Due to an association with various carriers, these extracellular or circulating miRNAs turned out to be extremely stable: resistant to the effects of ribonucleases (RNases), freeze – thaw cycles, and significant pH fluctua-

tions [5]. Additionally, the content of circulating miRNAs did not significantly change during prolonged incubation of plasma at room temperature [6, 7].

It was initially shown that various extracellular vesicles (EVs) serve as carriers of circulating miRNAs. Indeed, almost all types of cells form and secrete several types of EVs, including microvesicles and exosomes [4, 8]. Microvesicles form by protrusion of the plasma membrane from the cell with subsequent separation of the formed vesicle from the membrane and have a size of 100–1000 nm. Exosomes, on the other hand, are much smaller (40–100 nm) and are released after fusion of the multivesicular body fraction with the plasma membrane [9]. Consequently, the sizes of some microvesicles and exosomes can be very similar.

Therefore, many authors use the term “extracellular vesicles” to refer to a mixed population of microvesicles and exosomes. In addition to EVs, biological fluids also contain apoptotic bodies of 1–4 μm, which also consist of miRNAs. Circulating miRNAs were also found in high-density lipoproteins (HDL), which range in size from 9 to 12 nm. Finally, a large proportion of circulating miRNAs was found in the form of complexes with argonaute-2 proteins (Ago2-miRNAs) [4, 10].

An ideal biomarker should be accessible by non-invasive methods, cheap to quantify, specific to a disease

or physiological condition, and, in case of pathological conditions, it should be a reliable predictor of the disease even before clinical symptoms appear. Since circulating miRNAs have these characteristics, they are very promising for development of new non-invasive biomarkers. In addition, compared with DNA and mRNA, the small size of miRNAs makes them more stable and, therefore, less prone to degradation, which makes them a good option for biomarker development [5].

Another factor that plays a key role in their stability is Ago2 proteins, which are a catalytic component of the RNA-induced silencing complex (RISC). Interaction of Ago2 proteins with mature miRNAs makes them much more resistant to degradation [10]. For these reasons, and taking into account the fact that miRNAs exhibit different expression profiles depending on the tissue or the studied fluid, this characteristic was studied not only for diagnosing various pathological conditions, but also as a method for identifying tissues or biological fluids in a forensic medical examination [11].

During a forensic medical examination, correct identification of possible detected tissues or biological fluids is crucial for detecting possible sources of DNA that will be subsequently used for identifying a donor of biological material. Over the years, several methods have been developed to identify biological material, such as serological tests [11]. However, these methods show low levels of sensitivity and specificity. Therefore, due to the characteristics of miRNAs, they have recently been studied as an alternative to traditional methods of identifying biological material in forensic medical examinations.

CIRCULATING miRNAs AND BIOLOGICAL FLUIDS

In 2009, E.K. Hanson et al. were the first to present results on expression profiling of circulating miRNAs in the field of forensic medicine [12]. In a well-designed and comprehensive study, they demonstrated that circulating miRNAs could be extracted from a variety of human biological fluids and studied. They ultimately examined 452 circulating miRNAs using real-time polymerase chain reaction (qRT-PCR). For blood, saliva, semen, vaginal discharge, and menstrual blood, the authors developed a specific assay consisting of two differentially expressed circulating miRNAs, used to successfully identify and distinguish between a particular biological body fluid.

In a recent study, C. Courts et al. analyzed altered expression of 800 circulating miRNAs in venous blood and saliva using microarrays and identified six

differentially expressed circulating miRNAs (miR-126, miR-150, miR-451, miR-200c, miR-203, and miR-205) for potential identification of a specific biological body fluid [13]. Subsequently, the authors investigated the change in the expression of these six circulating miRNAs using qRT-PCR. The results demonstrated that even taking into account the fact that the panel of the analyzed circulating miRNAs in the study by E.K. Hanson et al. contained approximately half the amount of miRNAs, one of six candidate miRNAs for blood (miR-451) and saliva (miR-205) was also identified for the corresponding biological fluid.

In another study, seven circulating microRNAs were identified as potentially specific miRNAs for a particular biological fluid of the body, which can be used as significant markers in forensic science: miR-16 and miR-486 for venous blood, miR-888 and miR-891a for semen, miR-214 for menstrual blood, miR-124a for vaginal discharge, and miR-138-2 for saliva [14]. According to the results of the study by Z. Wang et al., the authors proposed five specific circulating miRNAs for biological body fluids (miR-16 and miR-486 for venous blood, miR-888 and miR-891a for semen and miR-214 for menstrual blood). Although current research confirms the potential use of these miRNAs to determine the origin of a particular body fluid for subsequent examinations, the authors were unable to identify circulating miRNAs specific to saliva and vaginal discharge.

E. Sauer et al. proved that miR-891a-5p was a semen-specific circulating miRNA among the five studied body fluids (venous blood, saliva, semen, vaginal discharge, and menstrual blood) [15]. In addition, circulating miR-10a-5p, miR-10b-5p, and miR-135-5p were overexpressed in semen samples compared with venous and menstrual blood, saliva, and vaginal discharge, which makes them additionally suitable markers. Besides, circulating miR-144-3p exhibited a specific expression profile for separating blood samples from non-blood samples.

E. Sauer et al. proposed a decision making algorithm to detect each of the five biological fluids, using as few combinations of circulating miRNAs as possible, in order to simplify the analysis procedure: miR-891a-5p was used to identify semen, miR-144-3p was applied for separating blood samples from non-blood samples, a combination of miR-144-3p and miR-203a-3p was used to distinguish between venous blood and menstrual blood samples, and a similar combination of miR-203a-3p and miR-124-3p was used to distinguish between saliva and vaginal discharge samples.

Table 1 presents the results of studies on differentially expressed circulating miRNAs in biological flu-

ids of the human body, which are most important for forensic research [12–20].

Table 1

Biological fluids and circulating microRNAs specific to them						
Research method	Venous blood	Menstrual blood	Vaginal discharge	Semen	Saliva	Ref.
qRT-PCR	miR-451 and miR-16	miR-451 and miR-412	miR-124a and miR-372	miR-135b, miR-10b	miR-658 and miR-205	12
Microarray; qRT-PCR	miR-126, miR-150, and miR-451	–	–	–	miR-200c, miR-203, and miR-205	13
Microarray; qRT-PCR	miR-16 and miR-486	miR-214	–	miR-888, miR-891a	–	14
qRT-PCR	miR-144-3p and miR-144-3p + miR-203a-3p	miR-144-3p	miR-124-3p	miR-891a-5p,	miR-203a-3p + miR-124-3p	15
qRT-PCR	–	–	–	–	miR-200c-3p, miR-203a, and miR-205-5p	16
qRT-PCR	miR-16 and miR-451	–	miR-1280 and miR-4286	miR-10b	–	17
qRT-PCR	miR-451	miR-412	miR-124a	miR-891a	miR-205	18
qRT-PCR	miR-451	–	–	–	miR-205	19
qRT-PCR	miR-144-3p and miR-451a-5p	–	miR-1260b	miR-888-5p and miR-891a-5p	miR-203a-3p and miR-223-3p	20

Note: miR – microRNA; qRT-PCR – real-time polymerase chain reaction (here and in Table 3,4); “–” – biological fluids that were not investigated in this work or specific circulating miRNAs to them were not found; “+” – a combination of circulating miRNAs.

MicroRNAs AND THEIR TISSUE SPECIFICITY

It was found that some tissue- or organ-specific circulating miRNAs could be identified mainly in the bloodstream [21–23]. In particular, organs with a high degree of vascularization, such as the kidneys, liver, brain, and lungs, secrete miRNAs specific to them into the bloodstream, and their detection seems to be important in forensic medical examination.

Y. Sun et al. found five miRNAs (miR-192, miR-194, miR-204, miR-215, and miR-216) which were predominantly expressed in human kidney tissues compared with other organs / tissues, such as the heart, spleen, lungs, striated muscle tissue, and prostate [24].

Later, K. Chandrasekaran et al. also identified these five miRNAs in kidney tissues and concluded that these miRNAs are some of the few miRNAs that have been recognized as specific to the kidneys [25]. In addition, these miRNAs were found in a stable form in the plasma / serum, and since they are predominantly highly expressed in the kidneys, this organ can secrete them into the bloodstream; therefore, they are of re-

nal origin. MiR-124 and miR-128 are considered to be brain-specific; these miRNAs were also found in the plasma and serum in healthy people [26, 27].

Similar miRNAs include miR-129-5p, miR-191, and miR-342-3p, because they can be found in the plasma and exhibit high expression in the brain tissue compared with other tissues, according to miRNAmap [28]. In the lungs, according to miRNAmap, miR-21, miR-30b, miR-30c-1, and miR-146b-5p are highly expressed in comparison with other organs and tissues [29]. These miRNAs are also present in the plasma and serum, and, based on their expression profile in the bloodstream, they probably originate from the lung tissue following whole-lung lavage [30]. MiR-122 is known to be a liver-specific miRNA, which can also be found in a stable form in the plasma, and, therefore, it originates from liver cells [31]. Likewise, miR-148, which can also be found in the plasma, is overexpressed in the liver, and experimental results confirmed its specificity to the liver [32].

Table 2 shows miRNAs with their specific expression profile in relation to a particular cell, tissue, or human organ [24–38].

Table 2

MiRNAs specific to a certain type of cell, organ, or tissue		
Cell, organ, or tissue	miRNAs	Ref.
Kidney	miR-192, miR-194, miR-204, miR-215 и miR-216 miR-10b-5p, and miR-204-5p	24, 25 33
Brain	miR-124 miR-128 miR-129-5p, miR-191, and miR-342-3p miR-9-5p, miR-124-3p, and miR-219a-5p	26 27 28 33
Lung	miR-21, miR-30b, miR-30c-1, and miR-146b-5p miR-146b-5p, and miR-233-3p	29, 30 33
Liver	miR-122 miR-148	31, 33 32
Skeletal muscle	miR-1-3p, miR-133a-3p, and miR-206	33
Skin	miR-203a-3p and miR-205-5p miR-943	33 34
Heart	miR-208b-3p and miR-499a-5p	33
Endothelial cell	miR-126	35
Vascular smooth muscle cell	miR-143/145	36
Erythrocytes	miR-16	36, 37
Platelets	miR-223	36, 38

EXPERIMENTAL PART

Brain damage in various pathologies is a serious condition where many processes are involved [39]. For this reason, identification of specific circulating and / or tissue biomarkers that may indicate a particular process in brain damage is relevant. F. Sessa et al. studied a change in the expression profile of five miRNAs (miR-21, miR-34, miR-124, miR-132, and miR-200b) in the brain material obtained from autopsy in three selected groups: drug addicts (cocaine), deaths associated with ischemic stroke, and the elderly [40]. The results of this study were the following: higher expression of miR-132 and miR-34 can be used as a marker of brain damage caused by drug use (cocaine); overexpression of miR-200b and miR-21 may indicate cases of age-related cognitive impairment; and, finally, the consequences of ischemic stroke may be associated with changes in the expression level of miR-200b, miR-21, and miR-124, with miR-124 having a significantly higher expression level. Changes in the expression profile of these miRNAs can be very useful as available tissue biomarkers in forensic studies to establish the exact cause of death in cases of brain pathology. These results suggest that changes

in the expression profile of these circulating miRNAs in human biological fluids can be studied in living people with brain damage associated with aging, drug abuse, and stroke.

In another preliminary study, C. Lux et al. suggested that differentiation between gunshot wounds to the head and other areas of the body could be accomplished by detecting miRNAs (or their absence) specifically or preferentially expressed in the brain matter (see the section “MiRNAs and their tissue specificity”) [41]. The aim of this study was to explore the possibilities and limitations of differentiating between brain tissue and blood, muscle, and adipose tissue by analyzing differentially expressed miR-124a and miR-124 in these samples, as well as to determine whether these miRNAs were resilient enough to withstand the physical impact associated with a gunshot wound.

A comparative analysis of the expression level of these miRNAs presented in [41] made it possible to reliably determine the brain tissue and differentiate it from blood, muscle, and adipose tissue samples. At the same time, these miRNAs not only demonstrated specificity to the brain tissue, but also, as expected, were much less susceptible to degradation compared with the *Clorf61* gene. If further studies can additionally establish specificity to the brain tissue, analysis of miR-124 / miR-124a expression can be used as an additional tool for identifying the brain tissue in forensic examinations.

P. Menathung et al. found that circulating miR-133a, miR-208b, and miR-499 are detectable and stable in human blood after death from myocardial infarction (MI) for 18 hours [42]. This study was based on already detected miRNA data in the tissue samples from the infarct zone, formalin-fixed and paraffin-embedded, the expression of which persisted for a week after death. It is known that miR-133a performs the antiapoptotic function of a damaged cardiomyocyte [43]. Overexpression of this miRNA inhibits the function of proapoptotic genes, for example, caspase-9 and apoptotic protease activating factor 1 (*APAF1*), protecting damaged cells from apoptosis [43]. The authors observed suppression of miR-133a expression in cardiomyocytes during the acute phase of MI, but its expression in the bloodstream was high (possibly within the apoptotic bodies). It is known that miR-208b is located in the intron of the *MYH7* gene [44].

M.F. Corsten et al. found that in patients with MI, the expression level of circulating miR-208b was increased by 1,600 times. P. Menathung et al. noted that this expression level was rather low after 18 hours,

which may be considered as the effect of post-mortem degradation of this circulating miRNA [44]. Recent studies demonstrated that miR-499 is specific to myocardial injury, and its expression level in the circulation after acute MI was high [45]. Therefore, circulating miR-499 was also present with a high level of expression in post-mortem blood samples of patients after MI. This study highlights the stability of some miRNAs in the post-mortem tissue, advocating their use in post-mortem examination. In addition, this result was further confirmed by the use of miR-499 to diagnose or define MI as a cause of death in post-mortem blood.

Drowning presents a dilemma for forensic science. There is no doubt that a significant proportion of drowning cases are associated with homicide. However, there are cases where drowning is the result of an accident. There are studies that demonstrated the relationship between changes in cellular microenvironment, especially activation of the ion channel / transport, and certain levels of miRNA expression [46]. In their study, S. Yu et al. identified 158 differentially expressed miRNAs in mouse brain tissue using microarrays in the experimental drowning model [47]. Among them, the authors classified four miRNAs (miR-6394, miR-706, miR-30c-1-3p, and miR-6238), which expression was significantly elevated in fresh water drowning models, and four miRNAs (miR-494-3p, miR-669h-3p, miR-135a-1-3p, and miR-5109) with reduced expression in salt water drowning models.

These results suggest that different drowning patterns induce changes in miRNA expression profiles in mouse brain tissue. Another question was why these miRNAs were expressed differently in different drowning models. To answer this question, the authors analyzed the target genes of differentially expressed miRNAs and studied their expression in the brain of mice. Among eight candidate miRNAs, aquaporin 4 (*AQP4*) was identified as a target for miR-30c-1-p and *HCN1* – as a target for miR-706 using *TargetScan*, *miRDB*, and *MGI* databases. However, only the change in miR-706 expression was statistically different ($p < 0.01$) between the fresh water and salt water drowning models, as measured by qRT-PCR.

The expression of the *HCN1* gene was tested by qRT-PCR. It was decreased in the brain tissue of mice in the fresh water drowning model and elevated in the salt water drowning model. Hyperpolarization-activated cyclic nucleotide-gated (HCNs) channels belong to the family of pore-loop cation channels and consist of four members (HCN1–4). HCN channels

are activated by membrane hyperpolarization and are permeable to Na^+ and K^+ .

In the brain, *HCN1* is expressed in the neocortex, hippocampus, cerebellar cortex, and brainstem [48]. In fact, the concentration of sodium in the blood increases with drowning in salt water, and under conditions of hypernatremia, brain osmolarity and intracellular sodium concentration increase. Increased expression of *HCN1* and differences in the expression of miR-706 can be explained by the changes in the microenvironment in this drowning model. Finally, miR-706 was overexpressed in neurons in the cortex and hippocampus, which are common regions of *HCN1* expression. All of this confirms that miR-706 can contribute to crime scene investigations and help understand the pathobiological processes in a dead body.

Traumatic brain injury (TBI) is the most common cause of disability and death among people under 45 years of age worldwide [49]. Detection of TBI is becoming one of the main tasks in clinical forensic medicine. Many TBI survivors have some functional impairment of various degree of severity, especially cognitive impairment, including memory disorders. The hippocampus is critical for learning and memory, and it is quite vulnerable in TBI, showing more significant and earlier pathological changes than other areas of the brain [49]. In one of the studies, a microarray analysis showed that 205 miRNAs exhibited significant changes in the expression in the rat hippocampus in a controlled cortical impact (CCI) model [50].

This indicates that TBI dramatically alters a large number of mature miRNA transcripts, showing extremely sensitive responses to changes in miRNA expression in TBI. In addition, microarray analysis data showed that miR-142-3p and miR-221 were the only miRNAs which expression either increased or decreased in all three time intervals after TBI (1, 2, and 5 days). Specific microRNAs, such as miR-142-3p and miR-221, can be potentially used as sensitive and informative biomarkers in forensic examination of TBI and improve research on cognitive disorders associated with TBI.

Another group of researchers also used a rat CCI model to analyze changes in miRNA expression at day 1 and day 7 after TBI [51]. In this experimental work, 60-day-old rats were subjected to CCI and then sacrificed after 1 and 7 days to extract total RNA from neurons of the dorsal hippocampus. Research methods, such as next-generation sequencing (NGS) and bioinformatics analysis, were used to detect miRNA signatures altered after CCI and signaling pathways

regulated by these miRNAs. In particular, it was predicted that miRNAs (for example, miR-21 and miR-23b), which exhibited a change in expression during the acute post-trauma period (1 day after CCI), would target genes involved in apoptosis, protein folding, and aerobic glycolysis. On the contrary, it was assumed that the same miRNAs, the expression of which would be altered 7 days after injury, would target genes associated with the repair processes.

Forensic medical examination of spontaneous abortions is complex and insufficiently studied and, therefore, poses significant difficulties for experts in resolving a number of specific issues. The etiology of spontaneous abortion is complex and multifactorial. Although researchers identified several causes of this phenomenon, including chromosomal abnormalities in the fetus, maternal immune response, endocrine factors, reproductive tract abnormalities and infections, and environmental risk, approximately half of these cases remain unexplained [52]. The emergence and development of spontaneous abortions also include epigenetic disorders, namely, dysregulation of miRNAs [53].

H. Chen et al. identified several genes and miRNAs that could potentially be involved in the development and progression of spontaneous abortion, including *FCGR1A*, *FCGR3A*, *CXCL8*, *HCK*, *PLEK*, *IL10*, *EVI2A*, *GMFG*, *ESR1*, *MMP10*, miR-498, and miR-4530 [54]. Although further studies *in vivo* and *in vitro* are required, these results provide important information for elucidating the pathological process of spontaneous abortion and may provide a theoretical basis for future research in forensic medicine.

In the field of biomedicine, human aging is of great interest, in particular, in terms of identifying diagnostic predictors of age-related diseases, such as cancer. There is potential for translating biomedical research results into forensic medicine to estimate the age of a donor of a specific biological sample using miRNA analysis. H. Noren Hooten et al. profiled the expression of 800 miRNAs in the peripheral blood using microarray analysis and qRT-PCR in both young (~30 years old) and old (~64 years old) cohorts and reported that the expression of most miRNAs decreased with age [55]. This suggests that changes in circulating miRNA expression may have potential as an indicator of donor age.

In another study, changes in the expression of miRNAs (miR-122 and miR-133a in heart tissues; miR-122 in liver tissues; and miR-133a in striated muscle tissues) *in vivo* were studied to determine post-mortem intervals (PMIs). [56]. PMI is defined as the time elapsed since an individual's death. Currently, various methods are being used to estimate PMI (e.g. physical, physicochemical, biochemical, microbiological, and entomological); however, they all have their limitations.

As mentioned, miRNAs are highly stable in various types of tissues, including post-mortem ones. This suggests that miRNAs are less sensitive to environmental conditions and, therefore, are suitable as stable markers. While the results of this study are promising, there are many variables to consider when estimating PMI, which are much more complex in real-world scenarios with humans than in laboratory settings using animal models.

A summary of the main experimental studies is presented in Table 3.

Table 3

Research devoted to the study of miRNA in forensic medical examination						
Authors	Research direction	Research methods	Research model	miRNAs	Sample type	Expression
Sessa et al. [40]	Brain damage	qRT-PCR	Humans	miR-132, miR-34, miR-124, miR-200b, and miR-21	Autopsy brain material	Increased
Lux et al. [41]	Identifying headshots by detecting miRNAs predominantly expressed in the brain tissue	qRT-PCR	Humans	miR-124a and miR-124	Autopsy material: peripheral blood, muscle and adipose tissue from the maxillofacial region, brain tissue	Increased
Menathung et al. [42]	Myocardial infarction	qRT-PCR	Humans	miR-133a, miR-208b, and miR-499	Post-mortem peripheral blood	Increased
Yu et al. [47]	Drowning	Microarray; qRT-PCR	<i>In vivo</i>	miR-6394, miR-706, miR-30c-1-3p, and miR-6238 miR-494-3p, miR-669h-3p, miR-135a-1-3p, and miR-5109	Brain tissue	Increased Increased

Table 3 (continued)

Authors	Research direction	Research methods	Research model	miRNAs	Sample type	Expression
Sun et al. [50]	TBI	Microarray; qRT-PCR	<i>In vivo</i>	miR-142-3p miR-221	Brain tissue (hippocampus)	Decreased (1 st and 3 rd day) and increased (5 th day) Increased (1 st , 3 rd , and 5 th day)
Martinez et al. [51]	TBI	Microarray; qRT-PCR	<i>In vivo</i>	miR-21 miR-23b	Brain tissue (hippocampus)	Increased (1 st and 7 th day) Decreased (1 st and 7 th day)
Chen et al. [54]	Spontaneous abortion	Microarray; Bioinformatics analysis	Humans	miR-498 and miR-4530	Chorionic villi	Increased
Noren Hooten et al. [55]	Donor age	qRT-PCR	Humans	miR-103, miR-107, miR-128, miR-130a, miR-155, miR-24, miR-221, miR-496, and miR-1538	Peripheral blood	Decreased
Tu et al. [56]	Post-mortem assessment	qRT-PCR	<i>In vivo</i>	miR-122, miR-133a, miR-122, and miR-133a	Heart, liver, and skeletal muscle	Increased

MiRNAs AND MENTAL STATE IN FORENSIC SCIENCE

It was confirmed that miRNAs contribute to the development of the nervous system and its functions [2]. Research showed that they are also involved in neuropsychiatric disorders, including schizophrenia [57]. A forensic psychiatric assessment of schizophrenia includes an evaluation in many aspects, however, there is no effective biological identification of schizophrenia. There is some evidence that the etiology of schizophrenia may include both genetic and environmental factors [58].

Therefore, the environmental contribution to the progression of schizophrenia is implemented through epigenetic mechanisms, and the study of mental illnesses

from the point of view of epigenetics informs and elucidates their pathogenesis [58]. It was shown that some aberrantly expressed miRNAs were isolated from the brain tissue, whole peripheral blood, serum, and plasma and were recognized as potential biomarkers in the diagnosis of schizophrenia [59–65]. These studies confirm that changes in the expression profile of some miRNAs in biological fluids can be used to identify biomarkers of schizophrenia and differentiate it from other mental diseases. It may also help to elucidate the etiology of schizophrenia with ambiguous clinical symptoms and identify potential differences between other psychiatric disorders with similar clinical symptoms [66].

Table 4 presents the studies examining circulating miRNAs as potential biomarkers in schizophrenia, mostly published over the past five years.

Table 4

Circulating miRNAs as potential biomarkers in schizophrenia					
miRNAs	Sample	Expression	Number of participants Schizophrenia / Control	Research methods	Ref.
miR-22-3p, miR-92a-3p, and miR-137	Peripheral blood	Increased	10/10	qRT-PCR	[60]
miR-34a-5p miR-432-5p miR-449a	Serum	Increased Decreased Increased	40/40	qRT-PCR	[61]
miR-30a-5p	Peripheral blood mononuclear cells	Decreased	38/50	qRT-PCR	[62]

Table 4 (continued)

miRNAs	Sample	Expression	Number of participants Schizophrenia / Control	Research methods	Ref.
miR-223	Plasma	Increased	21/21	Microarray; qRT-PCR	[63]
miR9-5p, miR29a-3p, miR106b-5p, miR125a-3p, and miR125b-3p	Peripheral blood	Increased	16/16	qRT-PCR	[64]
miR-132, miR-195, miR30e, miR-212, miR-34a, miR-30e, and miR-7	Peripheral blood mononuclear cells	Increased	25/13	qRT-PCR	[65]

CONCLUSION

MiRNAs as tissue and / or fluid biomarkers have tremendous potential in forensic science and several applications. In determination of body fluids, detection of specific circulating miRNAs can be very useful in identifying the donor of the sample. Additionally, miRNAs are highly stable and can be detected in damaged samples in the late post-mortem period. Moreover, miRNAs with differentiated expression profiles between healthy individuals and individuals with certain pathologies can be very useful in identifying suspects and / or victims, helping to narrow down the list of suspects.

Although miRNA expression analysis has been widely studied around the world, this research mainly focused on its applications in biomedical / cancer research. To truly unleash the full potential of miRNAs in forensic medicine, it is critical to uncover the molecular complexity of the available samples. Incorporating miRNA analysis into the forensic practice will help address several challenges that forensic experts are currently facing. Ensuring stable performance in adverse conditions, miRNA analysis provides reliable access to information that was previously impossible.

As mentioned earlier, miRNA analysis has just started being implemented. The number of published studies in this area is limited, but it is growing. In forensic medicine, methods that once seemed modern are now obsolete. It is essential that researchers develop methods for use in forensic investigations that produce results in record time, provide a higher degree of discrimination, and are fairly reliable.

REFERENCES

- Lu T.X., Rothenberg M.E. MicroRNA. *Journal of Allergy and Clinical Immunology*. 2018; 141 (4): 1202–1207. DOI: 10.1016/j.jaci.2017.08.034.
- Van Meter E.N., Onyango J.A., Teske K.A. A review of currently identified small molecule modulators of microRNA function. *European Journal of Medicinal Chemistry*. 2019; 188: 112008. DOI: 10.1016/j.ejmech.2019.112008.
- Vishnoi A., Rani S. MiRNA biogenesis and regulation of diseases: an overview. *Methods in Molecular Biology*. 2017; 1509: 1–10. DOI: 10.1007/978-1-4939-6524-3_1.
- Valihrach L., Androvic P., Kubista M. Circulating miRNA analysis for cancer diagnostics and therapy. *Molecular Aspects of Medicine*. 2019; 72: 100825. DOI: 10.1016/j.mam.2019.10.002
- Sanz-Rubio D., Martin-Burriel I., Gil A., Cubero P., Forner M., Khalyfa A., Marin J.M. Stability of circulating exosomal miRNAs in healthy subjects. *Scientific Reports*. 2018; 8 (1): 10306. DOI: 10.1038/s41598-018-28748-5.
- Matias-Garcia P.R., Wilson R., Mussack V., Reischl E., Waldenberger M., Gieger C., Anton G., Peters A., Kuehn-Stein A. Impact of long-term storage and freeze-thawing on eight circulating microRNAs in plasma samples. *PLoS One*. 2020; 15 (1): e0227648. DOI: 10.1371/journal.pone.0227648.
- Ward Gahlawat A., Lenhardt J., Witte T., Keitel D., Kaufhold A., Maass K.K., Pajtlar K.W., Sohn C., Schott S. Evaluation of storage tubes for combined analysis of circulating nucleic acids in liquid biopsies. *International Journal of Molecular Sciences*. 2019; 20 (3): 704. DOI: 10.3390/ijms20030704.
- Desmond B.J., Dennett E.R., Danielson K.M. Circulating extracellular vesicle microRNA as diagnostic biomarkers in early colorectal cancer – a review. *Cancers (Basel)*. 2019; 12 (1): 52. DOI: 10.3390/cancers12010052.
- Lu Y., Tan J., Miao Y., Zhang Q. The role of microvesicles and its active molecules in regulating cellular biology. *Journal of Cellular and Molecular Medicine*. 2019; 23 (12): 7894–7904. DOI: 10.1111/jcmm.14667.
- Fuji T., Umeda Y., Nyuya A., Taniguchi F., Kawai T., Yasui K., Toshima T., Yoshida K., Fujiwara T., Goel A., Nagasaka T. Detection of circulating microRNAs with Ago2 complexes to monitor the tumor dynamics of colorectal cancer patients during chemotherapy. *International Journal of Cancer*. 2019; 144 (9): 2169–2180. DOI: 10.1002/ijc.31960.
- Silva S.S., Lopes C., Teixeira A.L., Carneiro de Sousa M.J., Medeiros R. Forensic miRNA: potential biomarker for body fluids? *Forensic Science International: Genetics*. 2015; 14: 1–10. DOI: 10.1016/j.fsigen.2014.09.002.
- Hanson E.K., Lubenow H., Ballantyne J. Identification of forensically relevant body fluids using a panel of differentially expressed microRNAs. *Analytical Biochemistry*. 2009; 387 (2): 303–314. DOI: 10.1016/j.ab.2009.01.037.

13. Courts C., Madea B. Specific micro-RNA signatures for the detection of saliva and blood in forensic body-fluid identification. *Journal of Forensic Sciences*. 2011; 56 (6): 1464–1470. DOI: 10.1111/j.1556-4029.2011.01894.x.
14. Wang Z., Zhang J., Luo H., Ye Y., Yan J., Hou Y. Screening and confirmation of microRNA markers for forensic body fluid identification. *Forensic Science International: Genetics*. 2013; 7 (1): 116–123. DOI: 10.1016/j.fsigen.2012.07.006.
15. Sauer E., Reinke A.K., Courts C. Differentiation of five body fluids from forensic samples by expression analysis of four microRNAs using quantitative PCR. *Forensic Science International: Genetics*. 2016; 22: 89–99. DOI: 10.1016/j.fsigen.2016.01.018.
16. Wang Z., Zhang J., Wei W., Zhou D., Luo H., Chen X., Hou Y. Identification of saliva using microRNA biomarkers for forensic purpose. *Journal of Forensic Sciences*. 2015; 60 (3): 702–706. DOI: 10.1111/1556-4029.12730.
17. Sirker M., Fimmers R., Schneider P.M., Gomes I. Evaluating the forensic application of 19 target microRNAs as biomarkers in body fluid and tissue identification. *Forensic Science International: Genetics*. 2017; 27: 41–49. DOI: 10.1016/j.fsigen.2016.11.012.
18. O'Leary K.R., Glynn C.L. Investigating the isolation and amplification of microRNAs for forensic body fluid identification. *Microna*. 2018; 7 (3): 187–194. DOI: 10.2174/2211536607666180430153821.
19. Lewis C.A., Layne T.R., Seashols-Williams S.J. Detection of microRNAs in DNA extractions for forensic biological source identification. *Journal of Forensic Sciences*. 2019; 64 (6): 1823–1830. DOI: 10.1111/1556-4029.14070.
20. Fujimoto S., Manabe S., Morimoto C., Ozeki M., Hamano Y., Hirai E., Kotani H., Tamaki K. Distinct spectrum of microRNA expression in forensically relevant body fluids and probabilistic discriminant approach. *Scientific Reports*. 2019; 9 (1): 14332. DOI: 10.1038/s41598-019-50796-8.
21. Sehgal A., Chen Q., Gibbings D., Sah D.W., Bumcrot D. Tissue-specific gene silencing monitored in circulating RNA. *RNA*. 2014; 20 (2): 143–149. DOI: 10.1261/rna.042507.113.
22. Thomou T., Mori M.A., Dreyfuss J.M., Konishi M., Sakaguchi M., Wolfrum C., Rao T.N., Winnay J.N., Garcia-Martin R., Grinspoon S.K., Gorden P., Kahn C.R. Adipose-derived circulating miRNAs regulate gene expression in other tissues. *Nature*. 2017; 542 (7642): 450–455. DOI: 10.1038/nature21365.
23. Siracusa J., Koulmann N., Banzet S. Circulating myomiRs: a new class of biomarkers to monitor skeletal muscle in physiology and medicine. *Journal Cachexia Sarcopenia Muscle*. 2018; 9 (1): 20–27. DOI: 10.1002/jcsm.12227.
24. Sun Y., Koo S., White N., Peralta E., Esau C., Dean N.M., Perera R.J. Development of a micro-array to detect human and mouse microRNAs and characterization of expression in human organs. *Nucleic Acids Research*. 2004; 32 (22): e188. DOI: 10.1093/nar/gnh186.
25. Chandrasekaran K., Karolina D.S., Septhamian S., Arumugam A., Wintour E.M., Bertram J.F., Jeyaseelan K. Role of microRNAs in kidney homeostasis and disease. *Kidney International*. 2012; 81 (7): 617–627. DOI: 10.1038/ki.2011.448.
26. Sun Y., Luo Z.M., Guo X.M., Su D.F., Liu X. An updated role of microRNA-124 in central nervous system disorders: a review. *Frontiers in Cellular Neuroscience*. 2015; 9: 193. DOI: 10.3389/fncel.2015.00193.
27. McSweeney K.M., Gussow A.B., Bradrick S.S., Dugger S.A., Gelfman S., Wang Q., Petrovski S., Frankel W.N., Boland M.J., Goldstein D.B. Inhibition of microRNA 128 promotes excitability of cultured cortical neuronal networks. *Genome Research*. 2016; 26 (10): 1411–1416. DOI: 10.1101/gr.199828.115.
28. Wang K., Yuan Y., Cho J.H., McClarty S., Baxter D., Galas D.J. Comparing the microRNA spectrum between serum and plasma. *PLoS One*. 2012; 7 (7): e41561. DOI: 10.1371/journal.pone.0041561.
29. Ludwig N., Leidinger P., Becker K., Backes C., Fehlmann T., Pallasch C., Rheinheimer S., Meder B., Stähler C., Meese E., Keller A. Distribution of miRNA expression across human tissues. *Nucleic Acids Research*. 2016; 44 (8): 3865–3877. DOI: 10.1093/nar/gkw116.
30. Weber J.A., Baxter D.H., Zhang S., Huang D.Y., Huang K.H., Lee M.J., Galas D.J., Wang K. The microRNA spectrum in 12 body fluids. *Clinical Chemistry*. 2010; 56 (11): 1733–1741. DOI: 10.1373/clinchem.2010.147405.
31. Stojkovic S., Koller L., Sulzgruber P., Hülsmann M., Huber K., Mayr M., Hengstenberg C., Wojta J., Niessner A. Liver-specific microRNA-122 as prognostic biomarker in patients with chronic systolic heart failure. *International Journal of Cardiology*. 2020; 303: 80–85. DOI: 10.1016/J.IJCARD.2019.11.090.
32. Ponsuksili S., Trakooljul N., Hadlich F., Haack F., Murani E., Wimmers K. Genetic architecture and regulatory impact on hepatic microRNA expression linked to immune and metabolic traits. *Open Biology*. 2017; 7 (11): 170101. DOI: 10.1098/rsob.170101.
33. Sauer E., Extra A., Cachée P., Courts C. Identification of organ tissue types and skin from forensic samples by microRNA expression analysis. *Forensic Science International: Genetics*. 2017; 28: 99–110. DOI: 10.1016/j.fsigen.2017.02.002.
34. Sirker M., Fimmers R., Schneider P.M., Gomes I. Evaluating the forensic application of 19 target microRNAs as biomarkers in body fluid and tissue identification. *Forensic Science International: Genetics*. 2017; 27: 41–49. DOI: 10.1016/j.fsigen.2016.11.012.
35. Van Solingen C., Bijkerk R., de Boer H.C., Rabelink T.J., van Zonneveld A.J. The role of microRNA-126 in vascular homeostasis. *Current Vascular Pharmacology*. 2015; 13 (3): 341–351. DOI: 10.2174/15701611113119990017.
36. Gareev I., Yang G., Sun J., Beylerli O., Chen X., Zhang D., Zhao B., Zhang R., Sun Z., Yang Q., Li L., Pavlov V., Safin S., Zhao S. Circulating microRNAs as potential noninvasive biomarkers of spontaneous intracerebral hemorrhage. *World Neurosurgery*. 2020; 133: e369–e375. DOI: 10.1016/j.wneu.2019.09.016.
37. Gareev I.F., Beylerli O.A. Circulating microRNA as biomarkers: what are the prospects? *Preventive Medicine*. 2018; 21 (6): 142–150 (in Russ.). DOI: 10.17116/profmed201821061142.
38. Taghizadeh M., Ahmadizad S., Naderi M. Effects of endurance training on hsa-miR-223, P2RY12 receptor expression and platelet function in type 2 diabetic patients. *Clinical*

- Hemorheology and Microcirculation*. 2018; 68 (4): 391–399. DOI: 10.3233/CH-170300.
39. Corraliza-Gomez M., Sanchez D., Ganfornina M.D. Lipid-binding proteins in brain health and disease. *Frontiers in Neurology*. 2019; 10: 1152. DOI: 10.3389/fneur.2019.01152.
 40. Sessa F., Maglietta F., Bertozzi G., Salerno M., Di Mizio G., Messina G., Montana A., Ricci P., Pomara C. Human Brain Injury and miRNAs: An Experimental Study. *International Journal of Molecular Sciences*. 2019; 20 (7): 1546. DOI: 10.3390/ijms20071546.
 41. Lux C., Schyma C., Madea B., Courts C. Identification of gunshots to the head by detection of RNA in backspatter primarily expressed in brain tissue. *Forensic Science International*. 2014; 237: 62–69. DOI: 10.1016/j.forsciint.2014.01.016.
 42. Menathung P., Saengkaeotrakul P., Rasmeepaisarn K., Vongpaisarnsin K. Circulatory microRNA in acute myocardial infarction: A candidate biomarker for forensic investigation. *Forensic Science International: Genetics Supplement Series*. 2017; 6: e294–e295. DOI: 10.1016/j.fsigss.2017.09.136.
 43. Li N., Zhou H., Tang Q. miR-133: A suppressor of cardiac remodeling? *Frontiers in Pharmacology*. 2018; 9: 903. DOI: 10.3389/fphar.2018.00903.
 44. Corsten M.F., Dennert R., Jochems S., Kuznetsova T., Devaux Y., Hofstra L., Wagner D.R., Staessen J.A., Heymans S., Schroen B. Circulating microRNA-208b and microRNA-499 reflect myocardial damage in cardiovascular disease. *Circulation: Cardiovascular Genetics*. 2010; 3 (6): 499–506. DOI: 10.1161/CIRCGENETICS.110.957415.
 45. Chen X., Zhang L., Su T., Li H., Huang Q., Wu D., Yang C., Han Z. Kinetics of plasma microRNA-499 expression in acute myocardial infarction. *Journal of Thoracic Disease*. 2015; 7 (5): 890–896. DOI: 10.3978/j.issn.2072-1439.2014.11.32.
 46. Gomes A., da Silva I.V., Rodrigues C.M.P., Castro R.E., Soveral G. The emerging role of microRNAs in aquaporin regulation. *Frontiers in Chemistry*. 2018; 6: 238. DOI: 10.3389/fchem.2018.00238.
 47. Yu S., Na J.Y., Lee Y.J., Kim K.T., Park J.T., Kim H.S. Forensic application of microRNA-706 as a biomarker for drowning pattern identification. *Forensic Science International*. 2015; 255: 96–101. DOI: 10.1016/j.forsciint.2015.06.011.
 48. Luo P., He G., Liu D. HCN channels: new targets for the design of an antidepressant with rapid effects. *Journal of Affective Disorders*. 2019; 245: 764–770. DOI: 10.1016/j.jad.2018.11.081.
 49. Capizzi A., Woo J., Verduzco-Gutierrez M. Traumatic brain injury: an overview of epidemiology, pathophysiology, and medical management. *Medical Clinics of North America*. 2020; 104 (2): 213–238. DOI: 10.1016/j.mcna.2019.11.001.
 50. Sun T.Y., Chen X.R., Liu Z.L., Zhao L.L., Jiang Y.X., Qu G.Q., Wang R.S., Huang S.Z., Liu L. Expression profiling of microRNAs in hippocampus of rats following traumatic brain injury. *Journal of Huazhong University of Science and Technology (Medical Sciences)*. 2014; 34 (4): 548–553. DOI: 10.1007/s11596-014-1313-1.
 51. Martinez B., Peplow P.V. MicroRNAs as diagnostic markers and therapeutic targets for traumatic brain injury. *Neural Regeneration Research*. 2017; 12 (11): 1749–1761. DOI: 10.4103/1673-5374.219025.
 52. Inzani E., Marshall H.H., Thompson F.J., Kalema-Zikusoka G., Cant M.A., Vitikainen E.I.K. Spontaneous abortion as a response to reproductive conflict in the banded mongoose. *Biology Letters*. 2019; 15 (12): 20190529. DOI: 10.1098/rsbl.2019.0529.
 53. Barchitta M., Maugeri A., Quattrocchi A., Agrifoglio O., Agodi A. The Role of miRNAs as biomarkers for pregnancy outcomes: a comprehensive review. *International Journal of Genomics*. 2017; 2017: 8067972. DOI: 10.1155/2017/8067972.
 54. Chen H., Cheng S., Liu C., Fu J., Huang W. bioinformatics analysis of differentially expressed genes, methylated genes, and mirnas in unexplained recurrent spontaneous abortion. *Journal of Computational Biology*. 2019; 26 (12): 1418–1426. DOI: 10.1089/cmb.2019.0158.
 55. Noren Hooten N., Abdelmohsen K., Gorospe M., Ejiogu N., Zonderman A.B., Evans M.K. microRNA expression patterns reveal differential expression of target genes with age. *PLoS One*. 2010; 5 (5): e10724. DOI: 10.1371/journal.pone.0010724.
 56. Tu C., Du T., Ye X., Shao C., Xie J., Shen Y. Using miRNAs and circRNAs to estimate PMI in advanced stage. *Legal Medicine (Tokyo)*. 2019; 38: 51–57. DOI: 10.1016/j.legalmed.2019.04.002.
 57. Ivanova E., Bozhilova R., Kaneva R., Milanova V. The dysregulation of microRNAs and the role of stress in the pathogenesis of mental disorders. *Current Topics in Medicinal Chemistry*. 2018; 18 (21): 1893–1907. DOI: 10.2174/1568026619666181130135253.
 58. Cao T., Zhen X.C. Dysregulation of miRNA and its potential therapeutic application in schizophrenia. *CNS Neuroscience & Therapeutics*. 2018; 24 (7): 586–597. DOI: 10.1111/cns.12840.
 59. Santarelli D.M., Carroll A.P., Cairns H.M., Tooney P.A., Cairns M.J. Schizophrenia-associated microRNA-gene interactions in the dorsolateral prefrontal cortex. *Genomics Proteomics Bioinformatics*. 2019; 17 (6): 623–634. DOI: 10.1016/j.gpb.2019.10.003.
 60. Ma J., Shang S., Wang J., Zhang T., Nie F., Song X., Zhao H., Zhu C., Zhang R., Hao D. Identification of miR-22-3p, miR-92a-3p, and miR-137 in peripheral blood as biomarker for schizophrenia. *Psychiatry Research*. 2018; 265: 70–76. DOI: 10.1016/j.psychres.2018.03.080.
 61. He K., Guo C., Guo M., Tong S., Zhang Q., Sun H., He L., Shi Y. Identification of serum microRNAs as diagnostic biomarkers for schizophrenia. *Hereditas*. 2019; 156: 23. DOI: 10.1186/s41065-019-0099-3.
 62. Liu S., Zhang F., Shugart Y.Y., Yang L., Li X., Liu Z., Sun N., Yang C., Guo X., Shi J., Wang L., Cheng L., Zhang K., Yang T., Xu Y. The early growth response protein 1-miR-30a-5p neurogenic differentiation factor 1 axis as a novel biomarker for schizophrenia diagnosis and treatment monitoring. *Translational Psychiatry*. 2017; 7 (1): e998. DOI: 10.1038/tp.2016.268.
 63. Zhao Z., Jinde S., Koike S., Tada M., Satomura Y., Yoshikawa A., Nishimura Y., Takizawa R., Kinoshita A., Sakakibara E., Sakurada H., Yamagishi M., Nishimura F., Inai A., Nishioka M., Eriguchi Y., Araki T., Takaya A., Kan C., Umeda M., Shimazu A., Hashimoto H., Bundo M., Iwamoto K., Kakiuchi C.,

- Kasai K. Altered expression of microRNA-223 in the plasma of patients with first-episode schizophrenia and its possible relation to neuronal migration-related genes. *Translational Psychiatry*. 2019; 9 (1): 289. DOI: 10.1038/s41398-019-0609-0.
64. Camkurt M., Karababa F., Erdal M., Bayazit H., Kandemir S., Ay M., Kandemir H., Ay O., Cicek E., Selek S., Tasdelen B. Investigation of dysregulation of several microRNAs in peripheral blood of schizophrenia patients. *Clinical Psychopharmacology and Neuroscience*. 2016; 14 (3): 256–260. DOI: 10.9758/cpn.2016.14.3.256.
65. Sun X.Y., Lu J., Zhang L., Song H.T., Zhao L., Fan H.M., Zhong A.F., Niu W., Guo Z.M., Dai Y.H., Chen C., Ding Y.F., Zhang L.Y. Aberrant microRNA expression in peripheral plasma and mononuclear cells as specific blood-based biomarkers in schizophrenia patients. *Journal of Clinical Neuroscience*. 2015; 22 (3): 570–574. DOI: 10.1016/j.jocn.2014.08.018.
66. Kalia M., Costa E., Silva J. Biomarkers of psychiatric diseases: current status and future prospects. *Metabolism*. 2015; 64 (3): S11–S15. DOI: 10.1016/j.metabol.2014.10.026.

Authors information

Gareev Ilgiz F., Cand. Sci. (Med.), Researcher, Central Research Laboratory, BSMU, Ufa, Russian Federation. ORCID 0000-0002-4965-0835.

Beylerli Ozal A., Researcher, Central Research Laboratory, BSMU, Ufa, Russian Federation. ORCID 0000-0002-6149-5460.

Izmailov Adel A., Dr. Sci. (Med.), Professor, Chief Physician, Republican Clinical Oncological Dispensary, Ufa, Russian Federation. ORCID 0000-0002-8461-9243.

(✉) **Gareev Ilgiz F.**, e-mail: ilgiz_gareev@mail.ru

Received 28.04.2020

Accepted 28.12.2020

The role of calcium metabolism disorders in induction of hypersensitivity in cardiovascular diseases

Deeva N.S., Shabaldin A.V., Antonova L.V.

*Research Institute for Complex Issues of Cardiovascular Diseases (RICICD)
6, Sosnovy Blvd., Kemerovo, 650002, Russian Federation*

ABSTRACT

Impairment of extracellular and intracellular calcium homeostasis can have a damaging effect on the functioning of both individual organs and systems and the whole organism. In the cardiovascular system, disorders of calcium metabolism trigger a cascade of auto-inflammatory responses, leading to persistent morphological and functional disturbances. In this review, impaired calcium homeostasis is defined as a predictor of the development of hypersensitivity in cardiovascular diseases. We analyzed the development of a cardiovascular pathology (atherosclerosis, aortic valve calcification, and essential hypertension) with impaired calcium metabolism and pathological mechanisms that cause the disturbances. Calcium sensors, polymorphic variants of genes, and hormones involved in calcium metabolism can reveal the features of calcium metabolism and contribute to a personalized approach to treatment of cardiovascular diseases.

Key words: calcium metabolism, atherosclerosis, aortic valve calcification, auto-inflammation, essential hypertension, allergic reactions.

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

Source of financing. The study was supported by a comprehensive basic research program of the Siberian Branch of the Russian Academy of Sciences within the fundamental topic of the Research Institute for Complex Issues of Cardiovascular Diseases No. 0546-2019-0002 “Pathogenetic substantiation of the development of implants for cardiovascular surgery based on biocompatible materials with implementation of a patient-oriented approach using mathematical modeling, tissue engineering, and genomic predictors”.

For citation: Deeva N.S., Shabaldin A.V., Antonova L.V. The role of calcium metabolism disorders in induction of hypersensitivity in cardiovascular diseases. *Bulletin of Siberian Medicine*. 2021; 20 (3): 141–151. <https://doi.org/10.20538/1682-0363-2021-2-141-151>.

Роль нарушений обмена кальция в индукции иммунной гиперчувствительности при сердечно-сосудистых заболеваниях

Деева Н.С., Шабалдин А.В., Антонова Л.В.

*Научно-исследовательский институт комплексных проблем сердечно-сосудистых заболеваний (НИИКПССЗ)
Россия, 650002, г. Кемерово, Сосновый бульвар, 6*

РЕЗЮМЕ

Нарушение внеклеточного и внутриклеточного гомеостаза кальция способно оказывать повреждающее

действие на функционирование как отдельных органов и систем, так и на весь организм в целом. В сердечно-сосудистой системе дисбаланс кальциевого обмена запускает каскад реакций аутовоспалительного характера, приводящих к стойким морфологическим и функциональным нарушениям. В данном обзоре нарушение кальциевого гомеостаза рассматривается как предиктор развития иммунной гиперчувствительности при сердечно-сосудистой патологии. Проанализировано развитие патологии сердечно-сосудистой системы (атеросклероз, кальцификация клапанов сердца и эссенциальная гипертензия) при нарушении кальциевого обмена и патологических механизмах, которые данные нарушения вызывают. Кальциевые сенсоры, полиморфные варианты генов и гормоны, участвующие в обмене кальция, могут выявить особенности кальциевого обмена и внести свой вклад в персонализированный подход в лечение сердечно-сосудистой патологии.

Ключевые слова: метаболизм кальция, атеросклероз, кальцификация клапанов сердца, аутовоспаление, эссенциальная гипертензия, аллергические реакции.

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

Источник финансирования. Работа выполнена при поддержке комплексной программы фундаментальных научных исследований СО РАН в рамках фундаментальной темы НИИ КПССЗ № 0546-2019-0002 «Патогенетическое обоснование разработки имплантатов для сердечно-сосудистой хирургии на основе биосовместимых материалов с реализацией пациент-ориентированного подхода с использованием математического моделирования, тканевой инженерии и геномных предикторов».

Для цитирования: Деева Н.С., Шабалдин А.В., Антонова Л.В. Роль нарушений обмена кальция в индукции иммунной гиперчувствительности при сердечно-сосудистых заболеваниях. *Бюллетень сибирской медицины*. 2021; 20 (3): 141–151. <https://doi.org/10.20538/1682-0363-2021-3-141-151>.

INTRODUCTION

Calcium is a macronutrient that plays a pivotal role in many biochemical reactions contributing to the vital activity [1]. Transplantology studies the role of calcium metabolism, including its effect on heart valve and vascular prostheses [2, 3].

The biological role of calcium is extremely diverse, since it is a part of nearly all physiological processes. Membrane stabilization, enzyme activation, blood clotting, and cell death are the processes that involve calcium ions. Moreover, transport across membranes, cell – cell communication, information transfer, intracellular and extracellular signaling, bone and cardiac remodeling, and gene expression involve calcium as well [3, 4].

In intracellular signaling, calcium acts as a second and third messenger. In intercellular signaling, calcium coordinates biochemical processes. Through this mechanism, calcium affects the efficiency of the immune system, and its dysregulation induces hypersensitivity reactions, some of the manifestations of which include immune inflammatory responses [1, 5].

In the absence of abnormalities, nutritional disorders, and micro- and macroecological imbalance, calcium regulates routine cellular processes, such as

proliferation and differentiation. In case of any deviation from the norm, calcium metabolism can be significantly disrupted. The effect on the above-mentioned processes would be pathological, incorporating hypersensitivity / apoptosis. [1, 5, 6]. Therefore, calcium metabolism is essential for the body, and disorders of calcium metabolism underlie many chronic diseases, such as inflammatory responses that determine development of cardiovascular pathology [1, 3].

HYPERSENSITIVITY REACTIONS AS THE BASIS OF CARDIOVASCULAR PATHOLOGY

In 1968, P.G.H. Gell and R. Coombs proposed the following classification of hypersensitivity reactions: immediate hypersensitivity (IH, atopy), cytotoxic (cytolytic), immune complex-mediated, delayed hypersensitivity (DH, cellular), and antibody-mediated hypersensitivity [7, 8]. These reactions are important links in the pathogenesis of allergic and autoimmune diseases [7–9].

Hypersensitivity reactions include three parallel phases: 1) immunological (interaction of the antigen with antibodies and / or sensitized CD4+ T helper and CD8+ cytotoxic T lymphocytes); 2) pathochemical (release of inflammatory mediators); 3) patho-

physiological (stage of clinical manifestations). Hypersensitivity reactions are divided into five types due to the nature of the first, immunological phase. The effect of calcium metabolism is implemented via the pathochemical and pathophysiological stages [8].

In 1999, a new group of autoinflammatory diseases was introduced. Discovery of these diseases is associated with the identification of autosomal dominant and autosomal recessive disorders, as well as monogenic diseases manifested by familial periodic fevers, cold allergies, and non-infectious dermatitis [10]. The genes associated with these diseases encode the activity of inflammasomes and cause a hyperactive innate immune response [10].

Further studies made it possible to distinguish a group of polygenic autoinflammatory diseases that includes atherosclerosis [11]. Activation of innate immunity and development of a systemic inflammatory response and multi-organ damage are particularities of this pathology [12]. It is atherosclerosis that is the underlying pathology determining the cardiovascular disease continuum.

Acquired heart diseases are another significant pathology, formed due to immune inflammatory responses and constitutional symptoms. This pathology is manifested through acute rheumatic fever in children and rheumatic disease in adults. The etiology and pathogenesis of acquired heart diseases are being actively studied.

The concepts of specific rheumatogenic M types of streptococcus pyogenes, autoimmune damage to myocardial autoantigens, and immune complex-mediated inflammatory responses remain relevant [13]. According to the classification, this disease can be attributed to a mixed group [14]. High autoinflammatory activity is intrinsic in acquired heart diseases after infectious endocarditis [15]. Prerequisites for acquired heart diseases remain the same by the time of surgical treatment. Therefore, these factors can determine early and long-term consequences, such as calcification of the implant [16].

Multi-omics approaches to analysis of genomic, epigenomic, transcriptomic, and microbiome markers of immune inflammatory diseases create new prospects for their treatment [9]. At the same time, calcium metabolism disorders can make a significant contribution to the understanding of the molecular, cellular, and genetic mechanisms of this pathology.

The main focus of the study will be the role of calcium in maintaining hypersensitivity responses, implemented both through classical allergic (immunopathological) reactions and autoinflammatory reactions associated with activation of innate immunity. As mentioned above, the role of calcium in these reactions consists in its extracellular and intracellular regulatory functions.

EXTRACELLULAR CALCIUM METABOLISM

Calcium concentration in the blood is regulated by several hormones. Intracellular and extracellular concentration is controlled by a complex system of pumps, channels, exchangers, and a wide range of Ca^{2+} -binding proteins [6]. Parathyroid hormone (PTH), synthesized in the parathyroid gland (PTG), regulates extracellular calcium homeostasis. The calcium-sensing receptor (CaSR) expressed on the surface of PTG cells activates synthesis and secretion of PTH. In the bloodstream, PTH binds to the G protein-coupled parathyroid hormone 1 receptor (PTH1R), increasing osteolytic activity of osteoclasts and bone resorption. In the renal nephron, PTH promotes reabsorption of calcium [17].

By stimulating production of calcitriol in the kidneys, PTH promotes a vitamin D-mediated increase in calcium absorption in the intestine. Calcitriol (Vitamin D – $1,25(\text{OH})_2\text{D}$) is synthesized from the inactive precursor of vitamin D3 (cholecalciferol) through double subsequent hydroxylation by the enzyme 1α -hydroxylase in the kidneys and the liver. PTH stimulates CYP27B1 gene transcription, which encodes 1α -hydroxylase [18] and fibroblast growth factor-23 (FGF-23). In contrast, FGF-23 has an inhibitory effect on 1α -hydroxylase and stimulates synthesis of 24 -hydroxylase (CYP24), thus converting calcitriol to inactive metabolites [18, 19].

Vitamin D plays an important role in regulating calcium metabolism. Vitamin D stimulates expression of protein transporters (transient receptor potential cation channel subfamily V member 5 (TRPV 5), transient receptor potential cation channel subfamily V member 6 (TRPV 6), calcium-binding protein calbindin – CaBP-9k, CaBP-28k, etc.), which promote calcium absorption in the intestine, stimulate osteoclastic resorption, and enhance calcium reabsorption in the renal tubules [6, 18]. Another effect of PTH and vitamin D is an increase in the serum calcium concentration.

However, calcitonin has an opposite effect on calcium regulation. It is a calcium-lowering hormone secreted by parafollicular cells of the thyroid gland. The signal for its secretion is hypercalcemia. Calcitonin inhibits osteoclastic activity in the bone, reducing bone resorption and increasing excretion of calcium ions in the renal tubules [6, 17].

In addition to the three main hormones regulating calcium homeostasis, new regulators of calcium metabolism have been discovered, such as klotho. Klotho is a protein hormone that has two forms: membrane and secreted. It is synthesized by the kidneys, intestines, brain, and other organs. Membrane-bound klotho is a cofactor for FGF-23, synthesized by osteocytes. By binding together, klotho and FGF-23 interact with and biologically affect receptors of organs and tissues.

FGF-23 regulates vitamin D by inhibiting 1α -hydroxylase, thereby indirectly affecting calcium metabolism [18, 19]. Moreover, the association of klotho deficiency with atherosclerosis and vascular calcification was revealed. Decreased klotho levels are known to correlate with the severity of coronary artery stenosis. Decreased plasma klotho concentration and low klotho expression in the vascular wall are associated with high risk of coronary artery disease. At the same time, increased plasma klotho concentration is associated with low risk of developing cardiovascular diseases [20].

Besides calcium and phosphorus metabolism, vitamin D is involved in other physiological processes in the body. Vitamin D can have an extra-skeletal effect due to the presence of vitamin D receptors (VDR) in almost all cells and tissues of the body – cells of the immune system, vascular smooth muscle cells, endothelial cells, and cardiomyocytes [18]. Similar to thyroid and steroid hormone receptors, VDR is one of the transcription factors that regulates protein synthesis; thus, an active form of vitamin D is equivalent to steroid hormones [21].

Directly or indirectly, vitamin D regulates about 200 genes. It controls secretion of proinflammatory cytokines, adhesion molecules, proliferation of vascular smooth muscle cells and cardiomyocytes, exerting an inhibitory effect on the processes associated with atherosclerosis and vascular calcification and suppressing renin gene expression, thereby regulating the activity of the renin-angiotensin-aldosterone system (RAAS) [16, 18]. Indirectly, vitamin

D reduces synthesis of matrix metalloproteinases (MMPs), which significantly limits the growth of atherosclerotic plaque [22] and blocks synthesis of proinflammatory cytokines and cell adhesion molecules, preserving the integrity of the vascular wall and its components and limiting the process of vascular calcification [16].

Therefore, disorders of extracellular calcium metabolism can be associated with individual genomic and epigenomic characteristics that must be taken into account during assessment of hypersensitivity responses and cardiovascular pathology. In this regard, genetic polymorphisms in the PTH genes, calcitonin, CYP27B1, CYP24, TRPV 5, TRPV 6, cabp-9k, cabp-28k, FGF-23, VDR and others make an important contribution to calcium metabolism [15, 18, 21]. Moreover, chromosomal microdeletions, monogenic disorders, and thyroid surgery may affect extracellular calcium metabolism as well [23]. Disorders of extracellular calcium metabolism create prerequisites for changes in both intercellular and intracellular signaling.

INTRACELLULAR CALCIUM METABOLISM

In the cell, calcium can exist in three forms: 1) ionized (free); 2) localized in intracellular organelles (endoplasm, sarcoplasmic reticulum, nucleus, mitochondria); 3) chelated (bound). Intracellular calcium concentration is about 10,000 times less than extracellular calcium concentrations. Under resting conditions, the cytosolic free calcium concentration is 100–200 nM, which is facilitated by Ca^{2+} -ATPase and $\text{Na} / \text{Ca}^{2+}$ -exchangers. Moreover, many proteins in the cytoplasm bind calcium ions, acting as buffers [6].

More than 200 genes in the human genome encode Ca^{2+} -binding proteins [5]. The biochemical action of calcium in the cell is mediated by the EF-hand Ca^{2+} -binding motif and other Ca^{2+} -binding proteins (calcium sensors) associated with the regulation of ion currents, signal transmission, and cell proliferation and differentiation [24]. With calcium ions, troponin C regulates the interaction between actin and myosin in striated muscles. Mutations in troponin C are associated with some forms of congenital heart diseases [5, 25].

Calsequestrin, calregulin, and calreticulin regulate calcium transport and act as a buffer for calcium. Calcium ions and annexin V, which belongs to phos-

phospholipid-binding proteins, can selectively and affinely bind to phosphatidylserine, reaching the surface of the cell membrane during apoptosis. Annexin V is used as a specific marker of apoptosis [26]. Calmodulin is one of the most important calcium sensors, it has four high-affinity Ca^{2+} -binding centers, making it a multifunctional regulatory element [24].

Thus, calcium ions can regulate the activity of more than 100 enzymes, ion pumps, and cytoskeletal components. Increased serum calcium concentration triggers exocytosis of mediators in presynaptic nerve terminals, reduction of myocytes, hormone release of endocrine and exocrine glands, and migration of white blood cells, tumor cells, etc. A significant number of diseases have been described associated with defected Ca^{2+} transporters, channels, sensory proteins, as well as enzymes modulated by them [5]. Some Ca^{2+} -binding proteins are diagnostic markers for cancer [5, 27].

A complex system of intracellular calcium metabolism regulation plays a crucial role in regulating many biochemical processes and developing immune-mediated inflammatory and cardiovascular diseases [3, 28, 29]. Polymorphic variants of Ca^{2+} transporter genes and sensory proteins determine disorders of intracellular calcium metabolism. In the following parts of the work, the focus will be placed on the role of calcium in maintaining autoinflammatory diseases associated with activation of innate immunity through apoptosis and pyroptosis.

THE ROLE OF CALCIUM IN THE REGULATION OF APOPTOSIS

Certain individual characteristics can contribute to a significant increase in the serum calcium concentration in the cell up to 1000 nM. This results from Ca^{2+} channels opening in the plasma membrane or the endoplasmic reticulum via dihydropyridine and ryanodine receptors [30, 31].

The above-mentioned mechanisms lead to a drastic and short-term increase in the concentration of calcium in the cytoplasm, the so-called “calcium peaks” [5]. Certain signals increase the frequency of these peaks, leading to prolonged elevation of the level of calcium in the cytosol. Prolonged elevation has a cytotoxic effect on the cell, which is extremely sensitive to changes in the calcium concentration. Located on the inner membrane of mitochondria, phospholipase A2 is activated by cytosolic calcium,

damaging the phospholipids in the mitochondrial membranes and disrupting their work [30]. At the beginning of this process, the mitochondria successfully cope with an acute rise in cytosolic calcium, since they accumulate it in the form of an insoluble salt – hydroxyapatite. However, this is only a temporary measure. While the mitochondria “hide” calcium, they cannot synthesize adenosine triphosphate (ATP), a lack of which prevents excess calcium from leaving the cell through pumps. Following disruption in the respiratory chain, oxidative stress develops, forming pores in the mitochondrial membrane. Hydrolysis of membrane phospholipids leads to accumulation of free fatty acids in the mitochondria, which correlates with the degree of their damage. If this vicious circle is not broken, the cell will die [5].

Due to cytosolic calcium overload, the intrinsic (mitochondria-dependent) apoptotic pathway is triggered. The intrinsic pathway stimulates changes in the membrane potential and release of pro-apoptotic B-cell lymphoma-2 (Bcl-2) family proteins: Bcl-2-associated X-protein (BAX) and Bcl-2 homologous antagonist/killer (BAK) [5, 32]. These events disrupt the integrity of the mitochondrial membrane, forming apoptotic pores and promoting the release of mitochondrial proteins, such as cytochrome C and apoptosis-inducing factor (AIF), which is capable of initiating caspase-independent cell death [28, 30, 32]. Cytochrome C binds to the apoptosis protease-activating factor-1 (APAF-1), resulting in the formation of an apoptosome that converts procaspase-9 to active caspase-9. Then, the elements of the apoptotic cascade activate effector caspases (caspase-3, -6, -7) [5, 32].

Activated caspases break down plasma membrane calcium pumps (PMCA), overloading the cell with calcium and leading to even greater activation of proteases and caspases [5]. Thus, activated caspases trigger a chain of biochemical processes that lead to cell death.

Literature data suggest that the level of calcium determines what mechanism of cell death will be used – apoptosis or necrosis. A small increase in the level of calcium initiates apoptosis, however, a sudden rise in calcium is related to necrosis [28]. Progression of necrosis is associated with activation, increased levels of calcium, Ca^{2+} -dependent phospholipases and nucleases, as well as calpains, which cause destructive changes in cells. Energy supply

for the cell is another essential factor: with big ATP reserves, cell death happens via apoptosis, and with energy depletion – via necrosis [28, 30].

THE ROLE OF CALCIUM IN THE REGULATION OF PYROPTOSIS

Pyroptosis is a protective innate immune mechanism, a type of a programmed form of necrosis of leukocytes, more often, monocytes / macrophages [33]. However, unlike apoptosis, pyroptosis proceeds with development of inflammation, as it is accompanied by the release of proinflammatory cytokines. This process is possible due to the formation of a multiprotein complex – inflammasome. The inflammasome has sensory molecules, which bind caspase-1 to the apoptosis-associated speck-like protein containing a C-terminal caspase recruitment domain (PYCARD / ASC CARD). Activation of caspase-1 cleaves interleukin (IL)-1 β and IL-18 [34].

Moreover, the pore-forming protein gasdermin D is activated during pyroptosis [33]. Due to the difference in the osmotic pressure, Na⁺ ions and water rush into the cell. Cell lysis occurs, and all the content, including proinflammatory cytokines and damage-associated molecular patterns (DAMPs), burst into the intercellular space and potentiate local inflammation. As a type of programmed cell death, pyroptosis is activated by pathogen-associated molecular patterns (PAMPs) and aimed at eliminating infectious agents that have entered the cell [34, 35].

The NLRP3 inflammasome is the most studied and of the greatest interest for us. There are intracellular and extracellular pathways of NLRP3 inflammasome activation. The intracellular activation mechanism is related to calcium levels. When dehydration occurs due to changes in the osmotic pressure as potassium and chlorine ions leave the cell, calcium mobilization is stimulated through transient receptor potential channels (TRP channels) [36]. An increase in the intracellular concentration of calcium leads to activation of one of its targets – TGF- β -activated kinase 1 (TAK1), which is associated with deubiquitination of NLRP3 [5, 34].

Changes in the extracellular calcium concentration result in a decrease in cyclic adenosine monophosphate (cAMP) by inhibiting adenylate cyclase. Besides, due to these changes, intracellular calcium increases through activation of phospholipase C and synthesis of second messengers associated with cal-

cium mobilization. The effect of cAMP on the activation of the inflammasome remains controversial [37].

The peculiarity of the NLRP3 inflammasome is that it requires two signals to become activated. The first signal is products of microorganisms and cytokines and the second one is various crystalline substances, including cholesterol crystals. Cholesterol crystals and oxidized low-density lipoproteins (LDL) stimulate caspase-1, which, in turn, activates the NLRP3 inflammasome, resulting in inflammation [38–40].

LDL is oxidized by reactive oxygen species in endothelial cells. Macrophages not only actively capture oxidized LDL, but also play a pivotal role in the atherogenesis [39, 40]. NLRP3 activation results in synthesis of IL-1 β , associated with the atherosclerosis. IL-1 β stimulates adhesion of leukocytes and monocytes to endothelial cells and their procoagulant activity, increasing synthesis and activity of tumor necrosis factor (TNF), chemokines, nitric oxide, etc. [41]. Excessive activation of the NLRP3 inflammasome can cause uncontrolled systemic inflammation that underlies the pathogenesis of a wide range of inflammatory diseases, such as atherosclerosis, rheumatoid arthritis, Crohn's disease, obesity, diabetes mellitus, Alzheimer's disease, etc. [40].

Therefore, calcium ions can be attributed to non-protein mediators that initiate cell death, either through apoptosis or through pyroptosis. Selecting the mechanism of cell death is quite complex and multifaceted, since it depends on the type and strength of the stimulus that activates cell death. Moreover, calcium ions affect the activity of the inflammasome and, hence, the autoinflammatory process. Currently, the most significant autoinflammatory disease is atherosclerosis [40].

THE ROLE OF CALCIUM METABOLISM DISORDERS IN THE PATHOGENESIS OF CALCIFICATION AND ATHEROSCLEROSIS

The role of calcium metabolism disorders in the development of atherosclerosis, arterial hypertension, ischemic heart disease, and cerebrovascular diseases has been established, making it possible to refer these diseases to the “calcium deficiency” category [29]. Genetic mechanisms associated with the intracellular calcium concentration both in cardiomyocytes and in smooth muscle cells and neurons of

the brain are similar in the pathogenesis of these diseases. The detected polymorphisms of genes responsible for calcium metabolism would explain genetic susceptibility to bioprosthetic heart valve calcification [15].

Coronary artery calcification and valvular calcification increase the risk of cardiovascular events, in addition to being a prognostic factor for coronary artery disease. Sclerosis of the vascular wall and calcium mineralization change the architectonics of the vessel resulting in narrowing of its lumen up to obliteration. This process is similar to osteogenesis, with the same participants and principles of progression [16].

The nature of calcium salt deposition is important in the calcification of atherosclerotic plaques. With homogeneous calcium deposition, the strength of the plaque cap increases, reducing the risk of its rupture. Uneven deposition has the opposite effect. Therefore, it is difficult to assess whether calcium has a stabilizing or destabilizing effect.

Vascular calcification may occur due to deficiency of calcification inhibitors (osteopontin, pyrophosphates) produced by vascular smooth muscle cells. The osteoprotegerin (OPG) / receptor activator of nuclear factor- κ B (NF- κ B) ligand (RANKL) / receptor activator of NF- κ B (RANK) system is also involved in the development of vascular calcification [42]. OPG is found in some tissues and organs, including the heart. OPG takes part in synthesis of monocyte chemoattractant protein-1 (MCP-1) and MMPs, thereby supporting inflammation in the atherosclerotic plaque [43]. Studies show that deletion of the *OPG* gene in mice causes aortic calcification.

Other studies showed that elevated OPG levels in the blood correlate with the severity of atherosclerosis, heart failure, unstable angina, and acute myocardial infarction [42, 43]. Bioprosthetic heart valve calcification can also be stimulated by the underlying immune inflammatory process that induced formation of acquired heart disease.

As mentioned above, rheumatic disease is not only a streptococcal infection, but also an autoimmune disease that develops according to the second and third types of hypersensitivity responses (allergic: cytotoxic and immune complex-mediated) [7]. The pathogenesis of this disease is associated with hypersensitivity developing according to these allergic patterns and linked to particular human leuko-

cyte antigen (HLA) genes and alleles. The immune response (Ir) genes of the HLA class II determine immune dysregulation via autoimmune T lymphocyte clones [8]. At the same time, disorders of calcium metabolism may influence the pathochemical and pathophysiological phases of hypersensitivity.

In particular, the process of degranulation of cells involved in allergic inflammation is associated with calcium ions. Thus, calcium ions and complement components C3a and C5a (anaphylotoxins) are secondary liberators of histamine and other active molecules of cell granules [44]. Through phosphorylation by spleen tyrosine kinase (Syk), phospholipase C γ (PLC γ) is activated, which catalyzes breakdown of phosphatidylinositol bisphosphate (PIP2) to inositol trisphosphate (IP3) and diacylglycerol (DAG).

In inflammatory cells, IP3 mediates an increase in intracellular calcium, depleting the intracellular calcium deposits. The increased amount of calcium activates transcription factors, leading to granule exocytosis. In the presence of DAG, calcium ions activate protein kinase C, which phosphorylates myosin light chains, reducing the cytoskeletal elements and initiating degranulation [7]. Calcium ions are involved in activation of an esterase, leading to hydrolysis of phospholipids through phospholipase D. These phospholipids comprise the basis of the cell wall. Thinning of the membrane facilitates exocytosis of granules and secretion of mediators [45]. Moreover, calcium ions and Syk activate phospholipase A2 (PLA2), which is involved in synthesis of arachidonic acid, followed by eicosanoid formation. Eicosanoids are known to mediate the late phase of allergic inflammation [7, 8].

Therefore, the precise mechanism of calcification is not fully understood. However, theories exist about an imbalance between stimulants and inhibitors of calcification that trigger this process. Many studies are dedicated to the role of disorders of intracellular calcium metabolism as a basis for the pathochemical phase in immune hypersensitivity. The latter should be taken into account in case of surgical correction of valvular heart disease resulting from rheumatic fever.

Belonging to the group of metabolic disorders, atherosclerosis is considered to be a result of sterile inflammation. Sterile inflammation consists of accumulation of DAMPs inside and outside the cell, inflammasome assembly, hyperproduction of reactive

oxygen species and autophagy, and local synthesis of proinflammatory cytokines IL-1 β and IL-18. These events contribute to local accumulation of immunocompetent cells – macrophages and lymphocytes, followed by tissue injury and the development of fibrosis [40].

Therefore, the basis of atherosclerosis is the inflammatory response of innate immunity, aimed at maintaining metabolic homeostasis via recognition of DAMPs. The literature describes extreme stages of sterile inflammation, where an adaptive immune response can be characterized by the presence of autoreactive T and B lymphocytes and antibodies [46].

Chronic inflammation within the vascular wall is maintained by mediators of cell – cell interaction, particularly by IL-1, IL-6, and TNF [22]. Proinflammatory and proatherogenic mediators are involved in the induction of apoptosis, contributing to the development and further progression of atherogenesis. In terms of physiology, apoptosis is not associated with inflammation. However, apoptosis can have a proinflammatory effect in the atherogenesis. Released from the apoptotic cells, DNA is immediately absorbed by phagocytes that release the proinflammatory cytokines [47].

An increase in the expression and activity of proinflammatory cytokines destabilizes the atherosclerotic plaque due to enlargement of the lipid core, thickening of the fibrous cap, and accumulation of macrophages [22], eventually leading to plaque rupture and triggering the mechanism of atherothrombosis.

An association of genetic variations in Toll-like receptor (TLR) genes in the innate immune response (particularly TLR4) with development of atherosclerosis and associated vascular complications was revealed [48]. The expression of these receptors is presented on the surface of cardiomyocytes, macrophages, smooth muscle cells, and vascular endothelial cells. Interaction with bacterial lipopolysaccharides and other proatherogenic ligands can activate the transcription factor NF- κ B, resulting in synthesis of a wide range of inflammatory mediators, including proatherogenic ones [22, 46].

The role of disorders of intracellular calcium metabolism that stimulate apoptosis, pyroptosis, and inflammation has been described above. These pathological changes in calcium metabolism contribute to maintenance of atherosclerosis. Moreover, an imbalance between stimulants and inhibitors of

calcification makes a significant contribution to the progression of atherosclerotic lesions.

Therefore, atherosclerosis is an inflammatory process that involves immune inflammatory mechanisms of innate and adaptive immunity. Atherosclerosis encompasses processes and factors that lead to growth and later destabilization of the atherosclerotic plaque, atherothrombosis, and other complications. In the meantime, calcium is involved in calcification, as well as in initiation and maintenance of chronic inflammation [22, 40, 47].

THE ROLE OF CALCIUM IN THE DEVELOPMENT OF ESSENTIAL HYPERTENSION

The development of essential hypertension is associated with disorders of intracellular calcium metabolism. The experiments on spontaneously hypertensive rats (SHR) showed the significance of hereditary defects of Na⁺ and Ca²⁺ ion channels in the membrane of resistance artery smooth muscle cells. These changes result in increased vascular tone and high sensitivity to various pressor stimuli, with further development of endothelial dysfunction [49]. Moreover, changes in the water and electrolyte balance with reduction of natriuretic peptide levels were discovered [50].

Genetic defects were detected in dihydropyridine receptors (DHPR) localized on the cardiomyocyte membrane. Allowing calcium to enter the cell, these receptors are closely related to ryanodine receptors (RyRs), which regulate the release of calcium from the sarcoplasmic reticulum. By triggering the mechanism of calcium-induced calcium release (CICR), these receptors participate in cardiac muscle contraction. Disruptions in the process can lead to a persistent increase in blood pressure [31]. Moreover, any mutation in the *RyR2* gene, which leads to a disorder of calcium balance in the cell, is associated with heart failure, arrhythmogenic right ventricular dysplasia, and an increased risk of sudden cardiac death [51].

Therefore, a disorder of calcium metabolism is associated with increased vascular tone. Disruption of intracellular calcium homeostasis affects not only the cardiovascular system, but other systems as well.

CONCLUSION

The biological role of calcium is extremely diverse. Calcium ions activate and suppress immune

and inflammatory responses and regulate extracellular and intracellular metabolism. A countless number of biochemical reactions involving calcium confirm its uniqueness.

According to some authors, up to 150 different diseases are associated with impaired calcium metabolism. In relation to atherosclerosis and bioprosthetic valve calcification, the role of calcium consists in the initiation of a hypersensitivity response via apoptosis and pyroptosis. From the perspective of a personalized approach to management of cardiovascular diseases, it is necessary to monitor the serum calcium concentration and hormones involved in extracellular calcium metabolism. Features of intracellular calcium metabolism can be predicted by analyzing soluble OPG in the blood serum, calcium-sensing receptor gene polymorphism, and calcium transport proteins.

REFERENCES

1. Ahmedzhanova Z.I., Zhiemuratova G.K., Danilova E.A., et al. Macro- and micronutrients in the life of the body and their relationship with the immune system. *Journal of Theoretical and Clinical Medicine*. 2020; 1: 16–21 (in Russ.).
2. Antonova L.V., Kudryavtseva Y.A. Development of a tissue engineered small-diameter vascular graft for cardiovascular surgery. *Complex Issues of Cardiovascular Diseases*. 2016; 5 (3): 6–9 (in Russ.).
3. Majlyan D.E., Kolomic V.V. The role of calcium dysmetabolism in the pathogenesis of cardiovascular diseases. *Russian Journal of Cardiology*. 2019; 9: 78–85 (in Russ.). DOI: 10.15829/1560-4071-2019-9-78-85.
4. Blaine J., Chonchol M., Levi M. Renal control of calcium, phosphate, and magnesium homeostasis. *Clin. J. Am. Soc. Nephrol.* 2015; 10 (7): 1257–1272. DOI: 10.2215/CJN.09750913.
5. Schaub M.C., Heizmann C.W. Calcium in health and disease. In: Kretsinger R.H., Uversky V.N., Permyakov E.A. (eds). *Encyclopedia of metalloproteins*. Springer, New York, 2013: 478–484. DOI: 10.1007/978-1-4614-1533-6.
6. Ahpolova V.O., Brin V.B. Calcium metabolism and its hormonal regulation. *Fundamental Medicine and Biology*. 2017; 2: 38–46 (in Russ.).
7. Yarilin A.A. Immunology. M.: Geotar – Media. 2010: 615–617 (in Russ.).
8. Haitov R.M., Garib F.Yu. Immunology. Atlas. M.: Geotar – Media. 2020: 387–402 (in Russ.).
9. Gudima G.O. Strategies of precision medicine in modern clinical immunology and allergology. *Immunology*. 2019; 40 (4): 83–87 (in Russ.).
10. Figueras-Nart I., Mascaró J.M. Jr., Solanich X. et al. Dermatologic and dermatopathologic features of monogenic autoinflammatory diseases. *Front. Immunol.* 2019; 10: 2448. DOI: 10.3389/fimmu.2019.02448.
11. Nasonov E.L., Popkova T.V. Anti-inflammatory therapy for atherosclerosis: contribution to and lessons of rheumatology. *Rheumatology Science and Practice*. 2017; 55 (5): 465–473 (in Russ.). DOI: 10.14412/1995-4484-2017-465-473.
12. Nasonov E.L., Eliseev M.S. The role of interleukin-1 in the development of human diseases. *Rheumatology Science and Practice*. 2016; 54 (1): 60–77 (in Russ.). DOI: 10.14412/1995-4484-2016-60-77.
13. Carapetis J.R., Beaton A., Cunningham M.W. et al. Acute rheumatic fever and rheumatic heart disease. *Nat. Rev. Dis. Primers*. 2016; 2: 15084. DOI: 10.1038/nrdp.2015.84.
14. Nasonov E.L., Aleksandrova E.N., Novikov A.A. Autoimmune rheumatic diseases: results and prospects for researches. *Rheumatology Science and Practice*. 2015; 53 (3): 230–237 (in Russ.). DOI: 10.14412/1995-4484-2015-230-237.
15. Ponasenko A.V., Kutikhin A.G., Khutornaya M.V., et al. The association of rs13290979 polymorphism within Notch1 gene with severe bioprosthetic mitral valve calcification. *Fundamental and Clinical Medicine*. 2018; 3 (4): 12–21 (in Russ.). DOI: 10.23946/2500-0764-2018-3-4-12-21.
16. Wang J., Zhou J.J., Robertson G.R. et al. Vitamin D in vascular calcification: a double-edged sword? *Nutrients*. 2018; 10 (5): 652. DOI: 10.3390/nu10050652.
17. Centeno P.P., Herberger A., Mun H.C. et al. Phosphate acts directly on the calcium-sensing receptor to stimulate parathyroid hormone secretion. *Nat. Commun.* 2019; 10 (1): 4693. DOI: 10.1038/s41467-019-12399-9.
18. Christakos S., Dhawan P., Verstuyf A. et al. Vitamin D: metabolism, molecular mechanism of action, and pleiotropic effects. *Physiol. Rev.* 2016; 96 (1): 365–408. DOI: 10.1152/physrev.00014.2015.
19. Richter B., Faul C. FGF23 actions on target tissues-with and without Klotho. *Front Endocrinol. (Lausanne)*. 2018; 9: 189. DOI: 10.3389/fendo.2018.00189.
20. Navarro-González J.F., Donate-Correa J., Muros de Fuentes M. et al. Reduced Klotho is associated with the presence and severity of coronary artery disease. *Heart*. 2014; 100 (1): 34–40. DOI: 10.1136/heartjnl-2013-304746.
21. Bukhalko M.A., Skripchenko N.V., Skripchenko E.Yu., et al. Significance of gene polymorphism of vitamin D receptor in human pathology. *Russian Bulletin of Perinatology and Pediatrics*. 2017; 62 (6): 23–28 (in Russ.). DOI: 10.21508/1027-4065-2017-62-6-23-28.
22. Aimagambetova A.O. Atherogenesis and inflammation. *Science and Healthcare*. 2016; 1: 24–39 (in Russ.). DOI: 0000-0003-1496-3281.
23. Kim J.H., Shin Y.L., Yang S. et al. Diverse genetic aetiologies and clinical outcomes of paediatric hypo-

- parathyroidism. *Clin. Endocrinol. (Oxf.)*. 2015; 83 (6): 790–796. DOI: 10.1111/cen.12944.
24. Kolobynina K.G., Solov'eva V.V., Slepak V.Z., et al. The role of the EF-hand Ca^{2+} / Mg^{2+} -binding protein of tescalcin in cell proliferation and differentiation. *Genes and Cells*. 2015; 10 (1): 28–34 (in Russ.).
 25. Guhathakurta P., Prochniewicz E., Thomas D.D. Actin-myosin interaction: structure, function and drug discovery. *Int. J. Mol. Sci.* 2018; 19 (9): 2628. DOI: 10.3390/ijms19092628.
 26. Valencia-Núñez D.M., Kreutler W., Moya-Gonzalez J. et al. Endothelial vascular markers in coronary surgery. *Heart Vessels*. 2017; 32 (11): 1390–1399. DOI: 10.1007/s00380-017-1006-3.
 27. Chiang J.M., Tan R., Wang J.Y. et al. S100P, a calcium-binding protein, is preferentially associated with the growth of polypoid tumors in colorectal cancer. *Int. J. Mol. Med.* 2015; 35 (3): 675–683. DOI: 10.3892/ijmm.2015.2065.
 28. Bahar E., Kim H., Yoon H. ER stress-mediated signaling: action potential and Ca^{2+} as key players. *Int. J. Mol. Sci.* 2016; 17 (9): 1558. DOI: 10.3390/ijms17091558.
 29. Veronese N., Stubbs B., Crepaldi G. et al. relationship between low bone mineral density and fractures with incident cardiovascular disease: a systematic review and meta-analysis. *J. Bone Miner. Res.* 2017; 32 (5): 1126–1135. DOI: 10.1002/jbmr.3089.
 30. Orlova D.D., Tribulovich V.G., Garabadzhiu A.V., Barlev N.A., Martin S. The role of mitochondrial dynamics in cell death. *Cytology*. 2015; 57 (3): 184–191 (in Russ.).
 31. Zakharov E.A., Klyueva N.Z., Belostotskaya G.B. The mechanisms of Ca^{2+} -dependent hypertension formation on the model of rat cardiomyocytes in the culture. *Arterial Hypertension*. 2009; 15 (6): 683–687 (in Russ.). DOI: 10.18705/1607-419X-2009-15-6-683-687.
 32. Deev R.V., Bilyalov A.I., Zhampeisov T.M. Modern ideas about cell death. *Genes and Cells*. 2018; 8 (1): 6–19 (in Russ.). DOI: 10.23868/201805001.
 33. Shi J., Gao W., Shao F. Pyroptosis: gasdermin-mediated programmed necrotic cell death. *Trends Biochem. Sci.* 2017; 42 (4): 245–254. DOI: 10.1016/j.tibs.2016.10.004.
 34. Guo H., Callaway J.B., Ting J.P. Inflammasomes: mechanism of action, role in disease, and therapeutics. *Nat. Med.* 2015; 21 (7): 677–687. DOI: 10.1038/nm.3893.
 35. Feng S., Fox D., Man S.M. Mechanisms of gasdermin family members in inflammasome signaling and cell death. *J. Mol. Biol.* 2018; 430 (18 Pt. B): 3068–3080. DOI: 10.1016/j.jmb.2018.07.002.
 36. Pirozhkov S.V., Litvitskiy P.F. Inflammasomal diseases. *Immunology*. 2018; 39 (2-3): 158–165 (in Russ.). DOI: 10.18821/0206-4952-2018-39-2-3-158-165.
 37. Lee G.S., Subramanian N., Kim A.I. et al. The calcium-sensing receptor regulates the NLRP3 inflammasome through Ca^{2+} and cAMP. *Nature*. 2012; 492 (7427): 123–127. DOI: 10.1038/nature11588.
 38. Duewell P., Kono H., Rayner K.J. et al. NLRP3 inflammasomes are required for atherogenesis and activated by cholesterol crystals. *Nature*. 2010; 464 (7293): 1357–1361. DOI: 10.1038/nature08938.
 39. Victor V.M., Apostolova N., Herance R. et al. Oxidative stress and mitochondrial dysfunction in atherosclerosis: mitochondria-targeted antioxidants as potential therapy. *Curr. Med. Chem.* 2009; 16 (35): 4654–4667. DOI: 10.2174/092986709789878265.
 40. Strowig T., Henao-Mejia J., Elinav E. et al. Inflammasomes in health and disease. *Nature*. 2012; 481 (7381): 278–286. DOI: 10.1038/nature10759.
 41. Ridker P.M. From C-reactive protein to interleukin-6 to interleukin-1: Moving upstream to identify novel targets for atheroprotection. *Circ. Res.* 2016; 118 (1): 145–156. DOI: 10.1161/CIRCRESAHA.115.306656.
 42. Rattazzi M., Faggini E., Bertacco E. et al. RANKL expression is increased in circulating mononuclear cells of patients with calcific aortic stenosis. *J. Cardiovasc. Transl. Res.* 2018; 11 (4): 329–338. DOI: 10.1007/s12265-018-9804-2.
 43. Mogelvang R., Pedersen S.H., Flyvbjerg A. et al. Comparison of osteoprotegerin to traditional atherosclerotic risk factors and high-sensitivity C-reactive protein for diagnosis of atherosclerosis. *Am. J. Cardiol.* 2012; 109 (4): 515–520. DOI: 10.1016/j.amjcard.2011.09.043.
 44. Sidorovich O.I., Luss L.V. Food allergy: principles of diagnosis and treatment. *Medical Council*. 2016; (16): 141–147 (in Russ.). DOI: 10.21518/2079-701X-2016-16-141-147.
 45. Delves P.J., Martin S.J., Burton D.R. et al. Roitt's essential immunology. Wiley-Blackwell. 13th ed. 2017: 405–430.
 46. Land W.G. The role of damage-associated molecular patterns in human disease. Part 1 – Promoting inflammation and immunity. *Sultan Quaboos Univer. Med. J.* 2015; 15 (1): e9–21.
 47. Potapnev M.P. Immune mechanisms of sterile inflammation. *Immunology*. 2015; 36 (5): 312–317 (in Russ.).
 48. Barbarash O.L., Golovkin A.S., Ponasenko A.V., et al. The role of Toll-like receptor polymorphism in atherosclerosis complications development. *Russian Journal of Cardiology*. 2015; 20 (12): 72–79 (in Russ.). DOI: 10.15829/1560-4071-2015-12-72-79.
 49. Blagonravov M.L., Medvedeva E.V., Bryk A.A., et al. Features of electrolyte excretion in the early stages of hypertension in SHR rats. *Bulletin of Experimental Biology and Medicine*. 2017; 164 (7): 21–24 (in Russ.).
 50. Carey R.M. Blood pressure and the renal actions of AT2 receptors. *Curr. Hypertens. Rep.* 2017; 19 (3): 21. DOI: 10.1007/s11906-017-0720-7.
 51. Liu Y., Wei J., Wong King Yuen S.M. et al. CPVT-associated cardiac ryanodine receptor mutation G357S with reduced penetrance impairs Ca^{2+} release termination and diminishes protein expression. *PLoS One*. 2017; 12 (9): e0184177. DOI: 10.1371/journal.pone.0184177.

Authors information

Deeva Nadezhda S., Junior Researcher, Laboratory of Cellular Technologies, Research Institute for Complex Issues of Cardiovascular Diseases, Kemerovo, Russian. ORCID 0000-0002-6162-4808.

Shabaldin Andrey V., Dr. Sci. (Med.), Leading Researcher, Laboratory of Congenital Heart Diseases, Research Institute for Complex Issues of Cardiovascular Diseases, Kemerovo, Russian Federation. ORCID 0000-0002-8785-7896.

Antonova Larisa V., Dr. Sci. (Med.), Head of the Department of Cellular Technologies, Research Institute for Complex Issues of Cardiovascular Diseases, Kemerovo, Russian Federation. ORCID 0000-0002-8874-0788.

(✉) **Deeva Nadezhda S.**, e-mail: deevanadusha69@yandex.ru

Received 16.07.2020

Accepted 28.12.2020

The role of neutrophils in the pathogenesis of ischemic stroke

Dolgushin I.I.¹, Zaripova Z.Z.², Karpova M.I.¹

¹ South Ural State Medical University (SUSMU)
64, Vorovskogo St., Chelyabinsk, 454092, Russian Federation

² Chelyabinsk Regional Clinical Hospital
70, Vorovskogo St., Chelyabinsk 454048, Russian Federation

ABSTRACT

Background. Immune responses and inflammation play an important role in the pathogenesis of ischemic stroke.

Aim. To analyze the involvement of neutrophils in the pathogenesis of ischemic stroke.

Results. Data on the contribution of neutrophil granulocytes to the development of local sterile inflammation and secondary brain injury in acute ischemic stroke were summarized. Mechanisms of neutrophil influence on thrombosis, neutrophil extracellular trap formation (NETosis), protease release, and direct interaction with platelets with subsequent formation of platelet-leukocyte aggregates were discussed. Available information on the effectiveness of reperfusion therapy and an association of changes in neutrophil activity with development of infectious complications of stroke were presented. In addition, research data were presented on the contribution of neutrophils to atherogenesis, which is one of the most important etiological factors in ischemic stroke. The review showed that the contribution of neutrophils to the pathogenesis of ischemic stroke is associated with implementation of their secretory, regulatory, and phagocytic functions, as well as with NETosis.

Conclusion. It was shown that neutrophils are involved in the pathogenesis of ischemic stroke and modulate a response to treatment.

Key words: neutrophils, neutrophil extracellular traps, NETosis, ischemic stroke, post-stroke infections, thrombolysis.

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

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

For citation: Dolgushin I.I., Zaripova Z.Z., Karpova M.I. The role of neutrophils in the pathogenesis of ischemic stroke. *Bulletin of Siberian Medicine*. 2021; 20 (3): 152–160. <https://doi.org/10.20538/1682-0363-2021-2-152-160>.

Роль нейтрофилов в патогенезе ишемического инсульта

Долгушин И.И.¹, Зарипова З.З.², Карпова М.И.¹

¹ Южно-Уральский государственный медицинский университет (ЮУГМУ)
Россия, 454092, г. Челябинск, ул. Воровского, 64

² Челябинская областная клиническая больница (ЧОКБ)
Россия, 454048, г. Челябинск, ул. Воровского, 70

✉ Karpova Maria I., e-mail: kmi_2008@mail.ru

РЕЗЮМЕ

Актуальность. Имунные реакции и воспалительный процесс играют важную роль в патогенезе ишемического инсульта.

Цель. На основании научных публикаций проанализировать вовлеченность нейтрофилов в патогенез ишемического инсульта.

Результаты. Обобщены данные о вкладе нейтрофильных гранулоцитов в развитие локального асептического воспаления и вторичного повреждения мозга при остром инсульте. Обсуждены механизмы влияния нейтрофилов на процесс тромбообразования, образование нейтрофильных внеклеточных ловушек (нетоз), высвобождение протеаз и прямое взаимодействие с тромбоцитами с образованием лейкоцитарно-тромбоцитарных агрегатов. Приведены имеющиеся сведения об эффективности реперфузионной терапии, а также ассоциации изменений активности нейтрофилов с развитием инфекционных осложнений инсульта. Представлены данные исследований о вкладе нейтрофилов в атерогенез, являющийся одним из важнейших этиологических факторов ишемического инсульта. Показано, что участие нейтрофилов в патогенезе ишемического инсульта связано с реализацией их секреторных, регуляторных, фагоцитарных функций и с нетозом.

Заключение. Установлено, что нейтрофилы принимают активное участие в патогенезе ишемического инсульта и модулируют ответ на лечение.

Ключевые слова: нейтрофилы, нейтрофильные внеклеточные ловушки, нетоз, ишемический инсульт, инфекционные осложнения инсульта, тромболитическая терапия.

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

Источник финансирования. Авторы заявляют об отсутствии финансирования.

Для цитирования: Долгушин И.И., Зарипова З.З., Карпова М.И. Роль нейтрофилов в патогенезе ишемического инсульта. *Бюллетень сибирской медицины*. 2021; 20 (3): 152–160. <https://doi.org/10.20538/1682-0363-2021-3-152-160>.

INTRODUCTION

Stroke is one of the leading causes of mortality and persistent disability among the population both in the Russian Federation and in the world. About 450,000 stroke cases per year are registered in the Russian Federation [1]. The average rate of death during the first 30 days after stroke, according to different authors, ranges from 17 to 34%. Only half of patients who have experienced the first stroke live for 3 years or more, and 35% of the patients live 10 years or more [2]. In addition to mortality directly from stroke, a large number of deaths are caused by its infectious and inflammatory complications, primarily pneumonia [3, 4]. In more than 80% of patients, stroke leads to disability with persistent neurological deficiency [5].

Immune responses and inflammation initiated by ischemia are important pathogenetic phenomena causing destruction of the brain tissue [6]. It is assumed that features of the inflammatory response closely associated with depletion of antio-

xidant mechanisms and impaired microcirculation and blood – brain barrier (BBB) largely determine a course of the disease at all its stages [7]. At the same time, a dual role of immune system activation in acute stroke is noted.

On the one hand, the immune response is sanogenetic and aimed at removing necrotic tissue. On the other hand, it is able to aggravate the course of ischemic stroke, increasing the area of infarction [8]. During neuronal death, under the influence of gene expression, synthesis of proinflammatory cytokines (interleukin (IL)-1 α and IL-1 β , tumor necrosis factor α (TNF α)), cell – cell interaction molecules, and cyclooxygenase-2 is activated [9, 10]. The cascade of reactions initiated by ischemia inevitably involves factors of innate immunity, including the largest population of leukocytes – neutrophils.

From the moment I.I. Mechnikov discovered phagocytosis of microorganisms by neutrophil granulocytes in the 19th century, views on their role in immunological processes have changed significantly [11–14]. To date, basic research has convincingly

proved that the role of neutrophils is not limited to elimination of extracellular pathogens [13, 14]. They also perform a regulatory function by activating and modulating adaptive immunity, contributing to its full implementation [15]. Neutrophils, in the light of modern perceptions, are a pluripotent population of cells of the immune system related to innate immunity and playing an important role in maintaining immune homeostasis in the body [16].

Neutrophil population is heterogeneous and includes different subpopulations of cells [17]. Depending on mediators, receptors, and markers produced, they are divided into proinflammatory, inflammatory with antimicrobial potential, inflammatory with negative cytotoxic potential ("aggressive"), anti-inflammatory – regulating inflammation regression, anti-tumor, and hybrid, combining the properties of neutrophils and dendritic cells [13, 14]. A study of neutrophil subpopulations in inflammatory diseases revealed their predictive significance as markers of the severe course of acute infection and its adverse outcome [11].

In recent years, a large amount of new information has appeared that neutrophils are involved in the pathogenetic mechanisms of many noncommunicable diseases, including vascular ones [4, 13]. In this regard, it is of great interest to study the role of neutrophils in the development of ischemic stroke, which is one of the least studied aspects in the immunology of cerebrovascular disorders.

The aim of this review was to analyze currently available data on the role of neutrophils in the pathogenesis of ischemic stroke.

THE ROLE OF NEUTROPHILS IN STERILE INFLAMMATION IN CEREBRAL ISCHEMIA

Neutrophils are some of the most fast-reacting cells in the immune system. They migrate to the brain within minutes from the onset of stroke. The mechanism of intrathecal neutrophil entry in ischemic injury was studied in experimental models with lifetime imaging. Neutrophils migrate along the vessels even against the blood flow in order to exit the vascular bed and enter the infarct zone [18]. Neutrophils are capable of expressing various endothelial adhesion molecules during the first 15 minutes after ischemia [19].

Afterwards, 6–8 hours after stroke, neutrophils surround cerebral vessels and initiate infiltration of

surrounding tissue [20, 21]. In experimental models of ischemic stroke, it was shown that infiltration with neutrophils occurs already on the first day, reaching a peak on day 3, and then begins to decrease, but is present both 7 and 15 days after the development of cerebral ischemia.

M. Gelderblom et al. (2012) demonstrated that neutrophils are predominant immune cells in the ischemized hemisphere 3 days after middle cerebral artery occlusion in the experiment [22]. R.M. Weston et al. (2007) reported that neutrophil infiltration of ischemized tissues persists for up to 32 days, but the presence of neutrophils is disguised by the concentration of activated macrophages / microglia [19].

Penetrating into the ischemic area, neutrophils cause secondary damage to the brain tissue due to the release of proinflammatory factors, reactive oxygen species, proteases, and matrix peroxidases [23]. These factors damage the endothelial cell membrane and basal plate, leading to increased BBB permeability and post-ischemic brain edema [24].

NEUTROPHILS AND THROMBOSIS

The ability of neutrophils to participate in thrombosis through various mechanisms, such as formation of neutrophil extracellular traps (NETosis), release of proteases, and direct interaction with platelets to form leukocyte – platelet aggregates, deserves particular attention. An increase in the number of such aggregates was detected in the blood of patients with symptomatic carotid artery stenosis [25]. A decrease in the intensity of platelet – neutrophil aggregate formation occurs with inhibition of receptors to glycoprotein Iib / IIIa and selectins. The formation of leukocyte – platelet aggregates in acute ischemic stroke and reperfusion therapy can become a useful biomarker and target for treatment, since these aggregates increase intravascular thrombosis [26].

Due to great mobility, neutrophils, ahead of platelets, bind to the activated endothelium of damaged vessels using ICAM-1 receptors. This leads to activation and accumulation of platelets in the damaged vessel and an increase in thrombosis. There are a number of studies aimed at exploring possible ways to prevent this process [27, 28].

Proteases and cathepsin G, released by neutrophils, interact with coagulation factors, accelerating thrombus formation. Inhibition of cathepsin G in an experimental mouse model demonstrated improvement in the outcome of ischemic stroke. On the other

hand, protease ADAMTS13 released by neutrophils cleaves highly active von Willebrand factor and thereby reduces the activity of inflammation in the acute phase of ischemic stroke [29].

The prothrombotic activity of neutrophils is also explained by their ability to release molecules involved in the formation of a neutrophil extracellular trap (NET), release proteases, and directly interact with platelets. These processes can lead to increased brain ischemia in stroke [30].

NETs are formed in response to various stimuli, the key mechanism for their formation is activation of peptidylarginine deiminase 4 (PAD4). This enzyme is necessary for histone citrullination, which leads to chromatin decondensation, as a result of which separate strands of DNA are formed. Apart from DNA strands, the structure of NETs includes histones and specific protein granules, such as elastase and myeloperoxidase of neutrophils. This makes it possible to create a locally high concentration of cytotoxic substances in the DNA area. Initially, formation of NETs was known to be associated with their bactericidal effect [31], but lately, they have been increasingly referred to as the key participants in thrombosis [32].

Platelets can bind to released NETs and be activated due to their association with histones [33, 34]. Interaction with neutrophils occurs through the association of platelet P-selectin with neutrophil P-selectin glycoprotein ligand [35]. When activated, platelets are able to release HMGB-1 protein and present it on their surface, increasing neutrophil release of new DNA networks [36]. Histones associated with NETs also increase thrombin generation by platelets [35] and provoke the release of von Willebrand factor from Weibel – Palade bodies in endothelial cells, leading to leukocyte – platelet adhesion [37]. This process potentiates itself by activating the extrinsic coagulation pathway and leads to further thrombosis and inflammatory response in stroke [38].

The prothrombotic activity of NETs is also determined by their polyanionic surface, which activates the coagulation factor XII, participating in the activation of the intrinsic coagulation pathway [39]. The strategy of controlling the deoxyribonuclease activity in experimental models of stroke in mice resulted in dissolution of NETs and a protective effect *in vivo* [40].

Based on the accumulated data, it is assumed that the prothrombotic activity of neutrophils can both

increase the risk of ischemic stroke and contribute to further thrombosis in the acute phase of cerebrovascular disorder.

NEUTROPHILS AND SYSTEMIC THROMBOLYTIC THERAPY FOR STROKE

Currently, intravenous systemic thrombolysis using the recombinant tissue plasminogen activator (p-TAP) is a recognized and recommended method for treating patients with ischemic stroke, but its use is associated with an increased risk of intracranial hemorrhage [41], an inflammatory response in the brain tissues [42], and infiltration of the brain tissues with neutrophils [43]. These factors influence clinical effectiveness of thrombolytic therapy in ischemic stroke [44].

A number of studies investigated the role of neutrophils in the therapy using p-TAP [45]. It was shown that p-TAP therapy can affect the dynamics of changes in the neutrophil count in the brain tissues in stroke [44, 46]. There is evidence that an increase in the neutrophil count in peripheral blood in the first 24 hours after thrombolytic therapy is associated with an unfavorable prognosis [46]. This pattern may be explained by the formation of NETs. Thus, in acute myocardial infarction, an increase in the number of NETs in thrombolytic therapy was associated with a worse functional outcome and identified NETs as a promising therapeutic target for improving the effectiveness of p-TAP therapy [47].

Some studies note that when p-TAP and recombinant deoxyribonuclease I (DNase I) are used together to inhibit NETs, the outcome of thrombolytic therapy is improved; the duration and number of attempts during further endovascular reperfusion therapy are reduced compared with the use of p-TAP alone [46]. Similar conclusions were made in a number of studies which examined the possibility of potentiating p-TAP with DNase I in thrombolytic therapy of acute myocardial infarction and ischemic stroke [48, 49]. In addition, the ability of extracellular DNA and histones to modify fibrin structure, making it more resistant to mechanical and enzymatic effects, can be a possible reason for correlating the increase in the number of NETs with worse results of thrombolytic therapy [50].

Therefore, the study of neutrophil activity in thrombosis is of interest for further optimization of reperfusion therapy for acute ischemic stroke and development of new methods for treatment and prognosis in this disease.

NEUTROPHILS AND INFLAMMATORY COMPLICATIONS OF STROKE

Infectious inflammatory diseases are some of the most common and dangerous complications of stroke, which significantly affect the survival and recovery of patients [51]. In order to ensure optimal homeostasis in the body, nervous and immune systems are closely connected by multiple pathways, and serious damage to the brain tissue in stroke leads to multiple changes in these neuroimmune interactions [52].

Ischemized brain tissue affects peripheral immune cells, suppressing their activity, which is further manifested through lymphopenia, a decrease in proinflammatory cytokine production, and reduced monocyte activity [53]. This mechanism is probably adaptive and aimed at reducing the intensity of the inflammatory response in the ischemic area and lowering a risk of autoimmune diseases in response to neuroantigens under conditions of increased BBB permeability [54]. On the other hand, this leads to development of secondary immunodeficiency and can contribute to development of infectious complications [55]. The size of the ischemic area is an independent risk factor for immunosuppression and inflammatory complications after stroke [56].

In addition, development of secondary immunodeficiency in acute stroke is mediated by changes in the autonomic nervous system activity and hypothalamic – pituitary – adrenal (HPA) axis [57]. Mediators in this process are noradrenaline, acetylcholine, and glucocorticoids receptors to which are widely presented on cells of the immune system [58]. It is known that increased production of glucocorticoids upon activation of the HPA axis induces apoptosis in immune cells [59].

It should be noted that clinical studies on the role of neutrophils in the development of infectious complications of stroke are few. In one of them, conducted at the Hannover Medical School (Germany, 2015–2017), an attempt was made to determine an association between decreased neutrophil granulocyte function and the emergence of inflammatory complications in the acute phase of stroke.

The examination of 95 patients, no significant changes in the phagocytic activity of neutrophils were detected. However, peripheral blood neutrophils in the group of patients with early (first 7 days) infectious complications decreased their abil-

ity to release reactive oxygen species in response to N-formyl-methionyl-leucyl-phenylalanine (FMLP) stimulation, while maintaining the ability to release reactive oxygen species when stimulated by more active inducers, for example, *E. coli*.

In a clinical trial with anti-ICAM-1 monoclonal antibodies (Enlimomab) inhibiting neutrophil function and reducing the cerebral infarct volume in an experimental model, an increased incidence of infections, especially pneumonia, was observed. These inflammatory processes were associated with worsening of a stroke outcome, indicating a negative effect of neutrophil dysfunction in the development of infectious complications [61].

NEUTROPHILS AND ATHEROGENESIS

Apart from the direct impact on all stages of the ischemic cascade and the risk of developing infectious complications, neutrophils are actively involved in the processes that lead to stroke. Recently, more attention has been paid to the study of the role of neutrophils in the processes of atherogenesis [62, 63].

There is evidence that neutrophils synthesize and release cholesterol-binding peptides and proteins under conditions of close cell – cell interaction and / or stimulation with proinflammatory cytokines [64]. The ability of venous blood neutrophils to release protein – lipid complexes when cultured under conditions of close cell – cell interaction was called the lipid-releasing capacity of leukocytes (LRCL) [63, 64]. The following studies proved the clinical role of an increase in lipid-releasing capacity of leukocytes and its association with the course of coronary artery disease (CAD) [65].

The role of neutrophils in the development of atherosclerotic lesions is implemented in several directions. On the one hand, they are involved in early stages of atherogenesis, namely, in the development of endothelial dysfunction. On the other hand, neutrophils affect the emergence of complications in atherosclerosis: destabilization of the atherosclerotic plaque and formation of blood clots [66, 67].

When the vascular endothelium is damaged, fragments of the extracellular matrix, such as hyaluronan, fibronectin, etc., are released into the intercellular space and act as endogenous danger-associated molecular patterns (DAMPs). They are recognized by neutrophil pattern recognition receptors (TLR2/4) in the bloodstream and contribute to their attraction to

the focus of inflammation. In addition, chemotaxis and migration of neutrophils and other white blood cells are enhanced by production of proinflammatory cytokines, such as IL-1, IL-18, IL-8, chemokines, and cell adhesion molecules, by endothelial cells [68–70].

In the focus of inflammation, neutrophils synthesize cholesterol-binding proteins to form protein – lipid complexes accumulating in the vessel wall. In patients with CAD, neutrophil production of the following molecules was increased: C-reactive protein, von Willebrand factor, lipoprotein, and a brain natriuretic peptide precursor. Moreover, neutrophils in the endothelial lesion cause peroxidation of membrane lipids, DNA strand breaks, and endothelial cell damage [71].

The role of neutrophils in the atherogenesis may be also related to their ability to form NETs: extracellular network-like structures and released enzymes lead to endothelial damage [66, 71].

CONCLUSION

Therefore, neutrophils, being a pluripotent heterogeneous cell population, take an active part in the pathogenesis of ischemic stroke and are also able to modulate the response to treatment. Their further study may open up additional prospects for personalization of therapy and prediction of disease outcomes.

REFERENCES

1. Vilenskiy B.S. Stroke – current state of affairs. *Journal of Neurology*. 2008; 13 (2): 1–11 (in Russ.).
2. Wolf P.A., D'Agostino R.B., O'Neal M.A., Sytkowski P., Kase C.S., Belanger A., Kannel W.B. Secular trends in stroke incidence and mortality the Framingham Study. *Stroke*. 1992; 23 (11): 1551–1555. DOI: 10.1161/01.str.23.11.1551.
3. Katzan I.L., Cebul R.D., Husak S.H., Dawson N.V., Baker D.W. The effect of pneumonia on mortality among patients hospitalized for acute stroke. *Neurology*. 2003; 60 (4): 620–625. DOI: 10.1212/01.wnl.0000046586.38284.60.
4. Heuschmann P.U., Kolominsky-Rabas P.L., Misselwitz B., Hermanek P., Leffmann C., Janzen R.W., Rother J., Buecker-Nott H.J., Berger K. Predictors of in-hospital mortality and attributable risks of death after ischemic stroke. *Arch. Intern. Med.* 2004; 164 (16): 1761–1768. DOI: 10.1001/archinte.164.16.1761.
5. Stakhovskaya L.V., Klyuchikhina O.A., Bogatyreva M.D., Kovalenko V.V. Epidemiology of stroke in the Russian Federation: results of territorial population registry (2009–2019). *Korsakov's Journal of Neurology and Psychiatry*. 2013; 113 (5): 4–10 (in Russ.).
6. Gusev E.I., Skvortsova V.I., Stakhovskaya L.V. Stroke in the Russian Federation: time for combined efforts. *Korsakov's Journal of Neurology and Psychiatry*. 2007; 107 (8): 1–11 (in Russ.).
7. Skvortsova V.I., Stakhovskaya L.V., Sherstnev V.V., Gruden M.A., Myasoedov N.F., Efremova N.M. et al. The role of autoimmune diseases in the damaging effects of cerebral ischemia. *Korsakov's Journal of Neurology and Psychiatry, Stroke. Appendix to the Journal*. 2001; 101 (1): 46–54 (in Russ.).
8. Chamorro A., Hallenbeck J. The harms and benefits of inflammatory and immune responses in vascular disease. *Stroke*. 2006; 37 (1): 291–293. DOI: 10.1161/01.STR.0000200561.69611.f8.
9. Gusev E.I., Skvortsova V.I. Cerebral ischemia. M.: Medicine, 2001: 328 (in Russ.).
10. Kryzhanovskij G.N., Magaeva S.V., Makarov S.V., Sepiashvili R.I. Neuroimmunopathology. Guidelines. M., 2003: 282 (in Russ.).
11. Paltsev M.A., Kvetnoj M.I. Neuroimmunoendocrinology guidelines. M.: Medicine, 2008: 497–499 (in Russ.).
12. Dolgushn I.I., Savochkina A. Yu., Kurnosehko I.V., Dolgushina V.F., Savel'eva A.A., Samuseva I.V., Majakova V.B. The role of extracellular DNA traps in protective and pathological reactions of the body. *Russian Journal of Immunology*. 2015; 9 (18) 2: 164–170 (in Russ.).
13. Dolgushn I.I., Mesentseva E.A., Savochkina A. Yu., Kuznetsova E.K. Neutrophil as a multifunctional relay in the immune system. *Infection and Immunity*. 2019; 9 (1): 9–38 (in Russ.). DOI: 10.15789/2220-7619-2019-1-9-38.
14. Nesterova I.V., Kolesnikova N.V., Chudilova G.A. et al. The new look at neutrophilic granulocytes: rethinking old dogmas. Part 1. *Russian Journal of Infection and Immunity*. 2017; 7 (3): 219–230. DOI: 10.15789/2220-7619-2017-3-219-230.
15. Nesterova I.V., Kolesnikova N.V., Chudilova G.A. et al. The new look at neutrophilic granulocytes: rethinking old dogmas. Part 2. *Russian Journal of Infection and Immunity*. 2018; 8 (1): 7–18. DOI: 10.15789/2220-7619-2018-1-7-18.
16. Nesterova I.V., Shvydchenko I.N., Romenskaya V.A., Fomicheva E.V., Bykovskaya E.Yu. Neutrophil granulocytes – the key cells of the immune system. *Allergy and Immunology*. 2008; 9 (4): 432–435 (in Russ.).
17. Berezhnaja N.M. Neutrophils and immunological homeostasis. Kiev: Naukova Dumka, 1988: 205 (in Russ.).
18. Neumann J., Riek-Burchardt M., Herz J. et al. Very-late antigen-4 (VLA-4)-mediated brain invasion by neutrophils leads to interactions with microglia, increased ischemic injury and impaired behavior in experimental stroke. *Acta Neuropathologica*. 2015; 129 (2): 259–277. DOI: 10.1007/s00401-014-1355-2.
19. Weston R.M., Jones N.M., Jarrott B., Callaway J.K. Inflammatory cell infiltration after endothelin-1-induced cerebral ischemia: histochemical and myeloperoxidase correlation with temporal changes in brain injury. *J. Cerebral. Blood Flow and Metab.* 2007; 27 (1): 100–114. DOI: 10.1038/sj.jcbfm.9600324.
20. Watcharotayangul J., Mao L., Xu H., Vetri F., Baughman V.L., Paisansathan C. et al. Post-ischemic vascular adhesion protein-1 inhibition provides neuroprotection in a rat temporary middle cerebral artery occlusion model. *J. Neurochem.* 2012; 123 (Suppl. 2): 116–124. DOI: 10.1111/j.1471-4159.2012.07950.x.
21. Perez-de-Puig I., Miry-Mur F., Ferrer-Ferrer M., Gelpi E., Pedragosa J., Justicia C. et al. Neutrophil recruitment to the brain

- in mouse and human ischemic stroke. *Acta Neuropathol.* 2015; 129 (2): 239–257. DOI: 10.1007/s00401-014-1381-0.
22. Gelderblom M., Leypoldt F., Steinbach K., Behrens D., Choe C.-U., Siler D.A. et al. Temporal and spatial dynamics of cerebral immune cell accumulation in stroke. *Stroke.* 2009; 40 (5): 1849–1857. DOI: 10.1161/STROKEAHA.108.534503.
 23. Martynov M.Y., Gusev E.I. Current knowledge on the neuroprotective and neuroregenerative properties of citicoline in acute ischemic stroke. *J. Exp. Pharmacol.* 2015; 7: 17–28. DOI: 10.2147/JEP.S63544.
 24. Jickling G.C., Liu D., Ander B.P., Stamova B., Zhan X., Sharp F.R. Targeting neutrophils in ischemic stroke: translational insights from experimental studies. *J. Cereb. Blood Flow and Metab.* 2015; 35 (6): 888–901. DOI: 10.1038/jcb-fm.2015.45.
 25. McCabe D.J.H., Harrison P., Mackie I.J., Sidhu P.S., Purdy G., Lawrie A.S. et al. Increased platelet count and leucocyte-platelet complex formation in acute symptomatic compared with asymptomatic severe carotid stenosis. *Journal of Neurology, Neurosurgery and Psychiatry.* 2005; 76 (9): 1249–1254. DOI: 10.1136/jnnp.2004.051003.
 26. Ritter L.S., Stempel K.M., Coull B.M., McDonagh P.F. Leukocyte-platelet aggregates in rat peripheral blood after ischemic stroke and reperfusion. *Biological Research for Nursing.* 2005; 6 (4): 281–288. DOI: 10.1177/1099800405274579.
 27. Kim J.Y., Park J., Chang J.Y., Kim S.H., Lee J.E. Inflammation after ischemic stroke: the role of leukocytes and glial cells. *Exp. Neurobiol.* 2016; 25 (5): 241–251. DOI: 10.5607/en.2016.25.5.241.
 28. Hallevi H., Hazan-Halevy I., Paran E. Modification of neutrophil adhesion to human endothelial cell line in acute ischemic stroke by dipyridamole and candesartan. *European Journal of Neurology.* 2007; 14 (9): 1002–1007. DOI: 10.1111/J.1468-1331.2007.01847.X.
 29. Khan M.M., Motto D.G., Lentz S.R., Chauhan A.K. ADAMTS13 reduces VWF-mediated acute inflammation following focal cerebral ischemia in mice. *Journal of Thrombosis and Haemostasis.* 2012; 10 (8): 1665–1671. DOI: 10.1111/j.1538-7836.2012.04822.x.
 30. Brinkmann V., Zychlinsky A. Beneficial suicide: why neutrophils die to make NETs. *Nature Reviews Microbiology.* 2007; 5 (8): 577–582. DOI: 10.1038/NRMICRO1710.
 31. Martinod K., Wagner D.D. Thrombosis: tangled up in NETs. *Blood.* 2014; 123 (18): 2768–2776. DOI: 10.1182/blood-2013-10-463646.
 32. Fuchs T.A., Brill A., Duerschmied D. Extracellular DNA traps promote thrombosis. *Proceedings of the National Academy of Sciences of the United States of America.* 2010; 107 (36): 1580–1588. DOI: 10.1073/pnas.1005743107.
 33. Semeraro F., Ammollo C.T., Morrissey J.H., Dale G.L., Friese P., Esmon N.L., Esmon C.T. Extracellular histones promote thrombin generation through platelet-dependent mechanisms: involvement of platelet TLR2 and TLR4. *Blood.* 2011; 118 (7): 1952–1961. DOI: 10.1182/blood-2011-03-343061.
 34. Sreeramkumar V., Adrover J.M., Ballesteros I. et al. Neutrophils scan for activated platelets to initiate inflammation. *Science.* 2014; 346 (6214): 1234–1238. DOI: 10.1126/science.1256478.
 35. Maugeri N., Campana L., Gavina M. et al. Activated platelets present high mobility group box 1 to neutrophils, inducing autophagy and promoting the extrusion of neutrophil extracellular traps, *Journal of Thrombosis and Haemostasis.* 2014; 12 (12): 2074–2088. DOI: 10.1111/jth.12710.
 36. Brill A., Fuchs T.A., Savchenko A.S., Thomas G.M., Martinod K., De Meyer S.F., Bhandari A.A., Wagner D.D. Neutrophil extracellular traps promote deep vein thrombosis in mice. *J. Thromb. Haemost.* 2012; 10 (1): 136–144. DOI: 10.1111/j.1538-7836.2011.04544.x.
 37. De Meyer S.F., Denorme F., Langhauser F., Geuss E., Fluri F., Kleinschnitz C. Thromboinflammation in stroke brain damage. *Stroke.* 2016; 47 (4): 1165–1172. DOI: 10.1161/STROKEAHA.115.011238.
 38. Von Bruhl M.L., Stark K., Steinhart A., Chandraratne S., Konrad I., Lorenz M., Khandoga A., Tirniceru A., Coletti R., Kollnberger M., Byrne R.A., Laitinen I., Walch A., Brill A., Pfeiler S., Manukyan D., Braun S., Lange P., Riegger J., Ware J. et al. Monocytes, neutrophils, and platelets cooperate to initiate and propagate venous thrombosis in mice *in vivo*. *The Journal of Experimental Medicine.* 2012; 209 (4): 819–835. DOI: 10.1084/jem.20112322fio.
 39. De Meyer S.F., Suidan G.L., Fuchs T.A., Monestier M., Wagner D.D. Extracellular chromatin is an important mediator of ischemic stroke in mice. *Arteriosclerosis, Thrombosis, and Vascular Biology.* 2012; 32 (8): 1884–1891. DOI: 10.1161/ATVBAHA.112.250993.
 40. Yaghi S., Boehme A.K., Dibu J. et al. Treatment and Outcome of Thrombolysis-Related Hemorrhage: A Multicenter Retrospective Study. *JAMA Neurol.* 2015; 72 (12): 1451–1457. DOI: 10.1001/jamaneurol.2015.2371.
 41. Gill D., Sivakumaran P., Wilding P., Love M., Veltkamp R., Kar A. Trends in C-reactive protein levels are associated with neurological change twenty-four hours after thrombolysis for acute ischemic stroke. *J. Stroke Cerebrovasc. Dis.* 2016; 25 (8): 1966–1999. DOI: 10.1016/J.JSTROKECEREBROVASC-DIS.2016.05.003.
 42. Uhl B., Zuchtriegel G., Pühr-Westerheide D. et al. Tissue plasminogen activator promotes postischemic neutrophil recruitment via its proteolytic and nonproteolytic properties. *Arterioscler. Thromb. Vasc. Biol.* 2014; 34 (7): 1495–1504. DOI: 10.1161/ATVBAHA.114.303721.
 43. Dong Q., Dong Y., Liu L., Xu A., Zhang Y., Zheng H., Wang Y. The Chinese Stroke Association scientific statement: intravenous thrombolysis in acute ischaemic stroke. *Stroke and Vascular Neurology.* 2017; 2 (3): 147–159. DOI: 10.1136/svn-2017-000074.
 44. Guo Z., Yu S., Xiao L. et al. Dynamic change of neutrophil to lymphocyte ratio and hemorrhagic transformation after thrombolysis in stroke. *J. Neuroinflammation.* 2016; 13 (1): 199. DOI: 10.1186/S12974-016-0680-X.
 45. Shi J., Peng H., You S., Liu Y., Xu J., Xu Y., Liu H., Shi R., Cao Y., Liu C.F. Increase in neutrophils after recombinant tissue plasminogen activator thrombolysis predicts poor functional outcome of ischaemic stroke: a longitudinal study. *Eur. J. Neurol.* 2018; 25 (4): 687–745. DOI: 10.1111/ene.13575.
 46. Mangold A., Alias S., Scherz T., Hofbauer T., Jakowitsch J., Panzenbücker A. et al. Coronary neutrophil extracellular trap

- burden and deoxyribonuclease activity in ST-elevation acute coronary syndrome are predictors of ST-segment resolution and infarct size. *Circ. Res.* 2015; 116 (7): 1182–1192. DOI: 10.1161/CIRCRESAHA.116.304944.
47. Ducroux C., Di Meglio L., Loyau S., Delbosc S., Boisseau W., Deschildre C., Maacha M.B., Blanc R. et al. Thrombus neutrophil extracellular traps content impair tpa-induced thrombolysis in acute ischemic stroke. *Stroke.* 2018; 49 (3): 754–757. DOI: 10.1161/STROKEAHA.117.019896.
 48. Laridan E., Denorme F., Desender L., Francois O., Anderson T., Deckmyn H. et al. Neutrophil extracellular traps in ischemic stroke thrombi. *Ann. Neurol.* 2017; 82 (2): 223–232. DOI: 10.1002/ana.24993.
 49. Longstaff C., Varjú I., Sytonyi P., Szaby L., Krumrey M., Hoell A. et al. Mechanical stability and fibrinolytic resistance of clots containing fibrin, DNA, and histones. *J. Biol. Chem.* 2013; 288 (10): 6946–6956. DOI: 10.1074/jbc.M112.404301.
 50. Westendorp W.F., Nederkoorn P.J., Vermeij J.-D., Dijkgraaf M.G., van de Beek D. Post-stroke infection: A systematic review and metaanalysis. *BMC Neurol.* 2011; 11: 110. DOI: 10.1186/1471-2377-11-110.
 51. Chavan S.S., Pavlov V.A., Tracey K.J. Mechanisms and therapeutic relevance of neuro-immune communication. *Immunity.* 2017; 46 (6): 927–942. DOI: 10.1016/j.immuni.2017.06.008.
 52. Meisel C., Schwab J.M., Prass K. et al. Central nervous system injury-induced immune deficiency syndrome. *Nat. Rev. Neurosci.* 2005; 6 (10): 775–786. DOI: 10.1038/NRN1765.
 53. Rumer C., Engel O., Winek K. et al. Blocking stroke-induced immunodeficiency increases CNS antigen-specific autoreactivity but does not worsen functional outcome after experimental stroke. *J. Neurosci.* 2015; 35 (20): 7777–7794. DOI: 10.1523/JNEUROSCI.1532-14.2015.
 54. Dirnagl U., Klehmet J., Braun J.S. et al. Stroke-induced immunodepression: experimental evidence and clinical relevance. *Stroke.* 2007; 38 (2 Suppl.): 770–783. DOI: 10.1161/01.STR.0000251441.89665.BC.
 55. Hug A., Dalpke A., Wiczorek N. et al. Infarct volume is a major determiner of post-stroke immune cell function and susceptibility to infection. *Stroke.* 2009; 40 (10): 3226–3232. DOI: 10.1161/STROKEAHA.109.557967.
 56. Chamorro A., Meisel A., Planas A.M., Urra X., van de Beek D., Veltkamp R. The immunology of acute stroke. *Nat. Rev. Neurol.* 2012; 8 (7): 401–410. DOI: 10.1038/NRNEUROL.2012.98.
 57. Szaby C., Thiernemann C., Wu C.C. et al. Attenuation of the induction of nitric oxide synthase by endogenous glucocorticoids accounts for endotoxin tolerance *in vivo*. *Proc. Natl. Acad. Sci. USA.* 1994; 91 (1): 271–275. DOI: 10.1073/PNAS.91.1.271.
 58. Zhang J., Shi K., Li Z. et al. Organ- and cell-specific immune responses are associated with the outcomes of intracerebral hemorrhage. *Faseb. J.* 2018; 32 (1): 220–229. DOI: 10.1096/fj.201700324R.
 59. Van Gemmeren T., Schuppner R., Grosse G.M., Fering J., Gabriel M.M., Huber R., Worthmann H., Lichtinghagen R., Weissenborn R. early post-stroke infections are associated with an impaired function of neutrophil granulocytes. *J. Clin. Med.* 2020; 9 (3): 872. DOI: 10.3390/jcm9030872 IO.
 60. Krams M., Lees R.R., Hacke W., Grieve A.P., Orgogozo J.-M., Ford G.A. Acute stroke therapy by inhibition of neutrophils (ASTIN): an adaptive dose-response study of UK- 279,276 in acute ischemic stroke. *Stroke.* 2003; 34 (11): 2543–2548. DOI: 10.1161/01.STR.0000092527.33910.89.
 61. Smedly L.A., Tonnesen M.G., Sandhaus R.A. et al. Neutrophil-mediated injury to endothelial cells. *J. Clin. Invest.* 1986; 77 (4): 1233–1243. DOI: 10.1172/JCI112426.
 62. Mishlanov V.Yu., Tuyev A.V., Shutov A.A., Baidina T.V. Method for determining leukocyte lipid-releasing capacity in the diagnosis of mechanisms of atherogenesis in patients with coronary heart disease and ischemic stroke. *Clinical Laboratory Diagnostics.* 2006; 5: 9–12 (in Russ.).
 63. Higazi A.A., Nassar T., Ganz T., Rader D.J., Udassin R., Bdeir K., Hiss E., Sachais B.S., Williams K.J., Leitersdorf E., Cines D.B. The alpha-defensins stimulate proteoglycan-dependent catabolism of low-density lipoprotein by vascular cells: a new class of inflammatory apolipoprotein and a possible contributor to atherogenesis. *Blood.* 2000; 96 (4): 1393–1398.
 64. Obukhova O.V. Lipid-releasing capacity of leukocytes in patients with coronary heart disease. *Cardiovascular Therapy and Prevention.* 2009; 8 (6S1): 259 (in Russ.).
 65. Qi H., Yang S., Zhang L. Neutrophil extracellular traps and endothelial dysfunction in atherosclerosis and thrombosis. *Front. Immunol.* 2017; 8: 928. DOI: 10.3389/fimmu.2017.00928.
 66. Chistiakov D.A., Bobryshev Y.V., Orekhov A.N. Neutrophil's weapons in atherosclerosis (review). *Experimental and Molecular Pathology.* 2015; 99 (3): 663–671. DOI: 10.1016/j.yexmp.2015.11.011.
 67. Rahman A., Fazal F. Blocking NF-kappaB: an inflammatory issue. *Proc. Am. Thorac. Soc.* 2011; 8 (6): 497–503. DOI: 10.1513/pats.201101-009MW.
 68. Xiao L., Liu Y., Wang N. New paradigms in inflammatory signaling in vascular endothelial cells. *Am. J. Physiol. Heart Circ. Physiol.* 2014; 306 (3): H317–325. DOI: 10.1152/ajpheart.00182.2013.
 69. Chistiakov D.A., Grechko A.V., Myasoedova V.A. et al. The role of monocytosis and neutrophilia in atherosclerosis. *J. Cell Molec. Med.* 2018; 22 (3): 1366–1382. DOI: 10.1111/jcmm.13462.
 70. Hattar K., Heygster D., Eul B. et al. Amplification of LPS and LTA induced cytokine synthesis in NSCLC/neutrophil cocultures. *J. Clin. Oncol.* 2008; 26 (15): 22198. DOI: 10.1200/jco.2008.26.15_suppl.22198.
 71. Zychlinsky A. NETs: a new strategy for using old weapons. *Trends in Immunology.* 2009; 30 (11): 513–521. DOI: 10.1016/j.it.2009.07.011.

Authors information

Dolgushin Ilia I., Dr. Sci. (Med.), Professor, Academician of the Russian Academy of Sciences, Head of Microbiology, Virology, Immunology, and Clinical Laboratory Diagnostics Department, South Ural State Medical University, Chelyabinsk, Russian Federation. ORCID 0000-0002-0901-8042.

Karpova Maria I., Dr. Sci. (Med.), Associate Professor, Academician of the Russian Academy of Sciences, Head of Neurology Department, South Ural State Medical University, Chelyabinsk, Russian Federation. ORCID 0000-0001-5848-7235.

Zaripova Zarina Z., Neurologist, Chelyabinsk Regional Clinical Hospital, Stroke Unit, Chelyabinsk, Russian Federation, ORCID 0000-0001-8419-9026.

(✉) **Karpova Maria I.**, e-mail: kmi_2008@mail.ru

Received 19.00.2020

Accepted 28.12.2020

Diagnostic markers of local allergic rhinitis

Klimov A.V., Klimov V.V., Koval S.A., Sviridova V.S., Koshkarova N.S.

*Siberian State Medical University
2, Moscow Trakt, Tomsk, 634050, Russian Federation*

ABSTRACT

Local allergic rhinitis is a new atopic disease recognized in modern allergy and rhinology. Its clinical symptoms are currently well described and compared with conventional allergic rhinitis, however, diagnostic and therapeutic protocols, including antigen-specific immunotherapy (ASIT), should be specified.

Therefore, local allergic rhinitis often remains underdiagnosed and by mistake is referred to non-allergic rhinitis. Yet, the pathogenesis and mechanisms of allergen tolerance breakdown in this endotype of allergic rhinitis remain understudied. Researchers continue to study the network of tolerogenic cells and biomolecules, such as regulatory T cells, tolerogenic dendritic cells, interleukin (IL)-10, IL-35, transforming growth factor- β (TGF- β), vegetative innervation of the nose, and neurotransmitters.

The review focuses on diagnostic markers of local allergic rhinitis.

Key words: local allergic rhinitis, nasal endoscopy, nasal allergen provocation test, nasal specific IgE, basophil activation test.

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

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

For citation: Klimov A.V., Klimov V.V., Koval S.A., Sviridova V.S., Koshkarova N.S. Diagnostic markers of local allergic rhinitis. *Bulletin of Siberian Medicine*. 2021; 20 (3): 161–167. <https://doi.org/10.20538/1682-0363-2021-2-161-167>.

Диагностические маркеры локального аллергического ринита

Климов А.В., Климов В.В., Коваль С.А., Свиридова В.С., Кошкарлова Н.С.

*Сибирский государственный медицинский университет (СибГМУ)
Россия, 634050, г. Томск, Московский тракт, 2*

РЕЗЮМЕ

Локальный аллергический ринит является новой атопической болезнью в современной аллергологии и ринологии. Его клинические симптомы в настоящее время хорошо описаны с проведением сравнения с классическим аллергическим ринитом, но диагностические и терапевтические протоколы, включая аллерген-специфическую иммунотерапию, еще уточняются.

Таким образом, локальный аллергический ринит остается недостаточно диагностированной патологией

и по ошибке часто относится к группе неаллергических ринитов. Однако патогенез и особенно механизмы срыва аллергической толерантности при этом эндотипе аллергического ринита остаются неизученными. Продолжаются исследования сети протолерогенных клеток и молекул, включая Т-регуляторные клетки, протолерогенные дендритные клетки, интерлейкин (IL)-10, IL-35, трансформирующий фактор роста β , вегетативную иннервацию носа и нейротрансмиттеры.

Цель настоящего обзора сфокусирована на диагностических маркерах локального аллергического ринита.

Ключевые слова: локальный аллергический ринит, видеориноскопия, назальный провокационный тест, специфические назальные иммуноглобулины, тест активации базофилов.

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

Источник финансирования. Авторы заявляют об отсутствии финансирования.

Для цитирования: Климов А.В., Климов В.В., Коваль С.А., Свиридова В.С., Кошкарлова Н.С. Диагностические маркеры локального аллергического ринита. *Бюллетень сибирской медицины*. 2021; 20 (3): 161–167. <https://doi.org/10.20538/1682-0363-2021-3-161-167>.

INTRODUCTION

Since D.G. Powe et al. [1] described “entopy” as a “local allergy paradigm”, local atopic disorders in the unified airway have become an interesting and hot topic in allergy. To date, local allergic asthma [2–5], local allergic rhinitis [6–10], dual allergic rhinitis [11, 12], and local allergic conjunctivitis [13] have been described. Conventional atopic disorders of the unified airway have been largely studied for many years and included in global consensus position statements on diagnosis and therapy [14–16]. However, diagnostic and proper therapeutic approaches to local allergic respiratory disorders are still being developed.

A comparative study of conventional allergic rhinitis (AR) and local allergic rhinitis (LAR) has been carried out both from theoretical and clinical viewpoints over the last decade [12, 17]. Patients with LAR have the same clinical symptoms as those with AR, such as nasal obstruction, sneezing, itching, and rhinorrhea. The difference is in comorbid conditions. Both endotypes of rhinitis develop in children and adults, share similar clinical characteristics, and are associated with sensitization to house dust mites (HDMs) *D. pteronissinus* and *D. farinae*, sometimes displaying severe persistent clinical presentation, often with ocular and asthma symptoms [8, 18, 19].

From a theoretical viewpoint, the pathogenesis of AR and LAR shares almost all features of allergic inflammation in the nose but differs in the mechanisms and level of allergen tolerance

breakdown. It is worth noting that the number of people with a family history of atopic diseases with simultaneous clinical allergic manifestations does not coincide with the number of persons with a family history of atopic diseases but without clinical symptoms (i.e. practically healthy people) due to the *phenomenon of allergen tolerance* [20–23]. Practically healthy, though sensitized, persons always prevail over patients with AR and LAR.

At the systemic level, maintenance of allergen tolerance seems to be associated with a network of tolerogenic cells and biomolecules, such as regulatory T cells, tolerogenic dendritic cells, interleukin (IL)-10, IL-35, transforming growth factor β (TGF β), etc. [20, 24]. At the local (nasal) level, allergen tolerance appears to depend on peculiarities of innervation of the nose and an appropriate set of neurotransmitters [25–27]. Local concentrations of pathogen-associated molecular patterns (PAMPs), generated from the microbiota, and allergen-associated molecular patterns (AAMPs) [28] can also matter.

In contrast to the skin and gastrointestinal tract, innervation of the unified airway is characterized by a distinctive feature. The unified airway is innervated by the somatic nervous system and the autonomic nervous system (ANS), the latter mediating symptoms of allergic inflammation.

The ANS consists of both parasympathetic neurons, whose cell bodies reside in the brainstem and act via the vagus nerve and sympathetic neurons,

whose cell bodies reside in the paravertebral ganglia. Conversely, the gastrointestinal tract has its own autonomic nervous system called the enteric nervous system that consists of intrinsic primary afferent neurons, interneurons, and myenteric and submucosal plexuses [25]. This system appears to support allergen tolerance in the gastrointestinal tract.

If systemic allergen tolerance is maintained at the systemic level but a neurotransmitter imbalance takes place in the nose, the ANS may be responsible for the autonomous allergen tolerance breakdown that results in LAR [27].

DIAGNOSTIC MARKERS OF LOCAL ALLERGIC RHINITIS

Initially, any suspected allergic rhinitis patient should be examined according to the consensus statement on rhinology / allergy [14]. This algorithm includes history taking, clinical examination, nasal endoscopy (video rhinoscopy), rhinomanometry and acoustic rhinometry, spiral computed tomography (CT) of the paranasal sinuses, nasal cytology or histology, allergic skin tests, and serum total IgE level.

An initial routine ENT examination of patients with unverified allergic rhinitis mainly includes video rhinoscopy and CT. Endoscopy does not allow to detect the difference between AR and LAR. Commonly, swelling and cyanosis of the nasal mucosa and nasal obstruction (Figure) are revealed, and in some patients, septal deviation, rhinosinusitis, and polyps are detected. CT specifies the anatomical features and inflammatory comorbidities, such as nasal crest, rhinosinusitis, polyps, turbinate hypertrophy, central compartment atopic disease, etc. [29, 30].

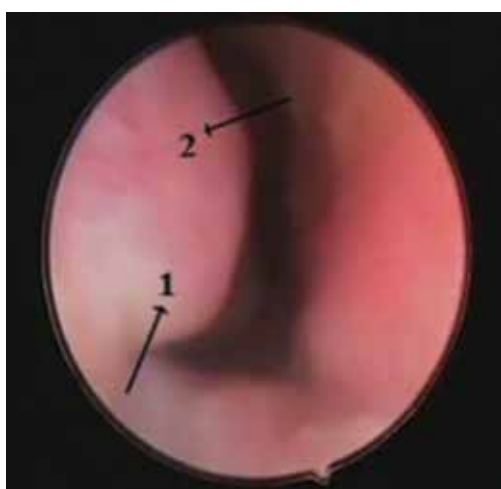


Figure. Endoscopic imaging in local allergic rhinitis. Edema (1) and cyanosis (2) of the mucosa in the inferior nasal turbinate

Allergic skin tests have been used in clinical allergy for about 150 years. There are skin prick tests [31] and intradermal tests. According to several prospective studies and systematic reviews, skin prick tests demonstrated to be a safe method of allergy testing. Besides, skin prick tests can be performed in any age group [14]. Given the limited penetration depth, skin prick tests are safe with very infrequent reports of anaphylaxis and no reported fatalities [32].

Intradermal testing is rarely used as a primary testing modality, and it is often a secondary method *in vivo* following skin prick tests. Interestingly, intradermal tests appear to be more sensitive and specific, when indoor allergens, such as HDMs, are being tested. However, it is unclear what such higher sensitivity depends on [14].

Performance and reliability of serum total IgE measurement depend on several factors, including the choice of reagents and the quality of equipment. Some authors demonstrated insufficient accuracy of this marker for allergen diagnosis, regardless of the concentration [32–34].

According to the ongoing scientific discussion [35], new techniques of IgE detection, such as new component-resolved diagnosis methods for specific IgE detection, cannot replace skin prick tests [36]. IgE is the only cytophilic antibody among other immunoglobulin isotypes. High-affinity FcεRI is expressed on basophils and mast cells and binds to the IgE – allergen complex triggering atopic inflammation [37].

DIAGNOSTIC MARKERS CONFIRMING LOCAL ALLERGIC RHINITIS

Confirmation of LAR identification is based on the following algorithm [17, 38]:

- 1) absence of systemic atopic disorders, such as food, insect allergy or atopic dermatitis;
- 2) negative skin prick tests;
- 3) absence of the elevated level of serum total IgE;
- 4) evidence of IgE sensitization at the local level.

A lack of systemic atopic diseases, elevated serum total IgE, and negative skin prick tests confirm maintenance of allergen tolerance at the systemic level in these patients. However, local atopic disorders in the unified airway, including LAR, may be manifested in some of the patients. Hence, the

evidence of IgE presence in the nose is essential for proper diagnosing and eliminating diagnostic errors.

There are three methods of nasal specific IgE detection:

(a) nasal allergen provocation test (NAPT), or allergen challenge test [14, 19, 39–41], using the Visual Analogue Scale (VAS) (a, i) or rhinomanometry;

(b) nasal specific IgE determination [14; 42–44] in the nasal secretion (b, i) or nasal tissue obtained by nasal mucosal brush biopsy (MBB) (b, ii);

(c) basophil activation test (BAT) [12, 14, 45, 46].

These methods are characterized by different specificity and sensitivity (Table).

Table

Specificity and sensitivity of HDM-specific IgE detection in local allergic rhinitis		
Tests	Specificity, %	Sensitivity, %
(a, i) nasal provocation test (NAPT) according to VAS;	90.6 [19]	73.4 [19]
(a, ii) nasal provocation test (NAPT) according to rhinomanometry	100 [41]	83.7 [41]
(b,i) nasal specific IgE detection in the nasal secretion;	> 90 [17]	22–40 [17]
(b, ii) nasal specific IgE detection using MBB	100 [47]	–
(c) basophil activation test (BAT)	93 [46]	50 [46]

The allergen for NAPT may be administered by some devices, including syringes, nasal sprays, nose droppers, micropipettes, etc. The result of NAPT can be evaluated 20 min after allergen application by several methods, such as VAS, rhinomanometry, acoustic rhinometry, inflammatory markers in the nasal secretion, and nasal NO concentration. NAPT is conducted separately for HDM allergens *D. pteronyssinus* and *D. farinae* at a dose of 5,000 standardized biological units (SBU) / ml for each allergen, with 0.2 ml administered via a calibration device in both nostrils at room temperature [40].

VAS allows to assess changes in the nasal symptoms, such as rhinorrhea, itching, sneezing, and nasal obstruction, and carry out differential diagnosis of LAR and non-allergic rhinitis [19]. Currently, special VAS-incorporating applications have been developed for smartphones to assess the disease control with high efficiency [39, 48].

Rhinomanometric interpretation of NAPT results is based on analyzing nasal airway resistance measured in the right and left nasal passage separately during normal breathing [40]. Due to high specificity

and sensitivity (Table), NAPT is recognized as the gold standard for the diagnosis of LAR [9, 12].

Evidence of the presence of nasal specific IgE in the nasal secretion is essential for LAR diagnosis but is difficult to obtain [14]. Using the immunoCAP technique [42], the concentration of IgE > 0.35 kU / l is considered positive [42]. Nasal secretions are collected via absorptive filter paper applied to the inferior nasal turbinates for 5 minutes [49], and HDM-specific IgE is then assayed using the immunoCAP technique. Different methods have been described for the most effective identification of nasal specific IgE, including nasal lavage, mucosal biopsy, and MBB [14, 43, 47]. However, in clinical practice, non-invasive methods are preferable [12]. Therefore, nasal allergen-specific IgE has high specificity and medium sensitivity for the diagnosis of LAR.

BAT is a flow cytometry-based assay performed on the peripheral blood, where the expression of activation markers like CD63 is measured following stimulation with HDM or other allergens [12]. BAT may be a useful additional diagnostic method when LAR is doubtful, when the allergen responsible for clinical symptoms is unknown, and it is necessary to assess the response to ASIT [14, 45]. As seen in Table, BAT has high specificity and medium sensitivity.

From a clinical viewpoint, eosinophil cationic protein, tryptase, cytokines, and chemokines are additional, specifying markers for both LAR and AR [7, 50]. According to the peculiarities of allergen tolerance breakdown, psychosomatic markers are more typical of LAR than of AR. For this reason, the questionnaire proposed by R.L. Spitzer et al. [51] can be a useful tool for the differential diagnosis of these endotypes of allergic rhinitis.

CONCLUSION

LAR and AR share similar clinical and endoscopic symptoms. However, the diagnosis of LAR is based on NAPT, the detection of nasal specific IgE, or a positive BAT in the absence of systemic IgE sensitization [14]. NAPT is the gold standard for LAR identification, as it displays optimal specificity and sensitivity [9]. Unfortunately, LAR often remains underdiagnosed and by mistake is referred to non-allergic rhinitis.

Currently, only protocol-based pharmacological treatment is available for LAR [40]. However, some investigations show encouraging results with

the use of ASIT in LAR [52]. On the whole, LAR remains an understudied disorder in modern allergy and rhinology requiring further research [7, 12, 14, 17, 19].

REFERENCES

1. Powe D.G., Bonnin A.J., Jones N.S. 'Entropy': local allergy paradigm. *Clin. Exp. Allergy*. 2010; 40 (7): 987–997. DOI: 10.1111/j.1365-2222.2010.03536.x.
2. Campo P., Eguiluz-Gracia I., Salas M. et al. Bronchial asthma triggered by house dust mites in patients with local allergic rhinitis. *Allergy*. 2019; 74 (8): 1502–1510. DOI: 10.1111/all.13775.
3. Froidure A., Mouthuy J., Durham S.R. Asthma phenotypes and IgE responses. *Eur. Respir. J.* 2016 Jan.; 47 (1): 304–319. DOI: 10.1183/13993003.01824-2014.
4. Kılıç E., Kutlu A., Hastalıkları G. et al. Does local allergy (entropy) exists in asthma? *J. Clin. Anal. Med.* 2016. Letters to Editors from 01.02.2016. DOI: 10.4328/JCAM.3272.
5. Klimov A.V., Isaev P.Yu., Klimov V.V., Sviridova V.S. Endotypes of allergic rhinitis and asthma accompanying food allergy. *Bulletin of Siberian Medicine*. 2019; 18 (2): 287–289. DOI: 10.20538/1682-0363-2019-2-287–289.
6. Rondón C., Canto G., Blanca M. Local allergic rhinitis: A new entity, characterization and further studies. *Curr. Opin. Allergy Clin. Immunol.* 2010; 10 (1): 1–7. DOI: 10.1097/ACI.0b013e328334f5fb.
7. Incorvaia C., Fuiano N., Martignago I. et al. Local allergic rhinitis: evolution of concepts. *Clin. Transl. Allergy*. 2017; 7: 38. DOI: 10.1186/s13601-017-0174-7.
8. De Mello J.F. Local allergic rhinitis. *Braz. J. Otorhinolaryngol.* 2016; 82 (6): 621–622. DOI: 10.1016/j.bjorl.2016.09.001.
9. Eguiluz-Gracia I., Pérez-Sánchez N., Bogas G. et al. How to diagnose and treat local allergic rhinitis: a challenge for clinicians. *J. Clin. Med.* 2019; 8 (7): 1062–1074. DOI: 10.3390/jcm8071062.
10. Zicari A.M., Occasi F., Di Fraia M. et al. Local allergic rhinitis in children: novel diagnostic features and potential biomarkers. *Am. J. Rhinol. Allergy* 2016; 30 (5): 329–334. DOI: 10.2500/ajra.2016.30.4352.
11. Eguiluz-Gracia I., Fernandez-Santamaria R., Almudena Testera-Montes A., Ariza A., Campo P. et al. Coexistence of nasal reactivity to allergens with and without IgE sensitization in patients with allergic rhinitis. *Allergy*. 2020; 75 (7): 1689–1698. DOI: 10.1111/all.14206.
12. Maoz-Segal R., Machnes-Maayan D., Veksler-Offengenden I. et al. Local allergic rhinitis: an old story but a new entity. *Rhinosinusitis*. London: IntechOpen; 2019: 1–9. DOI: 10.5772/intechopen.86212.
13. Yamana Y., Fukuda K., Ko R., Uchio E. Local allergic conjunctivitis: a phenotype of allergic conjunctivitis. *Int. Ophthalmol.* 2019; 39 (11): 2539–2544. DOI: 10.1007/s10792-019-01101-z.
14. Wise S.K., Lin S.Y., Toskala E. et al. International consensus statement on allergy and rhinology: allergic rhinitis. *Int. Forum Allergy Rhinol.* 2018; 8 (2): 108–352. DOI: 10.1002/alr.22073.
15. Global Initiative for Asthma. Global strategy for asthma management and prevention. 2020. URL: www.ginasthma.org.
16. Takamura E., Uchio E., Ebihara N. et al. Japanese guideline for allergic conjunctival diseases. *Allergol. Int.* 2017; 66 (2): 220–229. DOI: 10.1016/j.alit.2016.12.004.
17. Campo P., Eguiluz-Gracia I., Bogas G. et al. Local allergic rhinitis: implications for management. *Clin. Exp. Allergy*. 2019; 49 (1): 6–16. DOI: 10.1111/cea.13192.
18. Sennkamp J., Joest I., Filipiak-Pittroff B. et al. Local allergic nasal reactions convert to classic systemic allergic reactions: A long-term follow-up. *Int. Arch. Allergy Immunol.* 2015; 166 (2): 154–160. DOI: 10.1159/000380852.
19. Jung C.-G., Lee J.-H., Ban G.-Y., Park H.-S., Shin Y.S. Prevalence and clinical characteristics of local allergic rhinitis to house dust mites. *Yonsei Med. J.* 2017; 58 (5): 1047–1050. DOI: 10.3349/ymj.2017.58.5.1047.
20. Wisniewski J., Agrawal R., Woodfolk J.A. Mechanisms of tolerance induction in allergic disease: Integrating current and emerging concepts. *Clin. Exp. Allergy*. 2013; 43 (2): 164–176. DOI: 10.1111/cea.12016.
21. Abdel-Gadir A., Massoud A.H., Chatila T.A. Antigen-specific Treg cells in immunological tolerance: implications for allergic diseases. *F1000Research*. 2018; 7: 38. DOI: 10.12688/f1000research.12650.
22. Calzada D., Baos S., Cremades-Jimeno L., Cárdena B. Immunological mechanisms in allergic diseases and allergen tolerance: the role of Treg cells. *J. Immunol. Res.* 2018; 2018: 6012053. DOI: 10.1155/2018/6012053.
23. Calderón M.A., Linneberg A., Kleine-Tebbe J. et al. Respiratory allergy caused by house dust mites: What do we really know? *J. Allergy Clin. Immunol.* 2015; 136 (1): 38–47. DOI: 10.1016/j.jaci.2014.10.012.
24. Klimov V.V. *From basic to clinical immunology*. Springer Nature, Cham; 2019. DOI: 10.1007/978-3-030-03323-1.
25. Voisin T., Bouvier A., Chiu I.V. Neuro-immune interactions in allergic diseases: Novel targets for therapeutics. *Int. Immunol.* 2017; 29 (6): 247–261. DOI: 10.1093/intimm/dxx040.
26. Kerage D., Sloan E.K., Mattarollo S.R., McCombe P.A. Interaction of neurotransmitters and neurochemicals with lymphocytes. *J. Neuroimmunology*. 2019; 332: 99–111. DOI: 10.1016/j.jneuroim.2019.04.006.
27. Klimov V.V., Klimov A.V. Autonomous breakdown of the allergen tolerance in the nose. *Authorea*. June 13, 2020. DOI: 0.22541/au.159204964.43963580.
28. Pali-Scholl I., Jensen-Jarolim E. The concept of allergen-associated molecular patterns (AAMP). *Curr. Opin. Immunol.* 2016; 42: 113–118. DOI: 10.1016/j.coi.2016.08.004.
29. DelGaudio J.M., Loftus P.A., Hamizan A.W., Harvey R.J., Wise S.K. Central compartment atopic disease. *Am. J. Rhinol. Allergy*. 2017; 31 (4): 228–234. DOI: 10.2500/ajra.2017.31.4443.
30. Marcus S., Schertzer J., Roland L.T., Wise S.K., Levy J.M., DelGaudio J.M. Central compartment atopic disease: prevalence of allergy and asthma compared with other subtypes of chronic rhinosinusitis with nasal polyps. *Int. Forum Allergy Rhinol.* 2020; 10 (2): 183–189. DOI: 10.1002/alr.22454.

31. Heinzerling L., Mari A., Bergmann K.C., Bresciani M., Burbach G., Darsow U. et al. The skin prick test – European standards. *Clin. Transl. Allergy*. 2013; 3 (1): 3. DOI: 10.1186/2045-7022-3-3.
32. Bernstein I.L., Li J.T., Bernstein D.I. et al. Allergy diagnostic testing: an updated practice parameter. *Ann. Allergy Asthma Im.* 2008; 100 (Suppl. 3): S1–148. DOI: 10.1016/s1081-1206(10)60305-5.
33. Satwani H., Rehman A., Ashraf S., Hassan A. Is serum total IgE levels a good predictor of allergies in children? *J. Pak. Med. Assoc.* 2009; 59 (10): 698–702.
34. Tu Y.L., Chang S.W., Tsai H.J. et al. Total serum IgE in a population-based study of Asian children in Taiwan: reference value and significance in the diagnosis of allergy. *PLoS One*. 2013; 8 (11): e80996. DOI: 10.1371/journal.pone.0080996.
35. Larenas-Linnemann D., Luna-Pech J.A., Mösges R. Debates in allergy medicine: allergy skin testing cannot be replaced by molecular diagnosis in the near future. *W.A.O.J.* 2017; 10 (32): 1–7. DOI: 10.1186/s40413-017-0164-1.
36. Sastre J., Sastre-Ibanez M. Molecular diagnosis and immunotherapy. *Curr. Opin. Allergy Clin. Immunol.* 2016; 16 (6): 565–570. DOI: 10.1097/ACI.0000000000000318.
37. Sutton B.J., Davies A.M. Structure and dynamics of IgE-receptor interactions: FcεRI and CD23/FcεRII. *Immunol. Rev.* 2015; 268 (1): 222–235. DOI: 10.1111/imr.12340.
38. Krzych-Fałta E., Namysłowski A., Samoliński B. Dilemmas associated with local allergic rhinitis. *Adv. Dermatol. Allergol.* 2018; 35 (3): 243–245. DOI: 10.5114/ada.2018.76215.
39. Klimek L., Bergmann K.-C., Biedermann T. et al. Visual analogue scales (VAS): measuring instruments for the documentation of symptoms and therapy monitoring in cases of allergic rhinitis in everyday health care. *Allergo J. Int.* 2017; 26 (1): 16–24. DOI: 10.1007/s40629-016-0006-7.
40. Wojas O., Samoliński B., Krzych-Fałta E. Local allergic rhinitis: nasal allergen provocation testing as a good tool in the differential diagnosis. *Int. J. Occup. Environ. Health.* 2020; 33 (2): 241–246. DOI: 10.13075/ijomh.1896.01503.
41. De Blay F., Doyen V., Lutz C. et al. A new, faster, and safe nasal provocation test method for diagnosing mite allergic rhinitis. *Ann. Allergy Asthma Immunol.* 2015; 115 (5): 385–390. DOI: 10.1016/j.anai.2015.07.014.
42. Meng Y., Lou H., Wang Y., Wang C., Zhang L. The use of specific immunoglobulin E in nasal secretions for the diagnosis of allergic rhinitis. *Laryngoscope*. 2018; 128 (9): E311–E315. DOI: 10.1002/lary.27120.
43. Hamizan A., Alvarado R., Rimmer J. et al. Nasal mucosal brushing as a diagnostic method for allergic rhinitis. *Allergy Asthma Proc.* 2019; 40 (3): 167–172. DOI: 10.2500/aap.2019.40.4209.
44. De Schryver E., Devuyst L., Derycke L. et al. Local immunoglobulin E in the nasal mucosa: Clinical implications. *Allergy Asthma Immunol. Res.* 2015; 7 (4): 321–331. DOI: 10.4168/aair.2015.7.4.321.
45. Hemmings O., Kwok M., McKendry R., Santos A.F. Basophil activation test: old and new applications in allergy. *Curr. Allergy Asthma Rep.* 2019; 19 (12): 58. DOI: 10.1007/s11882-019-0889-8.
46. Gomez E., Campo P., Rondón C., Barrionuevo E., Blanca-Lopez N., Torres M.J. et al. Role of the basophil activation test in the diagnosis of local allergic rhinitis. *J. Allergy Clin. Immunol.* 2013; 132 (4): 975–976. DOI: 10.1016/j.jaci.2013.07.016.
47. Reisacher W.R. Detecting local immunoglobulin E from mucosal brush biopsy of the inferior turbinates using microarray analysis. *Int. Forum Allergy Rhinol.* 2013; 3 (5): 399–403. DOI: 10.1002/alr.21111.
48. Courbis A.-L., Murray R.B., Arnavielhe S. et al. Electronic clinical decision support system for allergic rhinitis management: MASK e-CDSS. *Clin. Exp. Allergy*. 2018; 48 (12): 1640–1653. DOI: 10.1111/cea.13230.
49. Hamed A., Palacios T., Khokhar D. et al. Local IgE production in allergic (AR) and non-allergic rhinitis (NAR). *J. Allergy Clin. Immunol.* 2017; 139 (Suppl. 2): AB155. DOI: 10.1016/j.jaci.2016.12.510.
50. Hoyte F.C.L., Nelson H.S. Recent advances in allergic rhinitis. *F1000Research*. 2018; 7: F1000. DOI: 10.12688/f1000research.15367.1.
51. Spitzer R.L., Kroenke K., Williams J.B.W., Lowe B. A brief measure for assessing generalized anxiety disorder. The GAD-7. *Arch. Intern. Med.* 2006; 166 (1): 1092–1097. DOI: 10.1001/archinte.166.10.1092.
52. Rondón C., Campo P., Salas M., Aranda A., Molina A., González M. et al. Efficacy and safety of *D. pteronyssinus* immunotherapy in local allergic rhinitis: a double-blind placebo-controlled clinical trial. *Allergy*. 2016; 71 (7): 1057–1061. DOI: 10.1111/all.12889.

Acknowledgements

The authors wish to express their gratitude to Prof. Oleg V. Kalyuzhin (I.M. Sechenov First Moscow State Medical University – Sechenov University) for advice and recommendations concerning this publication.

Authors contribution

All authors contributed to drafting and editing of the manuscript.

Authors information

Klimov Andrew V., Cand. Sci. (Med.), Associate Professor, Division of ENT Diseases, Immunology, and Allergy, Siberian State Medical University, Tomsk, Russian Federation. ORCID 0000-0002-2776-5834.

Klimov Vladimir V., Dr. Sci. (Med.), Head of the Division of ENT Diseases, Immunology, and Allergy, Siberian State Medical University, Tomsk, Russian Federation. ORCID 0000-0001-6673-7556.

Koval Stanislav A., Teaching Assistant, Division of ENT Diseases, Immunology, and Allergy, Siberian State Medical University, Tomsk, Russian Federation. ORCID 0000-0002-8298-7456.

Sviridova Valentina S., Cand. Sci. (Med.), Associate Professor, Division of ENT Diseases, Immunology, and Allergy, Siberian State Medical University, Tomsk, Russian Federation. ORCID 0000-0001-7594-6800.

Koshkarova Natalia S., Cand. Sci. (Med.), Associate Professor, Division of ENT Diseases, Immunology, and Allergy, Siberian State Medical University, Tomsk, Russian Federation. ORCID 0000-0002-6032-2402.

(✉) **Klimov Andrew V.**, e-mail: klimov.lor@mail.ru

Received 06.09.2020

Accepted 29.09.2020

Vascular catheterization in small laboratory animals in biomedical research: technological aspects of the method (review article)

Lapin K.N.¹, Ryzhkov I.A.¹, Maltseva V.A.², Udut E.V.³

¹*Federal Research and Clinical Center of Intensive Care Medicine and Rehabilitology
25/2, Petrovka Str., Moscow, 107031, Russian Federation*

²*Scientific Center "Signal"
8, Bolshaya Olenya Str., Moscow, 107014, Russian Federation*

³*E.D. Goldberg Research Institute of Pharmacology and Regenerative Medicine, Tomsk National Research Medical Center (NRMCC), Russian Academy of Sciences
3, Lenina Av., Tomsk, 634028, Russian Federation*

ABSTRACT

This review addresses the use of vascular catheterization in small laboratory animals in biomedical research with an emphasis on the technological aspects of the method. The use of vascular catheters for blood sampling, drug delivery or biomonitoring improves the quality of the study (reliability and reproducibility of results) and promotes compliance with modern bioethical standards. The key factors that determine the success of the surgery and the entire study are considered with an up-to-date approach. In particular, recommendations are given on the choice of the vessel and the type and size of the catheter, depending on the characteristics of the animal and the study objectives. Catheterization of the external jugular vein of a rat is described in detail, and the fundamental stages of the procedure are the same for all major vessels of rodents. Much attention is paid to potential complications of vascular catheterization, care for catheterized animals in the postoperative period, as well as measures for maintaining the patency of the catheter and its proper functioning. The main limitations for the widespread use of catheterization in research are insufficient qualification of the surgeon and the need to use surgical equipment and microsurgical instruments.

Key words: jugular vein catheterization, vascular catheter, chronic catheterization, experimental surgery, small laboratory animals.

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

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

For citation: Lapin K.N., Ryzhkov I.A., Maltseva V.A., Udut E.V. Vascular catheterization in small laboratory animals in biomedical research: technological aspects of the method (review article). *Bulletin of Siberian Medicine*. 2021; 20 (3): 168–181. <https://doi.org/10.20538/1682-0363-2021-2-168-181>.

Катетеризация сосудов мелких лабораторных животных при проведении биомедицинских исследований: технологические аспекты метода (обзор)

Лапин К.Н.¹, Рыжков И.А.¹, Мальцева В.А.², Удуд Е.В.³

¹ Научно-исследовательский институт (НИИ) общей реаниматологии имени В.А. Неговского, Федеральный научно-клинический центр реаниматологии и реабилитологии (ФНКЦ РР) Россия, 107031, г. Москва, ул. Петровка, 25/2

² Научный центр «Сигнал» Россия, 107014, г. Москва, ул. Большая Оленья, 8

³ Научно-исследовательский институт фармакологии и регенеративной медицины (НИИФРМ) им. Е.Д. Гольдберга, Томский национальный исследовательский медицинский центр (НИМЦ) Российской академии наук Россия, 634028, г. Томск, пр. Ленина, 3

РЕЗЮМЕ

Настоящий обзор посвящен применению катетеризации сосудов мелких лабораторных животных в практике биомедицинских исследований с акцентом на технологические аспекты метода. Использование сосудистых катетеров для отбора проб крови, введения препаратов или с целью биомониторинга позволяет повысить качество исследования (достоверность и воспроизводимость результатов) и способствует соблюдению биоэтических норм. С современных позиций рассмотрены ключевые факторы, от которых зависит успех операции и всего исследования. В частности, даны рекомендации по выбору сосуда, типа и размера катетера в зависимости от особенностей животного и задач исследования. Подробно описана операция катетеризации наружной яремной вены крысы, принципиальные этапы которой одинаковы для всех магистральных сосудов грызунов. Также большое внимание уделено потенциальным осложнениям катетеризации сосудов, особенностям ухода за катетеризированным животным в послеоперационном периоде и мероприятиям, направленным на поддержание проходимости катетера и его надлежащего функционирования. Основными ограничениями широкого применения метода катетеризации в научных исследованиях являются недостаточная квалификация хирурга и необходимость использования операционного оборудования и микрохирургических инструментов.

Ключевые слова: катетеризация яремной вены, сосудистый катетер, хроническая катетеризация, экспериментальная хирургия, мелкие лабораторные животные.

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

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

Для цитирования: Лапин К.Н., Рыжков И.А., Мальцева В.А., Удуд Е.В. Катетеризация сосудов мелких лабораторных животных при проведении биомедицинских исследований: технологические аспекты метода (обзор). *Бюллетень сибирской медицины*. 2021; 20 (3): 168–181. <https://doi.org/10.20538/1682-0363-2021-3-168-181>.

INTRODUCTION

Catheterization is a surgical manipulation of inserting a special tube (catheter) into a body cavity, duct, or vessel. For many decades, catheterization of blood vessels in humans and animals has been used for diagnostic and therapeutic purposes for multiple blood sampling, injections of various substances, as well as for registration of physiological parameters of

the body [1, 2]. For scientific purposes, it is used in experimental biology and medicine during physiological research, modeling of pathological processes, preclinical study of drugs, and for other tasks.

Catheterization of small laboratory animals, especially rodents, is a difficult procedure due to the small size of the animal. Historically, scientific experiments on laboratory animals began in the middle of the XIX

century [3, 4]. The method of vascular catheterization in humans developed rapidly in the 1930–1940s [5]. At the same time, the first publications on catheterization of vessels in small laboratory animals appeared later, in the second half of the XX century [6, 7], when it became possible to make small-diameter catheters due to the development of technologies for production of polymer materials.

Currently, generally accepted world standards for handling of laboratory animals require adherence to the bioethical concept, which includes the 3Rs [8]. Introduction of this concept into the practice of experimental research has shown that humane treatment of animals is one of the important factors in obtaining reliable results. A modern biomedical preclinical study is usually accompanied by drug infusions and / or sampling of biomaterial from laboratory animals. The method of vascular catheterization during these manipulations allows researchers to reduce discomfort and stress of animals and, therefore, avoid unwanted physiological changes that may affect the results of the experiment. Thus, comparing parameters of blood from catheterized animals and blood obtained by other conventional methods, for example, decapitation, showed significant differences between animals in terms of stress markers and blood composition [9–11]. In addition, a smaller number of animals could be included in pharmacokinetic studies, if vascular catheterization was used. Therefore, two of the 3Rs principles are observed in the vascular catheterization method. Firstly, the technique of working with animals is improved, thereby reducing negative, distressing, or painful effects (refinement); secondly, the study requires a smaller number of animals to obtain reliable results (reduction).

The main aim of this review was to summarize international and authors' experience of using vascular catheterization in small laboratory animals in the practice of biomedical research with an emphasis on the technological aspects of the method. Many modern publications are devoted to various catheterization modifications, which are used to solve a wide range of tasks. Therefore, in our opinion, the review provides the most typical examples of the method application and indicates the main aspects affecting the success of the operation and the overall study. It should be noted that in many cases, the recommendations for choosing the type of catheter, the catheterization technique, and care of an implanted catheter are similar for humans and animals, therefore, some clinical studies were also included in the review.

AREAS OF APPLICATION OF CATHETERIZATION AND THE RANGE OF RESEARCH PROBLEMS

Vascular catheterization in small laboratory animals is most often used to solve the following problems:

1. Single or multiple *infusion* of various solutions (drugs, biologically active substances, blood substitutes, genetic material, contrast agents, etc.).
2. Single or multiple *sampling of biomaterial* (for example, to study the pharmacokinetics of drugs or to conduct experiments with controlled blood loss).
3. *Biomonitoring*. Various sensors are implanted by catheterization, which makes it possible to continuously (online) measure temperature, systolic, diastolic, and pulse pressure, heart rate, glucose level, blood gases, etc. in a freely moving animal during a long period of time [12–16].

CHOOSING A VESSEL FOR CATHETERIZATION

When choosing a vessel for catheterization and a place for catheter insertion, the anatomical features of the vascular system in this animal species should also be taken into account in addition to research objectives. The place for biomaterial sampling (artery, central or peripheral veins) can affect the measured blood parameters [17], and the place for drug infusion can affect its pharmacokinetic parameters [18]. The direction of catheter insertion into the vessel is also of great importance: antegrade (towards the direction of the blood flow) or retrograde (against the blood flow). Thus, retrograde arterial catheters are usually placed to measure systemic arterial pressure and antegrade catheterization is used for targeted administration of substances into the cerebral blood flow or into the tissues of the limb. In most cases, central venous catheters (in the external jugular and femoral veins) are inserted towards the heart (antegrade) [13].

The choice of a vessel for implantation determines the choice of a catheter with certain characteristics [19]. For example, arteries and veins have different anatomy and different elastic properties. The arteries have thick walls, their muscle cells are able to contract actively, so introduction of a large-diameter catheter will be difficult. In addition, an arterial spasm can occlude a catheter that is too soft. In contrast, the vein wall is thinner and stretches more easily than the artery wall, so a softer and larger catheter can be implanted into the vein. It should be noted that when a catheter

Main Vessels for the Catheterization

Optimum Characteristics of the Catheter		For what Experiments	Catheter Location	For what Experiments	Optimum Characteristics of the Catheter	
Rat	Mouse				Rat	Mouse
OD: 0.7-1.0 mm ID: 0.4-0.7 mm Material: PVC, PU	OD: 0.3-0.5 mm ID: 0.15-0.30 mm Material: PVC, PU	Infusion + Blood Sampling ++ Biomonitoring +/- Chronic catheterization	Jugular Vein	Infusion +/- Blood Sampling + Biomonitoring ++ Chronic catheterization	OD: 0.5-0.9 mm ID: 0.3-0.6 mm Material: PE, PVC	OD: 0.3-0.4 mm ID: 0.15-0.20 mm Material: PE, PVC
OD: 0.8-1.2 mm ID: 0.4-0.7 mm Material: PVC, PU, Silicone	OD: 0.4-0.7 mm ID: 0.2-0.4 mm Material: PVC, PU, Silicone	Infusion +/- Blood Sampling + Biomonitoring ++ Chronic catheterization	Vena Cava	Infusion +/- Blood Sampling - Biomonitoring ++ Chronic catheterization	OD: 0.7-1.1 mm ID: 0.4-0.7 mm Material: PE, PVC, PU, Silicone	OD: 0.3-0.6 mm ID: 0.15-0.30 mm Material: PE, PVC, PU, Silicone
OD: 0.8-1.1 mm ID: 0.4-0.7 mm Material: PVC, PU	OD: 0.3-0.5 mm ID: 0.15-0.30 mm Material: PVC, PU	Infusion +/- Blood Sampling +/- Biomonitoring + Acute Catheterization	Femoral Vein	Infusion + Blood Sampling + Biomonitoring +/- Acute Catheterization	OD: 0.6-1.0 mm ID: 0.4-0.7 mm Material: PE, PVC	OD: 0.3-0.4 mm ID: 0.15-0.20 mm Material: PE, PVC
OD: 0.6-0.9 mm ID: 0.4-0.7 mm Material: PVC, PE	OD: 0.3-0.4 mm ID: 0.15-0.20 mm Material: PVC, PE	Infusion +/- Blood Sampling +/- Biomonitoring +/- Acute Catheterization	Tail Vein	Infusion + Blood Sampling + Biomonitoring +/- Acute Catheterization	OD: 0.4-0.8 mm ID: 0.4-0.7 mm Material: PE, PVC	OD: 0.3-0.4 mm ID: 0.15-0.20 mm Material: PE, PVC

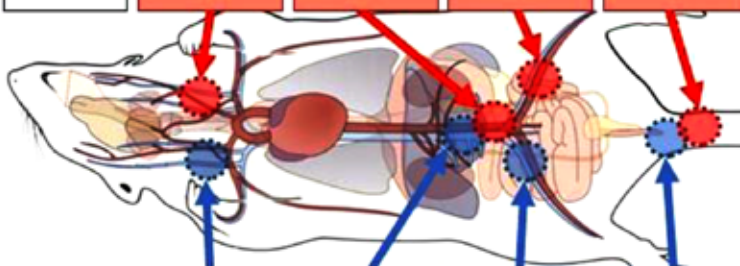


Fig. 1. The main vessels of rodents used for catheterization in biomedical research and catheter optimal characteristics: OD – catheter outer diameter, ID – catheter inner diameter, PE – polyethylene, PVC – polyvinyl chloride, PU – polyurethane, ++ – used often, + – used, +/- – used rarely

is implanted into the vein, its intravascular end should not attach to the vein walls. Figure 1 shows the main blood vessels most commonly used for catheterization, as well as the characteristics of catheters suitable for

implantation into these vessels. French (Ch, Fr) or Birmingham Gauge (G) units are often used to measure the outer diameter of the catheter. Their correspondence to the metric system is presented in Table 1.

Table 1

Correspondence of the French and Birmingham Gauge systems to the metric system									
Scale	Unit	Outer diameter of catheter							
French	Charriere (Ch) / French (Fr)	1	–	2	–	3	–	4	5
Birmingham	Gauge (G)	29	27	22	20	–	18	–	16
Metric	mm	0.33	0.4	0.67	0.9	1.00	1.25	1.35	1.67

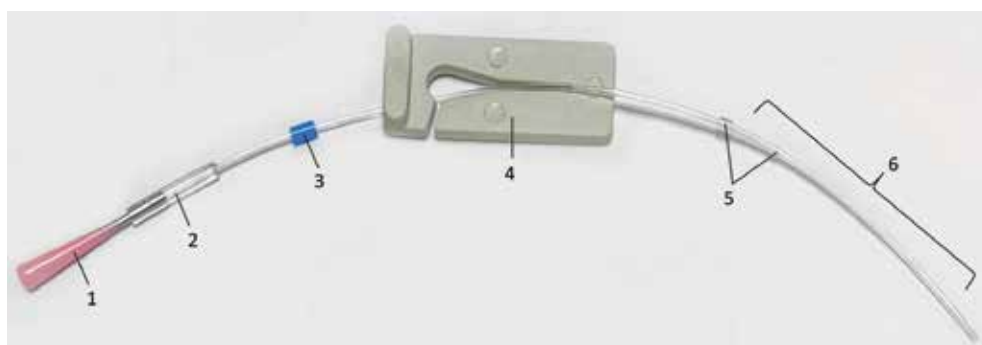


Fig. 2. Catheter components and their purpose: 1 – catheter plug, 2 – catheter adapter to connect external devices, 3 – fixing collar for the external catheter anchoring and color coding of the catheter type, 4 – slide clamp, 5 – fixing collar for anchoring of the intravascular part of the catheter in the vessel, 6 – intravascular part of the catheter

Types of catheters. When choosing a catheter, three principles should be taken into account. Firstly, each model should be designed for a specific animal species and type of vessels. Secondly, the catheter should ideally match the external device to which it would be attached. Thirdly, the configuration of the catheter should be simple and versatile enough to suit most surgeries and research. Figure 2 shows components of the catheter and their purpose.

The functional characteristics of the catheter primarily depend on the material. In addition, the catheter performance is affected by the size, tip geometry, and implantation depth. The optimal catheter material is selected based on the research objectives: the duration of the experiment, the chemical nature of infusion fluids, the size of the animal and its physical condition, as well as surgeon's proficiency in the catheterization technique. For example, in order to conduct a chronic experiment, it is better to use softer materials that are less damaging to the vascular endothelium during implantation. However, a soft catheter is more difficult to insert into the vessel. In preclinical and clinical practice, catheters made of silicone (Sil), polyurethane (PU), polyethylene (PE),

and polyvinyl chloride (PVC) are most often used [20]. They have different biological, physical, and chemical characteristics (Table 2). It should be noted that since old polymer materials are being actively improved and new ones (including combined or multilayer ones) are being developed, the characteristics given in Table 2 may vary depending on the manufacturer.

Biocompatibility of a catheter depends on its rigidity, the geometry of the intravascular part, as well as the chemical and physical characteristics of the surface: inertness, hydrophilicity, irregularities and mechanical defects, polar or ionic groups, etc. [20–23]. In addition, microorganisms of a certain type prefer to colonize a certain polymer [22]. Depending on the situation, any of these factors can be decisive and lead to complications during catheterization. Chemical inertness and flexibility are the most significant characteristics.

Rigid catheters are still widely used, especially in acute experiments, because they are easier to implant [20]. From this point of view, PU is of particular interest, since it is a thermoplastic polymer that softens in a vessel when heated by body heat.

Table 2

Characteristics of various polymers used in the production of catheters

Material properties	Material			
	Silicone	Polyurethane	Polyethylene	Polyvinyl chloride
Hemocompatibility (biocompatibility)	Very good	Good	Medium	Medium
Chemical inertness	Interaction possible	Interaction possible	Inert	Interaction possible
Rigidity	Very soft	Soft (intravascular part)	Rigid	Soft/Rigid
Ease of insertion into the vessel	Hard	Medium	Easy	Medium
Wall thickness	Thick	Medium	Thin	Medium
Ease of ligation	Very easy	Medium	Hard	Medium
Material memory	Very good	Medium	Poor	Medium
Breaking strength	Poor	Very good	Very good	Very good
Sterilization	Ethylene oxide or steam	Ethylene oxide	Ethylene oxide	Ethylene oxide

It is more durable than silicone, so a PU catheter can be made with thinner walls and a larger inner diameter, which can reduce the likelihood of a thrombus blocking the catheter lumen [24]. A PU catheter with a modified surface (including those with various coatings) almost does not cause thrombus formation [23, 25, 26], while uncoated PU has a rough surface that promotes thrombus formation [23].

CATHETERIZATION TECHNIQUE

Before performing experiments in which vascular catheterization is used in laboratory animals, it is necessary to obtain approval of the study protocol by the local bioethics committee.

The success of catheterization depends on careful planning of the three main stages: 1) preoperative preparation, 2) the surgery, and 3) postoperative care of the animal.

Preoperative preparation. The first stage includes preparation of the animal, the operating room, and the necessary equipment. A healthy, quarantined, and tamed animal is selected for catheterization. One day before the surgery, a clinical examination of the animal is carried out: weighing, measuring the body temperature, heart rate, assessing the condition of the coat, mucous membranes, the presence of secretions, and deviations in behavior. In addition, the animal is palpated to detect tumors and skin abnormalities. A day before the surgery or immediately before it, the hair in the area of the planned surgical wound is removed (by shaving, plucking, chemical epilation, etc.). The surface area of the shaved skin should be at least twice the estimated area of the surgical wound. Despite the fact that rodents do not have a gag reflex, it is advisable to deprive the animal of food a few hours before the surgery.

The room in which the surgery will take place must comply with the requirements of the state standard GOST R 55634-2013. In addition, it is advisable that the room is well ventilated and not walk-through. Before the surgery, the surfaces of the operating room, equipment, instruments, and furniture are treated with disinfectant and the air is disinfected using a UV germicidal lamp. The main equipment, surgical instruments, and consumables for vascular catheterization of small laboratory animals are shown in Figure 3. Additional surgical instruments and equipment (optical devices, ventilator, electric coagulator, pulse oximeter, etc.) are selected by the surgeon based on the tasks and capabilities.

Before the surgery, the surgeon puts on a cap and a mask, washes the hands, treats them with an antiseptic, then puts on a sterile gown and anatomical surgical gloves. The animal is anesthetized and anatomical landmarks are assessed to select a site for operative access to the catheterized vessel. To disinfect the operating field, the skin of the animal is treated with an antiseptic according to the principle “from the center to the periphery”. Then, during the surgery, it is important not to disrupt the created aseptic barrier and not to introduce pathogenic microorganisms that can cause postoperative complications.

Anesthesia. Vascular catheterization is an invasive procedure and needs to be performed using adequate anesthetic techniques. Anesthesia is necessary not only to relieve pain in a laboratory animal, but also to immobilize it and minimize unwanted physiological alterations caused by the stress response of the animal's body. Since in some cases anesthetics can significantly affect the course of physiological processes (for example, due to cardiorespiratory depression), this can significantly affect the results of experimental studies [27].

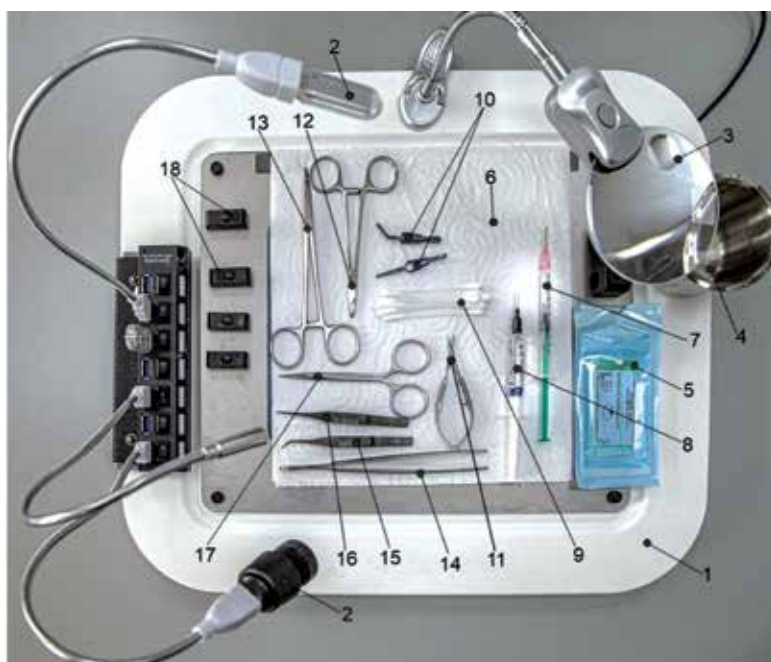


Fig. 3. Basic equipment, surgical instruments, and consumables for vascular catheterization in small laboratory animals: 1 – heated surgical platform; 2 – lighting; 3 – magnifier; 4 – procedure bowl; 5 – suture material; 6 – catheter; 7 – surgical syringe with heparinized normal saline; 8 – syringe with normal saline; 9 – cotton swabs; 10 – micro bulldog clamp; 11 – microscissors; 12 – needle holder forceps; 13 – mosquito clamp; 14 – general purpose tweezers; 15 – suture tying forceps, curved; 16 – suture tying forceps, straight; 17 – vertically curved scissors; 18 – magnetic multi-positional retractors.

Thus, it is necessary to choose such anesthetics that would not cause significant changes in the analyzed parameters of the animal. General anesthesia can be achieved via inhaled and injectable anesthetics. Among inhaled anesthetics, it is recommended to use isoflurane (3–5%) or sevoflurane (2–4%) in combination with oxygen or oxygen / air mixture [13]. When choosing injectable anesthetics, preference is given to modern combination drugs [28, 29]. These drugs include a mixture of tiletamine and zolazepam (Zoletil 100, dose for rats: 20–40 mg/kg, IM or IP), as well as its combination with xylazine (dose for rats: 5–10 mg/kg, IM or IP). With the combined use of anesthetics, the dose of each component is reduced (dose for rats: a mixture of tiletamine and zolazepam 20 mg/kg, xylazine 5 mg/kg). Unfortunately, some effective anesthetics (ketamine, barbiturates, and narcotic analgesics) are rarely used in experimental practice in the Russian Federation due to the need for their strict control and licensing of this kind of activities. Chloral hydrate (300 mg/kg, IP) and pentobarbital (40 mg/kg, IP) have a low analgesic potential; therefore, during their use, additional local anesthesia in the incision area and the catheterized vessel is recommended [27].

Catheterization technique. Before starting a surgical procedure, the surgeon should have a clear under-

standing of the anatomical structure of the operated area. Figure 4 shows a schematic anatomy of the rat neck.

Table 3 shows the stages of catheter implantation into the right external jugular vein of the rat. Figure 5 illustrates the critical stage of the surgery.

COMPLICATIONS OF VASCULAR CATHETERIZATION

During vascular catheterization, the most common complications include bleeding, damage to other tissues and organs in the operating field (trachea, esophagus, nerve trunks, salivary glands, and muscles), and respiratory and hemodynamic disturbances in the operated animal during prolonged and traumatic surgery. Adherence to the correct catheterization technique and the experience (qualifications) of the surgeon are of great importance to prevent the complications mentioned above. It should be noted that ligation of one of the carotid arteries usually does not cause cerebral ischemia [30].

After catheter implantation (especially a long-term one), the most common postoperative complications are as follows: 1) catheter-associated infection; 2) catheter occlusion; 3) pain and bleeding in the area of the surgery.

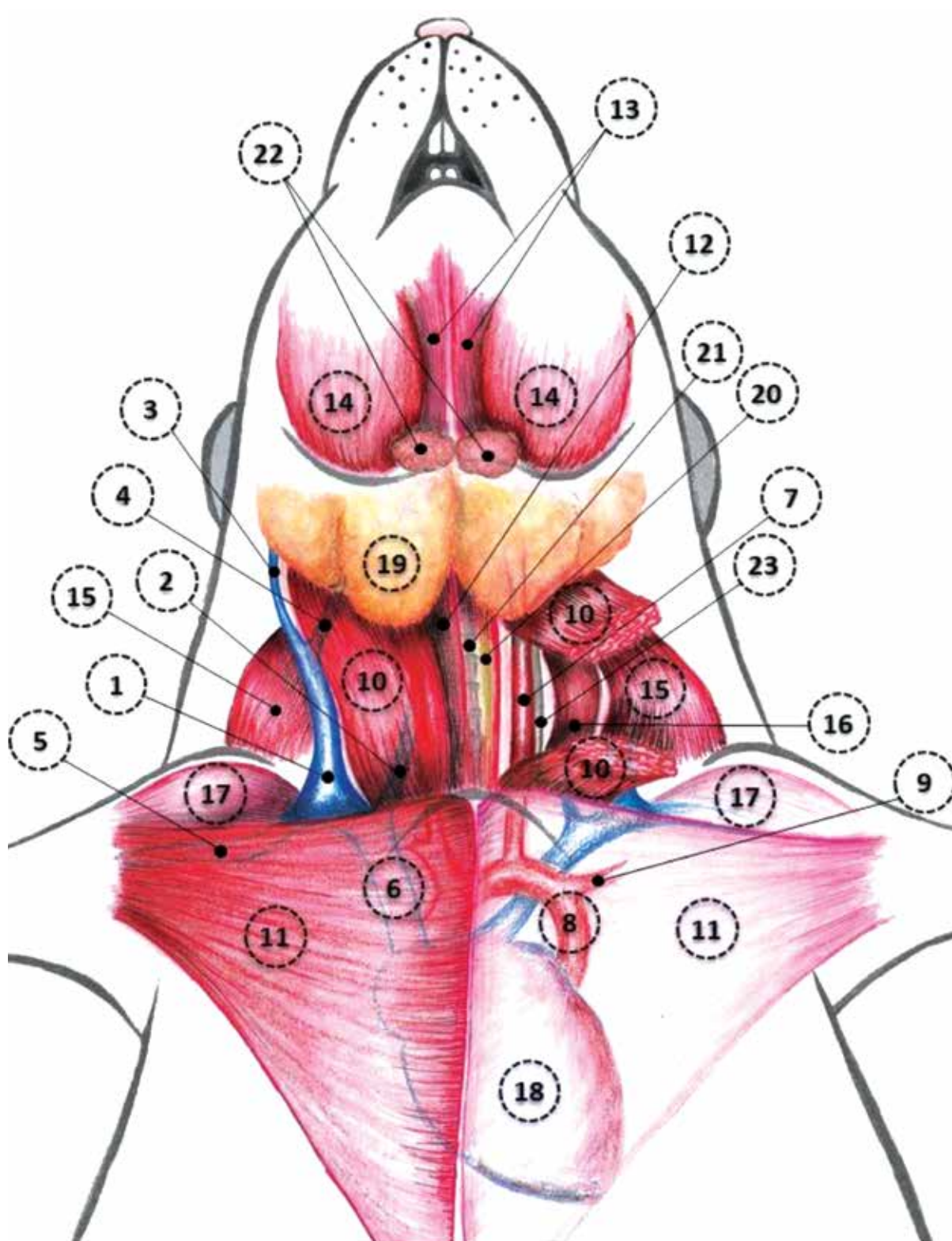


Fig. 4. Schematic illustration of the complex anatomical structures of the rat neck (illustration by K.N. Lapin): 1 – external jugular vein; 2 – internal jugular vein; 3 – anterior jugular vein; 4 – cranial jugular vein; 5 – subclavian vein; 6 – brachiocephalic vena cava; 7 – carotid artery; 8 – aortic arch; 9 – subclavian artery; 10 – sternomastoid muscle; 11 – pectoral muscle; 12 – sternohyoid muscle; 13 – digastric muscle; 14 – jaw muscle; 15 – cleidotrapezius; 16 – sternocleidomastoid muscle; 17 – deltoid muscle; 18 – heart; 19 – salivary gland; 20 – esophagus; 21 – trachea; 22 – lymph nodes; 23 – vagus nerve

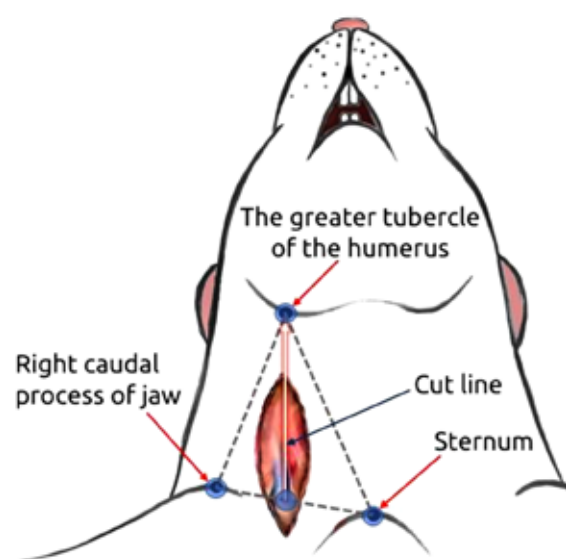


Fig. 5. Stages 1–3: 1) plan the incision line; 2) make a 1.5–2 cm incision. It should be as large as the surgeon needs and at the same time it should be as small as possible; 3) stop bleeding if necessary. During the surgery, flush the wound with normal saline to prevent the tissue from drying out

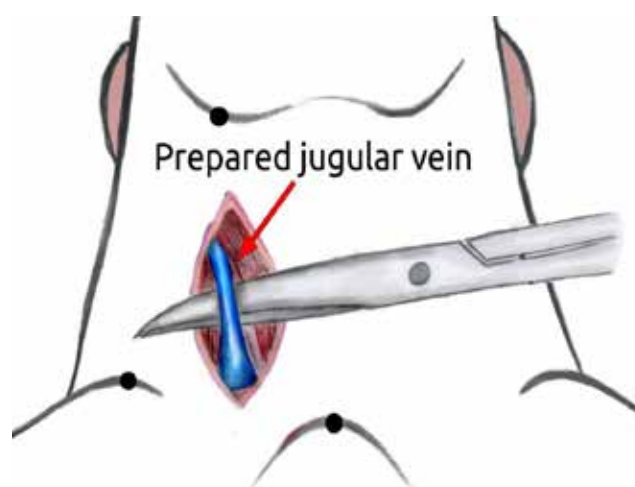


Fig. 6. Stages 4–5: 4) dissect the connective and adipose tissue with a blunt dissection using scissors until the external jugular vein is found; 5) prepare the jugular vein on all sides and separate it from the tissue pieces. The success of catheter implantation depends largely on careful isolation of the vein section

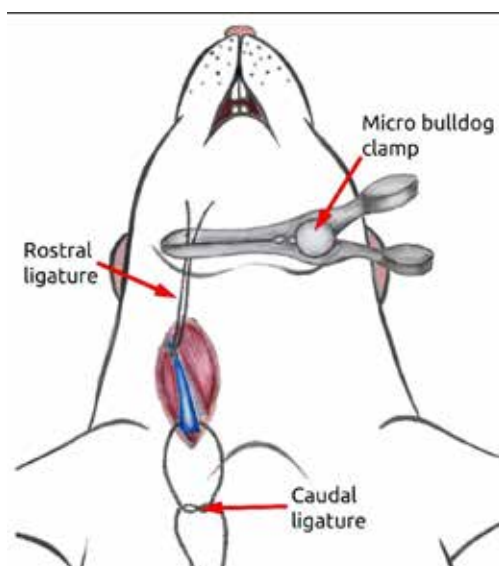


Fig. 7. Stages 6–10: 6) tie the vein with a rostral ligature (2–3 half-hitch knots) so as to stop blood flow to the heart; 7) fix the ends of the ligature with a micro bulldog clamp; 8) set a caudal ligature and stretch the vein slightly; 9) set a caudal ligature and tie it in one loose knot. Stretch the third ligature in the middle of the prepared vein section as a spare one if necessary; 9) dissect the vein wall with microscissors; pass the catheter tip caudally into the vein lumen

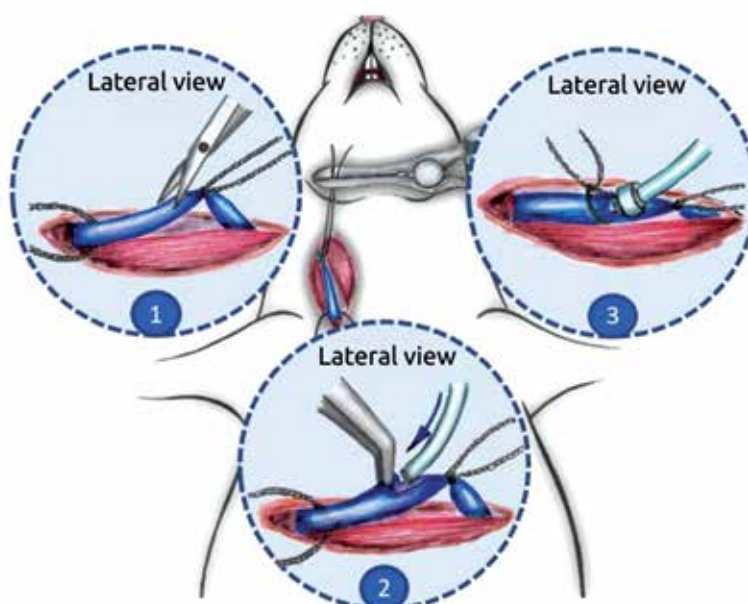


Fig. 8. Critical stage of catheter implantation into the right external jugular vein of a rat (illustration by K.N. Lapin)

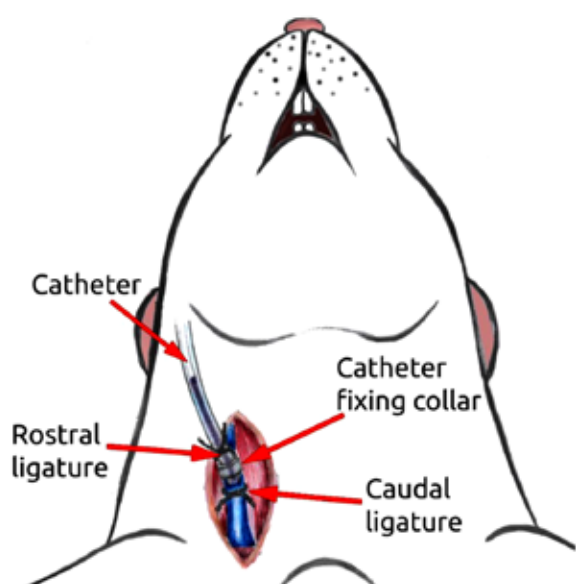


Fig. 9. Stages 11–13: 11) insert the catheter into the vein by 2–3 cm and tie the caudal ligature (2–3 half-hitch knots); 12) tie the rostral ligature tips around the catheter (2–3 half-hitch knots) so that the catheter fixing collar is positioned between the two ligatures. Tie a third ligature if necessary; 13) check the catheter for patency and the vein incision site for bleeding. Cut off the long ends of the ligatures

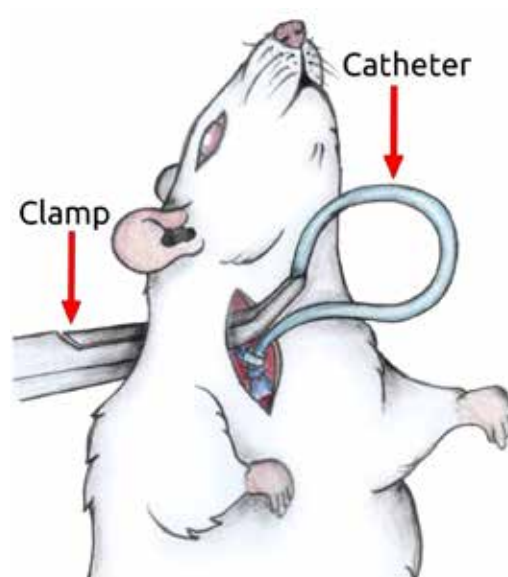


Fig. 10. Stages 14–18: 14) to tunnel the catheter subcutaneously, turn the rat to the side and grip the catheter with a clamp; 15) make a small dissection in the skin between the shoulder blades. Pass the mosquito clamp under the skin and exteriorize it out through the dissection; 16) grip the catheter outer end with a clamp and pass it under the skin. Extend the catheter fully. Connect it to the syringe and release it from the clamps; 17) check the patency of the catheter; 18) stitch up the incisions on the neck and back

Catheter-associated infection manifests itself through thrombophlebitis, sepsis, or a local infection that spreads into the tissues at the insertion site and / or along the catheter (tunnel infection). All these complications have a common pathogenesis and are closely related. For example, catheter-associated sepsis leads to the formation of blood clots in the catheterized vessel. It should be noted that vascular catheterization in small laboratory animals rarely leads to sepsis, and to prevent it, it is usually sufficient to adhere to the aseptic technique during surgical interventions [31], as well as to regularly treat the catheter insertion site with chlorhexidine-containing drugs.

The cause of catheter-associated infections is the formation of biofilms on the catheter surface when it is introduced into the bloodstream. First, the catheter surface is covered with a protein film of fibrin, collagen, elastin, etc. Then the adsorbed proteins are quickly colonized by pathogenic microorganisms. Bacteria and fungi enter the catheter from the skin and, less often, during the infusion of fluid contaminated with microorganisms [23, 32]. Enteral and parenteral administration of antibiotics to prevent catheter-associ-

ated infections is ineffective and may contribute to the development of antibiotic resistance [32, 33]. Therefore, in order to avoid colonization of catheters by microorganisms, new approaches are being actively developed, such as the use of catheter coatings that have antimicrobial, antiseptic, and / or anticoagulant effects (for example, rifampicin, silver sulfadiazine, chlorhexidine, heparin, EDTA). Numerous studies have proven the effectiveness of these coatings in reducing the incidence of catheter infections. The disadvantages of this approach include fragility of the coating, high cost, and the fact that the antimicrobial coating does not suppress the entire spectrum of pathogens [33, 34].

In addition to mechanical reasons (clamping, twisting, and improper placement in the vessel), catheter occlusion occurs mainly due to the formation of an intraluminal thrombus and, less often, due to the formation of sediment during infusion of suspensions or chemically incompatible drugs. According to the mechanism described above, a fibrin sheath (fibrin sleeve) is always formed around the intravascular part of the catheter, which can become

a focus of microbial colonization. Therefore, introduction of anticoagulants can also help prevent catheter-associated infection in addition to preventing occlusion [32, 35].

With long-term catheterization of animals and connection to automatic blood sampling systems, the most common complication is thromboembolism followed by secondary infection. A blood clot from the catheter or catheterized vessel is carried by the blood stream, most often to the kidneys or brain, which causes vascular occlusion and organ infarction, eventually leading to infection [31, 36, 37]. The most sensitive indicator of these pathological processes is weight loss of the animal in the postoperative period (more than 10% of the initial weight) [31].

CARE FOR ANIMALS WITH AN IMPLANTED CATHETER

Surgical intervention is stressful for the animal. Improper postoperative care can lead to the development of distress, which may lead to death of the animal. To minimize the suffering of the animal after the surgery, it is necessary to reduce the negative impact of external factors. The room should be warm, the light should not be too bright, noise and vibration from the laboratory equipment should be reduced, and some imitation of “shelter” should be placed in the cage, such as a few paper napkins. If the animal is in severe pain, analgesics should be given. It should be noted that severe stress can cause disturbances in hemodynamics and microcirculation, which will contribute to thrombus formation and catheter occlusion.

Long-term patency of the catheter is determined by the following factors: the place of implantation and the catheter insertion technique; biocompatibility of the material and catheter geometry; the frequency of flushing and the use of lock solutions; compliance with aseptic technique rules and antiseptics during the surgery and during postoperative care; individual physiological and biochemical characteristics of the animal. To maintain the catheter patency, it must be periodically flushed and filled with a preservation solution (catheter lock solution). The lock solution prevents blood clots from forming in the catheter when it is not in use. Ready-made solutions for locking catheters (for example, TauroLock™), in addition to their anticoagulant (or thrombolytic) effect, also have bactericidal and / or fungicidal activity. To prevent the lock solution from entering the systemic cir-

culatation of the animal, its volume should not exceed the volume of the catheter lumen and additional devices or tubes connected to the catheter. Before infusion or blood sampling, the lock solution is removed by aspiration.

During infusion or flushing of the catheter, attention should be paid to the fact that disconnection of the syringe leads to reflux of blood into the intravascular catheter end and may subsequently cause its occlusion. To prevent the backflow of blood, it is recommended to use the positive pressure technique: inject almost the entire volume of the solution with a syringe, close the catheter with a clamp before removing the syringe, and inject the remaining solution by disconnecting the syringe.

The most commonly used lock solution is sodium heparin in normal saline, although there is no clear evidence that this lock solution has more advantages than normal saline without heparin [38]. Depending on the study objectives, the final concentration of heparin can be different (usually 10–500 U/ml). It is more effective to use viscous fluids (dextrose solution, polyvinyl pyrrolidone, or glycerol) instead of normal saline, which avoids flushing of the lock solution from the catheter intravascular end. The disadvantage of heparin is that it can cause individual intolerance, as well as stimulate the formation of biofilms of *Staphylococcus aureus* [39]. Also, it cannot be used in some types of biomedical research, for example, in the analysis of mRNA [40]. Ethanol or solutions of chelating agents (sodium citrate and EDTA) are used instead of heparin [39–41]. Nevertheless, the most promising technology is creation of combined lock solutions that have both antithrombotic and antimicrobial properties with a wide spectrum of activity against bacteria and fungi [42–44].

To prevent occlusion of the catheter, it must be flushed regularly, at least every 30 minutes. In addition, flushing is always performed before and after infusion or blood sampling. During this procedure, blood clots, deposits of fibrin, lipids, and drugs, etc. are removed from the catheter lumen. Fluid flow rate plays an important role in flushing efficiency. Studies have shown that intermittent (bolus) flushing removes solid sediments from the catheter walls significantly better than continuous flow [39, 45]. Intermittent fluid delivery is most effective: 2–3 consecutive boluses at 0.4-second intervals that create turbulent flow. It should be noted that very abrupt introduction of fluid into the catheter can damage the vascular endothelium [45].

Usually, the catheter is flushed with normal saline or normal saline with heparin (1–10 U/ml). There is currently no clear evidence that flushing with heparin reduces the incidence of catheter occlusion compared with flushing with normal saline [46–49]. It should also be noted that regular long-term use of heparin can cause side effects in the animal and also affect the results of the experiment. Therefore, when flushing, it is advisable to consider the volume of the catheter lumen and aspirate the heparinized solution before using the catheter. When working with a catheter (connecting / disconnecting a syringe and infusion systems; flushing), it is very important to prevent air bubbles from entering the catheter and bloodstream to avoid air embolism and organ ischemia. This is especially important during repeated blood sampling and drug administration.

CONCLUSION

Currently, vascular catheterization in small laboratory animals, in particular, rats and mice, is widely used in research in laboratories around the world. Sampling blood by other methods often leads to hemolysis of the samples as well as to changes in the composition of the blood of animals caused by stress due to immobilization and manipulation. At the same time, in the study of biologically active substances, catheterization allows to perform infusion directly into the bloodstream and achieve maximum bioavailability. Thus, the catheterization method allows to obtain more reliable experimental data and adhere to the principles of ethical use of animals in research. In addition, when using the catheterization technique, the experiment involves a minimum number of animals, which significantly reduces the cost of the study.

It should be noted that the main limitation for the use of catheterization in research is insufficient qualification of the surgeon. Therefore, it is important to remember that the success of the surgery depends largely on how much time the surgeon devotes to developing the necessary practical skills and matrixing them into the muscle memory. This review should bridge possible gaps that prevent successful catheterization, as well as provide training in pre- and postoperative manipulations with the animal and care of the implanted catheter.

REFERENCES

1. Dudrick S.J. History of vascular access. *JPEN* 2006; 30 (1): S47–S56. DOI: 10.1177/01486071060300S1S47.
2. Kelly L.J. Pig bladders and feather quills: a history of vascular access devices. *Br. J. Nurs.* 2014; 23(19): S21–S25. DOI: 10.12968/bjon.2014.23.sup19.s21.
3. Bazin H. L'origine des rats de laboratoire, contribution à sa connaissance. *Bull. Acad. Vet. Fr.* 2001; 154: 145–150. DOI: 10.4267/2042/62578.
4. Franco N.H. Animal Experiments in Biomedical Research: A historical perspective animal. *Animals (Basel)*. 2013; 3: 238–273. DOI: 10.3390/ani3010238.
5. Nossaman B.D., Brittni M.D., Scruggs A., Vaughn B.S., Nossaman E., Subramanyam M.S., Murthy N., Kadowitz P.J. History of right heart catheterization: 100 years of experimentation and methodology development. *Cardiol. Rev.* 2010; 18 (2): 94–101. DOI: 10.1097/CRD.0b013e3181ceff67.
6. Zerati A.E., Wolosker N., de Luccia N., Puech-Leão P. Totally implantable venous catheters: history, implantation technique and complications. *J. Vasc. Bras.* 2017; 16 (2): 128–139. DOI: 10.1590/1677-5449.008216.
7. Park A.Y., Plotsky P.M., Pham T.D., Pacak K., Wynne B.M., Wall S.M., Lazo-Fernandez Y. Blood collection in unstressed, conscious, and freely moving mice through implantation of catheters in the jugular vein: a new simplified protocol. *Physiol. Rep.* 2018; 6 (21): e13904. DOI: 10.14814/phy2.13904.
8. Aske K.C., Waugh C.A. Expanding the 3R principles: more rigour and transparency in research using animals. *EMBO Rep.* 2017; 18 (9): 1490–1492. DOI: 10.15252/embr.201744428.
9. Grouzmann E., Cavadas C., Grand D., Moratel M., Aubert-J-F., Brunner H.R., Mazzolai L. Blood sampling methodology is crucial for precise measurement of plasma catecholamines concentrations in mice. *Pflugers Arch.* 2003; 447: 254–258. DOI: 10.1007/s00424-003-1140-x.
10. Vahl T.P., Ulrich-Lai Y.M., Ostrander M.M., Dolgas C.M., Elfers E.E., Seeley R.J., D'Alessio D.A., Herman J.P. Comparative analysis of ACTH and corticosterone sampling methods in rats. *Am. J. Physiol. Endocrinol. Metab.* 2005; 289 (5): E823–E828. DOI: 10.1152/ajpendo.00122.2005.
11. Karim N., Sanowar S. Jugular vein cannulation in rats – a mini review. *Canadian Journal of Pure and Applied Sciences.* 2009; 3 (3): 929–935.
12. Pacher P., Nagayama T., Mukhopadhyay P., Bátkai S., Kass D.A. Measurement of cardiac function using pressure – volume conductance catheter technique in mice and rats. *Nat. Protoc.* 2008; 3 (9): 1422–1434. DOI: 10.1038/nprot.2008.138.
13. Feng J., Fitz Y., Li Y., Fernandez M., Puch I.C., Wang D., Pazniokas S., Bucher B., Cui X., Solomon S.B. Catheterization of the carotid artery and jugular vein to perform hemodynamic measures, infusions and blood sampling in a conscious rat model. *J. Vis. Exp.* 2015; 95: 51881. DOI: 10.3791/51881.
14. Segreti J.A., Polakowski J.S., Blomme E.A., King A.J. Simultaneous measurement of arterial and left ventricular pressure in conscious freely moving rats by telemetry. *J. Pharmacol. Toxicol. Methods.* 2016; 79: 23–33. DOI: 10.1016/j.vascn.2016.01.003.
15. Zhang X.D., Pechter D., Yang L., Ping X., Yao Z., Zhang R., Shen X., Li N.X., Connick J., Nawrocki A.R., Chakravarthy M., Li C. Decreased complexity of glucose dynamics preceding the onset of diabetes in mice and rats. *PLoS One.* 2017; 12 (9): e0182810. DOI: 10.1371/journal.pone.0182810.

16. Bogdan S., Luca V., Ober C., Melega I., Pestean C., Codea R., Oana L. Comparison among different methods for blood pressure monitoring in rats: literature review. *Bull. Univ. Agric. Sci. Vet. Med. Cluj Napoca*. 2019; 76 (1): 5–19. DOI: 10.15835/buasvmcn-vm:2019.0007.
17. Higgins C. Lactate measurement: arterial versus venous blood sampling. Retrieved from *Acutecaretesting. Org*. 2017. URL: <https://acutecaretesting.org/en/articles/lactate-measurement-arterial-versus-venous-blood-sampling>
18. Guidelines for conducting preclinical studies of drugs. Part 1; ed. A.N. Mironov. M.: Grif and K, 2012: 944 (in Russ.).
19. Moureau N. Vessel health and preservation: the right approach for vascular access. *Springer Open*, 2019: 303. DOI: 10.1007/978-3-030-03149-7_23.
20. Jacobs B.R. Central venous catheter occlusion and thrombosis. *Crit. Care Clin*. 2003; 19 (3): 489–514. DOI: 10.1016/s0749-0704(03)00002-2.
21. Galloway S., Bodenham A. Long-term central venous access. *Br. J. Anaesth*. 2004; 92 (5): 722–734. DOI: 10.1093/bja/ae109.
22. Francolini I., Piozzi A. Antimicrobial polyurethanes for intravascular medical devices. In Book: *Advances in polyurethane biomaterials*; 1st ed., eds. Cooper S.L., Guan J. Cambridge, Massachusetts: Woodhead Publishing, 2016: 349–385. DOI: 10.1016/b978-0-08-100614-6.00012-3.
23. Neoh K.G., Li M., Kang E.-T., Chiong E., Tambyah P.A. Surface modification strategies for combating catheter-related complications: recent advances and challenges. *J. Mater. Chem. B*. 2017; 5: 2045–2067. DOI: 10.1039/c6tb03280j.
24. Teilmann A.C., Falkenberg M.K., Hau J., Abelson K.S.P. Comparison of silicone and polyurethane catheters for the catheterization of small vessels in mice. *Lab. Anim*. 2014; 43 (11): 397–403. DOI: 10.1038/labani.570.
25. Xu L.-C., Siedlecki C.A. Antibacterial polyurethanes. In Book: *Advances in polyurethane biomaterials*; 1st ed., eds. Cooper S.L., Guan J. Cambridge, Massachusetts: Woodhead Publishing, 2016: 247–284. DOI: 10.1016/b978-0-08-100614-6.00009-3.
26. Gunaratnam G., Spengler C., Trautmann S., Jung P., Mischo J., Wieland B., Metz C., Sören B.L., Hannig M., Jacobs K., Bischof M. Human blood plasma factors affect the adhesion kinetics of *Staphylococcus aureus* to central venous catheters. *Sci. Rep*. 2020; 10 (1): 20992. DOI: 10.1038/s41598-020-77168-x.
27. Flecknell P.A. Laboratory animal anaesthesia; 3rd ed. Laboratory animal anaesthesia; 3rd ed. DOI: 10.1016/B978-0-12-369376-1.X0001-9.
28. Heiser A., Liu J.H.K. Rat jugular vein and carotid artery catheterization for acute survival studies. New York: Springer, 2007: 116. DOI: 10.1007/0-387-49416-2.
29. Uhlig C., Krause H., Koch T., De Abreu M.G., Spieth P.M. Anesthesia and Monitoring in Small Laboratory Mammals Used in Anesthesiology, Respiratory and Critical Care Research: A Systematic Review on the Current Reporting in Top-10 Impact Factor Ranked Journals. *PLoS One*. 2015; 10 (8): e0134205. DOI: 10.1371/journal.pone.0134205.
30. Manual of stroke models in rat; 1st ed., ed. Wang-Fischer Y. Boca Raton: CRC Press; 2009: 352. DOI: 10.1201/9781420009521.
31. Allavena R.E., West H., Gale J., Debre M. Pathological and clinical analysis of vascular catheterization models in rats, with exploration of interventions to improve clinical tolerance. *Toxicol. Pathol*. 2016; 44 (8): 1095–1104. DOI: 10.1177/0192623316666197
32. Matveeva E.Yu., Vlasenko A.V., Yakovlev V.N., Alekseev V.G. Infectious complications of central venous catheterization. *General Reanimatology*. 2011; VII (5): 67–74 (in Russ.). DOI: 10.15360/1813-9779-2011-5-67.
33. Mendoza G., Regiel-Futyr A., Tamayo A., Monzon M., Irusta S., De Gregorio M.A., Kyzioł A., Arruebo M. Chitosan-based coatings in the prevention of intravascular catheter-associated infections. *J. Biomater. Appl*. 2017; 32 (6): 725–737. DOI: 10.1177/0885328217739199.
34. Viola G.M., Rosenblatt J., Raad I.I. Drug eluting antimicrobial vascular catheters: progress and promise. *Adv. Drug Deliv. Rev*. 2017; 112: 35–47. DOI: 10.1016/j.addr.2016.07.011.
35. Yang J., Maarek J.-M. I., Holschneider D.P. In vivo quantitative assessment of catheter patency in rats. *Lab. Anim*. 2005; 39 (3): 259–268. DOI: 10.1258/0023677054307033.
36. Fonseca U.N.K., Nielsen S.G., Hau J., Hansen A.K. Permanent catheterization of the carotid artery induces kidney infection and inflammation in the rat. *Lab. Anim*. 2010; 44: 46–53. DOI: 10.1258/la.2009.008122.
37. Teilmann A.C., Rozell B., Kalliokoski O., Hau J., Abelson K.S.P. Carotid catheterization and automated blood sampling induce systemic IL-6 secretion and local tissue damage and inflammation in the heart, kidneys, liver and salivary glands in NMRI mice. *PLoS One*. 2016; 11 (11): e0166353. DOI: 10.1371/journal.pone.0166353.
38. López-Briz E., Garcia V.R., Cabello J.B., Bort-Martí S., Sanchis R.C., Burls A. Heparin versus 0.9% sodium chloride locking for prevention of occlusion in central venous catheters in adults. *Cochrane Database Syst. Rev*. 2018; 7 (7): CD008462. DOI: 10.1002/14651858.CD008462.pub3.
39. Goossens G.A. Flushing and locking of venous catheters: available evidence and evidence deficit. *Nurs. Res. Pract*. 2015; 2015: 985686. DOI: 10.1155/2015/985686.
40. De Luca T., Szilágyi K.L., Hargreaves K.A., Collins K.S., Benson E.A. Improving the patency of jugular vein catheters in sprague – dawley rats by using an antiseptic nitrocellulose coating. *J. Am. Assoc. Lab. Anim. Sci*. 2018; 57 (5): 520–528. DOI: 10.30802/aalas-jaalas-18-000017.
41. Mermel L.A., Alang N. Adverse effects associated with ethanol catheter lock solutions: a systematic review. *J. Antimicrob. Chemother*. 2014; 69: 2611–2619. DOI: 10.1093/jac/dku182.
42. Rosenblatt J., Reitzel R.A., Vargas-Cruz N., Chaftari A.-M., Hachem R., Raad I.I. Comparative efficacies of antimicrobial catheter lock solutions for fungal biofilm eradication in an in vitro model of catheter-related fungemia. *J. Fungi (Basel)*. 2017; 3 (1): 7. DOI: 10.3390/jof3010007.
43. Chandra J., Long L., Isham N., Mukherjee P.K., DiSciullo G., Appelt K., Ghannoum M.A. In vitro and in vivo activity of a novel catheter lock solution against bacterial and fungal biofilms. *Antimicrob. Agents Chemother*. 2018; 62 (8): e00722–18. DOI: 10.1128/aac.00722-18.
44. Hernández M.J., Soriano A., Filella X., Calvo M., Coll E., Rebled J.M., Poch E., Graterol F., Compte M.T., Maduell F.,

- Fontser N. Impact of locking solutions on conditioning bio-film formation in tunnelled haemodialysis catheters and inflammatory response activation. *J. Vasc. Access.* 2020; 21: 1129729820942040. DOI: 10.1177/1129729820942040.
45. Tong C., Peng X., Hu H., Wang Z., Zhou H. The effect of different flushing methods in a short peripheral catheter. *Acta Cir. Bras.* 2019; 34 (8): e201900804. DOI: 10.1590/s0102-865020190080000004.
46. Mitchell M.D., Anderson B.J., Williams K., Umscheid C.A. Heparin flushing and other interventions to maintain patency of central venous catheters: a systematic review. *J. Adv. Nurs.* 2009; 65(10): 2007–2021. DOI: 10.1111/j.1365-2648.2009.05103.x.
47. Ueda Y., Odunayo A., Mann F.A. Comparison of heparinized saline and 0.9% sodium chloride for maintaining peripheral intravenous catheter patency in dogs. *J. Vet. Emerg. Crit. Care (San Antonio)*. 2013; 23 (5): 517–522. DOI: 10.1111/vec.12093.
48. Dos Santos E.J.F., Nunes M.M.J.C., Cardoso D.F.B., Apóstolo J.L.A., Queirós P.J.P., Rodrigues M.A. Effectiveness of heparin versus 0.9% saline solution in maintaining the permeability of central venous catheters: a systematic review. *Rev. Esc. Enferm. USP.* 2015; 49 (6): 995–1003. DOI: 10.1590/s0080-623420150000600017.
49. Vose J., Odunayo A., Price J.M., Daves M., Schildt J.C., Tolbert M.K. Comparison of heparinized saline and 0.9% sodium chloride for maintaining central venous catheter patency in healthy dogs. *Peer J.* 2019; 7: e7072. DOI: 10.7717/peerj.7072.

Authors information

Lapin Konstantin N., Researcher, Laboratory of Experimental Research, Negovsky Research Institute of General Resuscitation, Federal Research and Clinical Center of Intensive Care Medicine and Rehabilitology, Moscow, Russian Federation. ORCID 0000-0002-7760-3526.

Ryzhkov Ivan A., Cand. Sci. (Med.), Head of the Laboratory of Experimental Research, Negovsky Research Institute of General Resuscitation, Federal Research and Clinical Center of Intensive Care Medicine and Rehabilitology, Moscow, Russian Federation. ORCID 0000-0002-0631-5666.

Maltseva Valentina A., Researcher, Scientific Center “Signal”, Moscow, Russian Federation. ORCID 0000-0001-6669-6205.

Udut Elena V., Dr. Sci. (Med.), Leading Researcher, Laboratory of Pathological Physiology and Regenerative Medicine, E.D. Goldberg Research Institute of Pharmacology and Regenerative Medicine, Tomsk NRMC, Tomsk, Russian Federation. ORCID 0000-0002-6104-4782.

(✉) **Lapin Konstantin N.**, e-mail: knlapin@gmail.com

Received 24.12.2020

Accepted 28.12.2020

Molecular genetic aspects of prostate cancer radioresistance

Omelchuk E.P., Kutilin D.S., Dimitriadi S.N., Gusareva M.A., Timoshkina N.N.

*National Medical Research Center for Oncology
63, 14 Liniya Str., Rostov-on-Don, 344037, Russian Federation*

ABSTRACT

Radioresistance of prostate cancer is a complex therapeutic problem. Biochemical recurrence after radiation therapy occurs in 22–69% of patients with prostate cancer. Nearly half of these patients progress to a clinical relapse within 15 years, and a third progress to castration-resistant prostate cancer. This review analyzes literature data on radioresistance mechanisms in prostate cancer cells. We searched for literature published in eLibrary, PubMed, and Scopus databases by key words: prostate cancer, radioresistance, markers. In total, 568 foreign and 178 national articles published between 1975 and 2020 were found. Of these publications, 77 articles were selected (published in 2001–2020), which reveal the molecular basis of tumor radioresistance.

Modern understanding of the origin of radioresistant cancer cells focuses on processes leading to enhanced DNA repair, activation of anti-apoptotic signaling pathways, and a decrease in the level of endogenous and exogenous reactive oxygen species. The state of a tumor microenvironment, autophagy, and epithelial-mesenchymal transition also play an important role in radioresistance. Currently, the mechanisms of resistance to radiation therapy are explained by the existence of tumor stem cells, which provide genetic heterogeneity and activation of carcinogenesis signaling pathways. The tumor can also be protected from radiation by a hypoxic microenvironment. Since cancer stem cells can acquire plasticity in response to radiation therapy, search for markers of radioresistance for screening and identification of radioresistant prostate cancer is relevant.

Key words: prostate cancer, radioresistance, cancer stem cells, DNA repair, reactive oxygen species, epithelial-mesenchymal transition, microenvironment, autophagy.

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

Source of financing. The study was carried out within the state assignment “Search for molecular genetic predictors of radioresistant prostate cancer and development of personalized treatment approaches”.

For citation: Omelchuk E.P., Kutilin D.S., Dimitriadi S.N., Gusareva M.A., Timoshkina N.N. Molecular genetic aspects of prostate cancer radioresistance. *Bulletin of Siberian Medicine*. 2021; 20 (3): 182–192. <https://doi.org/10.20538/1682-0363-2021-3-182-192>.

Молекулярно-генетические аспекты радиорезистентности рака предстательной железы

Омельчук Е.П., Кутилин Д.С., Димитриади С.Н., Гусарева М.А., Тимошкина Н.Н.

*Национальный медицинский исследовательский центр (НМИЦ) онкологии
Россия, 344037, г. Ростов-на-Дону, ул. 14-я линия, 63*

РЕЗЮМЕ

Радиорезистентность рака предстательной железы представляет собой сложную терапевтическую проблему. После проведения лучевой терапии 22–69% больных раком предстательной железы

сталкиваются с биохимическим рецидивом. Почти половина таких пациентов прогрессирует до клинического рецидива в течение 15 лет, а у трети наблюдается прогрессия до кастрационно-резистентного рака. Настоящий обзор посвящен анализу данных литературы о механизмах развития радиорезистентности в онкотрансформированных клетках предстательной железы. Осуществлен поиск литературных источников, опубликованных в базах eLibrary, PubMed, Scopus по ключевым словам: рак предстательной железы, радиорезистентность, маркеры. Всего найдено 568 иностранных и 178 отечественных работ, опубликованных в период 1975–2020 гг., из которых отобрано 77 статей, раскрывающих молекулярную основу радиорезистентности и вышедших в печать в 2001–2020 гг.

Современные представления о происхождении устойчивых к радиации злокачественных клеток концентрируются на процессах, приводящих к усиленной репарации ДНК, активации антиапоптотических сигнальных путей, снижению уровня эндо- и экзогенных активных форм кислорода. Также немаловажную роль играют состояние микроокружения опухоли, аутофагия и эпителиально-мезенхимальный переход. Механизмы развития устойчивости к радиационному лечению на сегодняшний день объясняются наличием стволовых клеток опухоли, которые обуславливают генетическую гетерогенность и возможность ухода от воздействия терапии с помощью активации сигнальных путей канцерогенеза. Также опухоль может быть защищена от радиации гипоксической средой. Ввиду возникающей пластичности опухолевых стволовых клеток в ответ на лучевую терапию актуальным представляется поиск их маркеров с целью скрининга и идентификации радиорезистентного рака предстательной железы.

Ключевые слова: рак предстательной железы, радиорезистентность, опухолевые стволовые клетки, репарация ДНК, активные формы кислорода, эпителиально-мезенхимальный переход, микроокружение, аутофагия.

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

Источник финансирования. Исследование выполнено в рамках государственного задания «Поиск молекулярно-генетических предикторов радиорезистентного рака предстательной железы и разработка персонализированных терапевтических подходов».

Для цитирования: Омельчук Е.П., Кутилин Д.С., Дмитрияди С.Н., Гусарева М.А., Тимошкина Н.Н. Молекулярно-генетические аспекты радиорезистентности рака предстательной железы. *Бюллетень сибирской медицины*. 2021; 20 (3): 182–192. <https://doi.org/10.20538/1682-0363-2021-3-182-192>.

INTRODUCTION

Prostate cancer (PC) is the second most common malignant disease diagnosed in men worldwide. It is also the fifth leading cause of cancer death [1, 2]. According to GLOBOCAN estimates, 1,276,106 new cases of PC were reported worldwide in 2018, with higher prevalence in developed countries, which reflects differences in diagnostic capabilities. PC incidence and mortality rates are strongly related to age: the highest incidence is seen in men over the age of 65 [3].

PC may be asymptomatic at early stages [3]. 58.5% of patients are diagnosed with localized PC which is characterized by a low-to-intermediate risk [4]. Patients without distant metastases respond successfully to treatment, in particular, to radiation therapy [5]. Radiation therapy can be an alternative to surgical treatment as a primary monotherapy for low- and intermediate-risk PC, as well as for treatment of locally advanced PC in combination therapy. Patients diag-

nosed with locally advanced PC (pT3a or pT3b) often require additional adjuvant radiation therapy after radical prostatectomy. Radiation therapy for oligometastatic PC is possible, but at the moment it is used only in clinical trials and requires further discussion [6, 7].

Radioresistance of a subpopulation of prostate cancer cells may be one of the possible reasons for continued tumor growth after radiation therapy [2]. Tumor progression and spread of distant metastases disable the use of classical therapy regimens [5]. Treatment recommendations differ depending on the time of the biochemical relapse onset. When it occurs after radical prostatectomy, radiation therapy is an option. When the relapse is detected after radiation therapy, alternative treatments are used [8].

Deciphering the mechanisms of PC radioresistance onset seems to be necessary due to the recurrence risk. The main mechanisms underlying radioresistance include disruption of DNA repair processes, activation of anti-apoptotic signaling pathways, a decrease in

intracellular reactive oxygen species (ROS), and epithelial – mesenchymal transition (EMT). Hypoxia of a tumor microenvironment and autophagy also contribute to the development of radioresistance. Currently, it is assumed that these processes take place not in all cancer cells, but only in the so-called cancer stem cells [9]. Presumably, they promote recurrence and metastasis both due to the reasons stated above and due to their unique ability to reproduce the heterogeneity of the primary tumor [10]. Searching for prostate cancer stem cell markers for identification, prognosis, and targeted treatment is critical for improving therapeutic and clinical outcomes.

CANCER STEM CELLS

Cancer stem cells (CSCs) have fundamental properties that distinguish them from other malignant cells: the ability to initiate carcinogenesis, unlimited self-renewal, and the ability to differentiate into all cell populations present in the primary tumor. The last two features underlie growth and progression of malignant neoplasms [11]. More evidence is emerging to support the dynamic nature of cancer stemness. The epithelial – mesenchymal transition (EMT) reflects plasticity of CSCs and determines the degree of tumor invasion and metastasis. Activation of the Notch, Hedgehog, WNT / β -Catenin, JAK / STAT, and NF κ B signaling pathways is observed in regulation of plasticity of both healthy and cancer stem cells [12, 13].

There are two concepts in the CSC origin. The first concept postulates their formation from postnatal stem cells; another hypothesis explains their occurrence by reprogramming of differentiated tumor cells [13], and EMT is the most representative process of cell reprogramming [12]. CSCs are characterized by changes in the activity of many signaling cascades; however, the importance of transcription factors OCT3/4, SOX2, KLF4, and cMYC, which regulate the work of genes responsible for pluripotency, is especially emphasized [14].

In addition to genetic reprogramming, the mechanisms of epigenetic reprogramming similar to processes taking place in embryonic stem cells play an important role in CSC formation [11]. In particular, genes of the Polycomb repressive complex, which provide pluripotency of stem cells through histone post-translational modifications, are overexpressed in prostate cancer [15]. DNA methylation is also an epigenetic feature of CSCs. DNA methyltransferases DNMT1 and DNMT3, which are required to maintain existing patterns of methylation and *de novo* methyl-

ation in CpG islands, are factors in CSC reprogramming [16].

Radioresistant subpopulations of prostate cancer cells are proved to have many properties in common with prostate CSCs, in particular, increased expression of CD133, CXCR4, ABCG2, OCT4, and NANOG. In addition, exposure of prostate cells to ionizing radiation stimulates constitutive activation of stem cell markers, which reprogram them epigenetically and mediate radioresistance formation. In particular, increased methylation of the H3 histone in the *ALDH1A1* promoter stimulates its transcription [17]. Inhibition of methylation causes apoptosis and decreased radioresistance in prostate cancer cells [18].

The most well-known markers of prostate CSCs are α v β 3-integrin, E-cadherin, N-cadherin, vimentin, NANOG, OCT4 and SOX2, as well as markers of EMT, for example, CD44.

Experimental studies use CD44, CD133, and α 2 β 1 markers to isolate a population of prostate CSCs. Caveolin-1, a membrane protein involved in receptor-independent endocytosis [19], and aldehyde dehydrogenase 1A1 (*ALDH1A1*), which catalyzes oxidation of retinal to retinoic acid, a signaling molecule of cell differentiation and self-renewal of stem cells [9], are considered as potential predictors of PC radioresistance.

Radioresistance of CSCs is now believed to be a complex set of complementary mechanisms. The presence of CSCs in the total tumor mass partially explains the phenomenon of cell resistance to ionizing radiation. Processes that promote resistance to radiation therapy occur in CSCs and include enhanced DNA repair, activation of apoptosis suppressors, EMT, decreased ROS levels, autophagy, and the state of the tumor microenvironment.

DNA REPAIR

Activation of cell cycle checkpoints, which cause its intermittence to correct defects in the nucleotide sequence, is an integral part of a cellular response to DNA damage caused by radiation [20]. Adaptation to replication stress involves repairing single- and double-strand breaks in DNA, which leads to increased activation of replication after radiation therapy [21]. Cells use two main mechanisms to repair DNA damage: non-homologous end joining (NHEJ) and homologous recombination (HR). HR is induced in the S and G2 phases of the cell cycle, while NHEJ can be activated at any point in the cell cycle with the greatest efficiency at the G2 stage of mitosis and pre-

dominance in the G0, G1, and early S phases [23]. The greatest radioresistance is observed in the late S phase and explained by increased replication, which promotes HR [24].

Dysregulation of the signaling cascades EGFR, PI3K / Akt / mTOR, ATM-Chk2, WNT, Notch, and Hedgehog is associated with CSC radioresistance [25–27]. Radioresistant prostate stem cells are characterized by increased phosphorylation of Chk2 and AKT. These modifications cause arrest of cell proliferation through the ATM-Chk2 pathway and enhancement of DNA repair through activation of the PI3K signaling cascade [17]. In their model experiment, S. Yadav et al. showed overexpression of *SMC1A* in prostate cancer cell lines DU145 and PC3. Knock-down of *SMC1A* increased the efficiency of radiation therapy in these cells, which could be associated with ATM-mediated repair of DNA double-strand breaks [27]. Ionizing radiation causing this effect leads to a delay in the S phase of DNA repair [28]. In contrast to the rest of the tumor mass, stem cells are characterized by reduced activation of the p53 signaling pathway after radiation therapy. As a result, normal functioning of the cell cycle and apoptosis is disrupted and, ultimately, mutations accumulate. Over time, mutational load increases intratumoral heterogeneity and leads to the disease progression [29].

Radioresistance supported by a high level of DNA repair is also determined by indirect mechanisms, and one of them is a decrease in ROS production causing DNA damage and ROS-dependent apoptosis [30]. Other mechanisms include increased expression of APE1 / Ref-1 and activation of the NNMT pathway, which decreases intracellular ROS levels [30, 31].

ACTIVATION OF APOPTOSIS SUPPRESSORS

Radiation therapy involves death of tumor cells due to activation of apoptotic signaling pathways in response to single- and double-strand breaks in DNA. Apoptosis disruption can be one of the reasons for radioresistance. Activation of the Wnt / β -catenin signaling pathway is excessive in prostate CSCs. This allows cells to activate their repair mechanisms when avoiding apoptosis [32]. A model experiment on the DU145 cell line showed that overexpression of the *SOX2* gene increased resistance to apoptosis, delaying cleavage of caspase-3 and decreasing supply of Ca^{2+} ions. The subsequent knockdown of *SOX2* increased sensitivity to apoptosis, which suggests an association between *SOX2* overexpression and radioresistance [33]. In turn, resistance to apoptosis may be due to

the differentiation status of stem cell differentiation, external factors of the microenvironment, and hypoxic conditions [9].

PRODUCTION OF REACTIVE OXYGEN SPECIES

Exposure to ionizing radiation causes excessive production of ROS, which are formed either directly through water radiolysis or indirectly through mitochondrial damage or metabolic changes [34]. The produced ROS molecules damage not only DNA, but also proteins and lipids, which complicates the process of cell repair after radiation-induced damage. Cancer cells, in contrast to non-malignant cells, have higher ROS levels due to the increased metabolic rate and the use of glycolysis for energy production. Despite the cytostatic effect of ionizing radiation, the production of ROS creates a favorable environment for acquisition of stemness properties by cancer cells [18]. Given the low proliferation rate of CSCs, they demonstrate lower levels of endogenous ROS, and, therefore, can neutralize their production more efficiently [21]. Oxygen-dependent production of free radicals can contribute to resistance to radiation therapy [35].

Radioresistant prostate tumors are characterized by lower baseline levels of intracellular ROS, which indicates implementation of another mechanism of radioresistance through their increased absorption. This is confirmed by studies that demonstrated increased expression of ROS acceptors in prostate CSCs [36]. In the DU145 and PC3 PC cell lines, an increase in intracellular ROS levels and a decrease in glutathione levels during repression of *SMC1A* were demonstrated, which leads to radiosensitization of cells in response to radiation therapy [27]. In addition, some transcription factors can affect the level of antioxidant proteins. For example, NF- κ B is required to resist oxidative stress, while NRF2 in combination with ARE is capable of stimulating radioresistance [37, 38].

Hypoxia of the microenvironment contributes to the development of radioresistance of PC cells through impaired ROS production and activation of hypoxia-inducible factor (HIF). The HIF-2 α subunit regulates the transcriptional activity of genes responsible for tolerating hypoxia and maintaining an undifferentiated phenotype, which stimulates selection of a radioresistant population of cancer cells [39]. The effect of a hypoxic microenvironment on CSCs is currently a promising area of research.

EPITHELIAL – MESENCHYMAL TRANSITION

The phenotype of CSCs is quite plastic and associated with EMT, which can be caused by exposure to radiation and hypoxic conditions of the microenvironment [40]. EMT is characterized by cell motility, inhibition of adhesion, loss of polarity, and interaction with the extracellular matrix. The key features of EMT, namely, a decrease in E-cadherin and an increase in N-cadherin and vimentin, are significantly more pronounced after radiation therapy for PC [41]. Induction of EMT and acquisition of stemness properties by cells are associated with activation of the PI3K / AKT / mTOR pathway. Studies of CSCs isolated from a human PC cell line obtained after prostatectomy also showed the presence of CD44 expression, which is an EMT marker [33].

The mechanism of radioresistance acquisition through EMT is implemented mainly through activation of the TGF- β , Wnt / β -catenin, Hedgehog, Notch, NANOG, and STAT3 signaling pathways [42]. EMT mediated by ionizing radiation and signaling through the TGF- β pathway plays a crucial role in enhancing the migratory and invasive capabilities of cancer cells [43]. EMT partially contributes to maintenance of the CSC phenotype [44]. In particular, activation of the main transcription factors of EMT signaling cascades, such as Snail, HIF, ZEB1 / 2, and Twist1, mediates stemness features of the tumor [45]. Snail plays a decisive role not only in migration and invasion of cancer cells, but also in the radiation-induced EMT [46]. Constitutive activation of Snail, which mediated acquisition of mesenchymal properties by the tumor, was revealed in a model of human PC cells [47]. The transcription factor ZEB1 represses miR-183, miR200c, and miR203, which exhibit antitumor activity [48]. Finally, Twist1 positively regulates the proto-oncogene *BMII*, thereby provoking EMT and forming tumor microenvironment niches [49].

Thus, EMT is a dynamic process with many transients. The presence of stem cells is a favorable condition for maintaining the vital activity of the tumor and avoiding radiation therapy by balancing between the epithelial and mesenchymal stages.

AUTOPHAGY

Autophagy is a natural process that allows cells to cope with stress by recycling damaged cellular components. Prolonged autophagy induction can lead to cell death due to excessive degradation of key intracellular components [50]. Therefore, autophagy performs a double paradoxical function – it suppresses

tumor growth at early stages by removing damaged proteins, but promotes tumor growth and survival at later stages under conditions of nutrient deficiency and hypoxia [51].

The role of autophagy in the survival and death of cancer cells seems to differ depending on a disease and is not completely clear in PC [52]. The data on the effect of autophagy suppression in prostate CSCs are very contradictory. S. Paglin et al. found that inhibition of autophagy leads to radiosensitization of LNCaP cells [53]. In contrast, Yao et al. showed improvement of the viability of PC cells with inhibition of autophagy in PC3 and LNCaP cells and xenograft mouse models [54]. The unidentified role of autophagy determines the relevance of a more detailed study of its contribution to the development of PC radioresistance.

TUMOR MICROENVIRONMENT

In addition to the internal mechanisms of radioresistance, the fate of tumor cells after radiation therapy depends on a variety of signals emanating from the tumor microenvironment. The latter consists of components that include, in addition to the extracellular matrix, fibroblasts and inflammatory, immune, vascular, and endothelial cells. All these components interact through cytokines, chemokines, and growth factors, creating a hypoxic, inflammatory, and immunosuppressive microenvironment. Ionizing radiation can act as a modifier of mechanisms that cause release of growth factors, activation of tumor-associated fibroblasts, and induction of inflammation and hypoxia [11]. These conditions are favorable for tumor growth, progression, and metastasis [55].

Hypoxia causes significant changes in tumor metabolism due to nutrient deficiency, low oxygen concentration, and dysregulation of carrier proteins and metabolic enzymes [56]. Persistent hypoxia can trigger mechanisms including activation of the HIF-1 α signaling pathway, autophagy, and EMT. Their importance in the development of radioresistance is explained by the possibility to maintain the vital function of CSCs [57].

CSCs are located in specific areas of the microenvironment, called niches, that provide autocrine signaling, as well as signals from tumor-associated fibroblasts, immune and endothelial cells, and components of the extracellular matrix [58]. Despite poor understanding of the composition of niches and their signaling interaction with the tumor, the microenvironment is known to supply CSCs with oxygen and nutrients

and support their functions protecting them from such effects as radiation [59].

Perivascular and hypoxic CSC niches are the most studied types. The perivascular niche is located in the immediate vicinity of blood vessels, supplying cells with nutrients, growth factors, and cytokines. This environment can induce secretion of stem cell factors, such as OCT4 and NANOG, to initiate transformation of cancer cells into cancer stem cells [60].

Hypoxia is another mechanism used by the niche to protect CSCs. Oxygen is a potent radiosensitizer, and it is required for radiation-induced ROS production and, as a consequence, for cell death. A lack of oxygen increases radiation resistance of cells and is associated with an early relapse after radiation therapy [61]. In addition, ROS neutralization is increased in the hypoxic niches [11]. Radiation therapy induces production of PDGF, IL-1 β , IL-6, TNF α , TGF β , CXCL12, and MMPs in the tumor microenvironment, which leads to activation of ROS acceptors and the STAT3 signaling cascade, promoting self-renewal and survival of cancer cells [62, 63].

In addition to the above-mentioned mechanisms, CSCs are supposed to be able to exist in a quiescent state which provides the basis for new micrometastases or initiation of a tumor relapse [30].

MARKERS OF PROSTATE CANCER RADIORESISTANCE

Defining prognostic determinants of a response to radiation therapy is an important task of research on PC radioresistance. Signaling pathways PI3K / Akt, MAPK / ERK, and VEGF and glucose metabolism are essential for radioresistant PC [64, 65].

The metastatic capability of radioresistant PC cells is also explained by increased expression of heat shock protein 90 (HSP90) [36]. PC resistance to radiation therapy may arise due to nuclear translocation of β -catenin induced by HIF-1 overexpression [57]. The ineffectiveness of radiation therapy can most likely be indicated by expression of PCSC1- and PCSC2-RAN. The CXCR4 and CXCR12 chemokine receptors are also recognized as biomarkers of radioresistant PC cells [64]. A recent study revealed an association between differential expression of 14 genes and radioresistance of PC cell lines [66]. PC cells were shown to respond to ionizing radiation by increasing the expression of *BRCA1*, *FANCG*, and *RAD51* [67]. The epithelial cell adhesion molecule (EpCAM) is a potential biomarker, as its overexpression is associated with radioresistance both *in vitro* and *in vivo* [68].

S.G. Zhao et al. created a diagnostic panel of gene expression (PORTOS), which predicted the outcomes of postoperative radiation therapy. The results of this study could predict the risk of distant metastases [69]. *RAD9* is considered to be a potential biomarker of PC radioresistance. Its contribution to regulation of the cell cycle, repair, and apoptosis, as well as to migration and invasion was proven. The role of this molecule as a transcription factor for androgen receptors is also undeniable [70]. Increased expression of markers of neuroendocrine differentiation CD133 and CD138 is presumably associated with increased expression of pluripotency genes and, ultimately, with subsequent development of radioresistance [71].

The detection of specific circulating microRNAs, in addition to genetic markers, also seems to be extremely promising. The presence of some microRNAs in PC patients is a more informative indicator than measuring the level of the prostate-specific antigen (PSA) [72]. According to the literature, more than 50 known microRNAs are involved in the pathogenesis of PC. The pattern of microRNA expression is specific in the lines of radioresistant PC cells [73]. Increased clonogenic survival after radiation is associated with significant changes in the expression profiles of miR-221, miR-4284, miR-31, and miR-200c [74]. Inhibition of miR-521 in the PC cell line provokes a radioresistant phenotype. Overexpression of miR-548c-3p in differentiated cells induces stem-like properties and radioresistance [75]. In radioresistant PC cells, miR-106a expression is elevated, which provides resistance to radiation therapy due to a direct effect on LITAF and increased proliferation [76]. Experiments on PC cells showed an association between increased miR-301a and miR-301b expression and hypoxia, as well as autophagy, which provokes enhanced radioresistance [77]. Aberrant expression of miR-521, miR-95, miR-106b, miR-32, and miR-205 is observed in the establishment of radioresistance [78]. In PC cells, miR-32 suppresses the function of the DAB2IP protein, inducing autophagy and inhibiting radiation-induced apoptosis [79]. Tumor radioresistance is also enhanced by miR-620 and miRNA-95, which target HPGD and SGPP1, respectively [80, 81].

According to our studies, the copy number of genes regulating proliferation and apoptosis can be considered as a factor of resistance of PC cells to radiation therapy. Thus, in a model experiment on PC-3 cells, we found that cells that retained their viability after five days of radiation therapy were characterized by an increased copy number of the *CDK1*, *CDKN1B*,

H2AX, *PTEN*, *XRCC4*, *RBBP8*, and *EP300* genes and a decreased copy number of the *CCND3*, *BAX*, *TP53*, and *BCL2* genes [82]. Table 1 presents the main markers of PC radioresistance.

Table 1

Markers of prostate cancer radioresistance		
Markers	Method for determination	Reference
$\alpha 2\beta$ -, $\alpha v\beta 3$ -integrins, E-, N-cadherins, vimentin	IHC	[9]
CD44, CD133	IHC	[9]
NANOG, OCT4, SOX2	RT-PCR	[9]
ALDH1A1	microchip	[17]
Caveolin-1	western blot	[19]
HSP90	ELISA	[35]
HIF-1 α	RT-PCR	[56]
PCSC1- RAN, PCSC2-RAN CXCR4 / CXCL12	IHC	[64]
ADAMTS9, AKR1B10, FOXL1, FST, ITGA2, GRPR, SOX17, STARD4, VGF, FHL5, LYPLAL1, PAK7, TDRD6, CXXC5	microchip	[65]
BRCA1, FANCG, RAD51	RT-PCR	[67]
EpCAM	RT-PCR	[68]
DRAM1, KRT14, PTPN22, ZMAT3, BIN2, ARHGAP15, IL1B, ANLN, RPS27A, MUM1, TOP2A, GNG11, CDKN3, HCLS1, DTL, IL7R, UBA7, NEK1, CDKN2AIP, APEX2, KIF23, SULF2, PLK2, EME1	microchip	[69]
RAD9	IHC	[70]
CD133, CD138	RT-PCR	[71]
miR-221, miR-4284, MiR-31, miR-200c	microchip	[74]
miR-521, miR-548c-3p	microchip	[75]
miR-106a	RT-PCR	[76]
miR-301a, miR-301b	RT-PCR	[77]
miR-521, miR-95, miR-106b, miR-32, miR-205	RT-PCR, microchip	[78]
miR-32	RT-PCR	[79]
miRNA-95	RT-PCR	[80]
miR-620	RT-PCR	[81]
CDK1, CDKN1B, H2AX, PTEN, XRCC4, RBBP8, EP300, CCND3, BAX, TP53, BCL2	RT-PCR	[82]

Note: IHC – immunohistochemistry, RT-PCR – real-time polymerase chain reaction, ELISA – enzyme-linked immunosorbent assay.

CONCLUSION

The cancer stem cell model determined the direction of research to explain the mechanisms of prostate cancer radioresistance development. A high degree of tumor heterogeneity and difficult conditions

for the emergence of tumor resistance to ionizing radiation suggest detection of specific markers. Their diversity is determined by the main processes occurring during radioresistance development, including increased DNA repair, activation of anti-apoptotic signaling pathways, production of ROS, hypoxia of the tumor microenvironment, epithelial – mesenchymal transition, and autophagy. Assessment of the transcriptional profile of the tumor can serve as a basis for predicting the outcomes of radiation therapy. A combination of the molecular genetic approach with standard biochemical and instrumental diagnostic methods will help to identify patients with radioresistant prostate tumors and select a personalized therapeutic strategy.

REFERENCES

- Zin'kovich M.S., Maksimov A.Ju., Rozenko L.Ja., Gusareva M.A., Karnaukhova E.A., Faenson A.V., Timoshkina N.N., Kutilin D.S. Radioresistance as a factor of evolution of radiation therapy for prostate cancer. *Modern Problems of Science and Education*. 2019; 2: 107. URL: <http://science-education.ru/ru/article/view?id=28627> (entry date: 01.06.2020) (in Russ.).
- Kutilin D.S., Sagakjanc A.B., Zin'kovich M.S., Maksimov A.Ju., Gusareva M.A., Bondarenko E.S., Potemkin D.S., Vasil'chenko N.G. Effects of various radiation therapy doses on survival of PC-3 cells. *Modern Problems of Science and Education*. 2019; 2: 157. URL: <http://science-education.ru/ru/article/view?id=28740> (entry date: 01.06.2020) (in Russ.).
- Rawla P. Epidemiology of prostate cancer. *World Journal of Oncology*. 2019; 10 (2): 63– 89. DOI: 10.14740/wjon1191.
- Kaprin A.D., Starinskij V.V., Petrova G.V. Cancer in Russia in 2018 (morbidity and mortality). M.: P. Herzen Moscow Oncology Research Institute, branch of the National Medical Research Radiological Center of the Ministry of Health of Russia, 2019: 250 (in Russ.).
- Teo M.Y., Rathkopf D.E., Kantoff P. Treatment of advanced prostate cancer. *Annual Review of Medicine*. 2019; 70: 479– 499. DOI: 10.1146/annurev-med-051517-011947.
- Kamran S.C., D'Amico A.V. Radiation therapy for prostate cancer. *Hematology/Oncology Clinics of North America*. 2020; 34 (1): 45– 69. DOI: 10.1016/j.hoc.2019.08.017.
- Evans A.J. Treatment effects in prostate cancer. *Modern Pathology*. 2018; 31 (S1): S110– 121. DOI: 10.1038/mod-pathol.2017.158.
- Artibani W., Porcaro A.B., De Marco V., Cerruto M.A., Siracusano S. Management of biochemical recurrence after primary curative treatment for prostate cancer: a review. *Urologia Internationalis*. 2018; 100 (3): 251–262. DOI: 10.1159/000481438.
- Tsing T., Beretov J., Ni J., Bai X., Bucci J., Graham P., Li Y. Cancer stem cells in prostate cancer radioresistance. *Cancer Letters*. 2019; 465: 94–104. DOI: 10.1016/j.canlet.2019.08.020.
- Kreso A., Dick J.E. Evolution of the cancer stem cell model. *Cell Stem Cell*. 2014; 14 (3): 275– 291. DOI: 10.1016/j.stem.2014.02.006.

11. Schulz A., Meyer F., Dubrovskaya A., Borgmann K. Cancer stem cells and radioresistance: DNA repair and beyond. *Cancers*. 2019; 11 (6): 862. DOI: 10.3390/cancers11060862.
12. Batlle E., Clevers H. Cancer stem cells revisited. *Nature Medicine*. 2017; 23 (10): 1124–1134. DOI: 10.1038/nm.4409.
13. Plaks V., Kong N., Werb Z. The cancer stem cell niche: how essential is the niche in regulating stemness of tumor cells? *Cell Stem Cell*. 2015; 16 (3): 225–238. DOI: 10.1016/j.stem.2015.02.015.
14. Eun K., Ham S.W., Kim H. Cancer stem cell heterogeneity: origin and new perspectives on CSC targeting. *BMB Reports*. 2017; 50 (3): 117–125. DOI: 10.5483/bmbrep.2017.50.3.222.
15. Crea F., Hurt E.M., Mathews L.A., Cabarcas S.M., Sun L., Marquez V.E., Danesi R., Farrar W.L. Pharmacologic disruption of Polycomb Repressive Complex 2 inhibits tumorigenicity and tumor progression in prostate cancer. *Molecular Cancer*. 2011; 10: 40. DOI: 10.1186/1476-4598-10-40.
16. Tzelepi V., Logotheti S., Efstathiou E., Troncoso P., Aparicio A., Sakellakis M., Hoang A., Perimenis P., Melachrinou M., Logothetis C., Zolota V. Epigenetics and prostate cancer: defining the timing of DNA methyltransferase deregulation during prostate cancer progression. *Pathology*. 2020; 52 (2): 218–227. DOI: 10.1016/j.pathol.2019.10.006.
17. Cojoc M., Peitzsch C., Kurth I., Trautmann F., Kunz-Schughart L.A., Telegeev G.D., Stakhovsky E.A., Walker J.R., Simin K., Lyle S., Fuessel S., Erdmann K., Wirth M.P., Krause M., Baumann M., Dubrovskaya A. Aldehyde dehydrogenase is regulated by β -catenin/TCF and promotes radioresistance in prostate cancer progenitor cells. *Cancer Research*. 2015; 75 (7): 1482–1494. DOI: 10.1158/0008-5472.CAN-14-1924.
18. Peitzsch C., Cojoc M., Hein L., Kurth I., Mäbert K., Trautmann F., Klink B., Schröck E., Wirth M.P., Krause M., Stakhovsky E.A., Telegeev G.D., Novotny V., Toma M., Muders M., Baretton G.B., Frame F.M., Maitland N.J., Baumann M., Dubrovskaya A. An epigenetic reprogramming strategy to resensitize radioresistant prostate cancer cells. *Cancer Research*. 2016; 76 (9): 2637–2651. DOI: 10.1158/0008-5472.CAN-15-2116.
19. Mahmood J., Zaveri S.R., Murti S.C., Alexander A.A., Connors C.Q., Shukla H.D., Vujaskovic Z. Caveolin-1: a novel prognostic biomarker of radioresistance in cancer. *International Journal of Radiation Biology*. 2016; 92 (12): 747–753. DOI: 10.1080/09553002.2016.1222096.
20. Hur W., Yoon S.K. Molecular pathogenesis of radiation-induced cell toxicity in stem cells. *International Journal of Molecular Sciences*. 2017; 18 (12): 2749. DOI: 10.3390/ijms18122749.
21. Diehn M., Cho R.W., Lobo N.A., Kalisky T., Dorie M.J., Kulp A.N., Qian D., Lam J.S., Ailles L.E., Wong M., Joshua B., Kaplan M.J., Wapnir I., Dirbas F.M., Somlo G., Garberoglio C., Paz B., Shen J., Lau S.K., Quake S.R., Brown J.M., Weissman I.L., Clarke M.F. Association of reactive oxygen species levels and radioresistance in cancer stem cells. *Nature*. 2009; 458 (7239): 780–783. DOI: 10.1038/nature07733.
22. Mladenov E., Magin S., Soni A., Iliakis G. DNA double-strand-break repair in higher eukaryotes and its role in genomic instability and cancer: Cell cycle and proliferation-dependent regulation. *In Seminars in Cancer Biology*. 2016; 37–38: 51–64. DOI: 10.1016/j.semcancer.2016.03.003.
23. Hufnagl A., Herr L., Friedrich T., Durante M., Taucher-Scholz G., Scholz M. The link between cell-cycle dependent radiosensitivity and repair pathways: a model based on the local, sister-chromatid conformation dependent switch between NHEJ and HR. *DNA Repair*. 2015; 27: 28–39. DOI: 10.1016/j.dnarep.2015.01.002.
24. Karanam K., Kafri R., Loewer A., Lahav G. Quantitative live cell imaging reveals a gradual shift between DNA repair mechanisms and a maximal use of HR in mid S phase. *Molecular Cell*. 2012; 47 (2): 320–329. DOI: 10.1016/j.molcel.2012.05.052.
25. Krause M., Dubrovskaya A., Linge A., Baumann M. Cancer stem cells: Radioresistance, prediction of radiotherapy outcome and specific targets for combined treatments. *Advanced Drug Delivery Reviews*. 2017; 109: 63–73. DOI: 10.1016/j.addr.2016.02.002.
26. Chang L., Graham P., Hao J., Ni J., Deng J., Bucci J., Malouf D., Gillatt D., Li Y. Cancer stem cells and signaling pathways in radioresistance. *Oncotarget*. 2016; 7 (10): 11002–11017. DOI: 10.18632/oncotarget.6760.
27. Yadav S., Kowolik C.M., Lin M., Zuro D., Hui S.K., Riggs A.D., Horne D.A. SMC1A is associated with radioresistance in prostate cancer and acts by regulating epithelial-mesenchymal transition and cancer stem-like properties. *Molecular Carcinogenesis*. 2019; 58 (1): 113–125. DOI: 10.1002/mc.22913.
28. Peitzsch C., Tyutyunnykova A., Pantel K., Dubrovskaya A. Cancer stem cells: the root of tumor recurrence and metastases. *In Seminars in Cancer Biology*. 2017; 44: 10–24. DOI: 10.1016/j.semcancer.2017.02.011.
29. Peitzsch C., Kurth I., Kunz-Schughart L., Baumann M., Dubrovskaya A. Discovery of the cancer stem cell related determinants of radioresistance. *Radiotherapy and Oncology*. 2013; 108 (3): 378–387. DOI: 10.1016/j.radonc.2013.06.003.
30. Vitale I., Manic G., Maria R.D., Kroemer G., Galluzzi L. DNA damage in stem cells. *Molecular Cell*. 2017; 66 (3): 306–319. DOI: 10.1016/j.molcel.2017.04.006.
31. D'Andrea F.P., Safwat A., Kassem M., Gautier L., Overgaard J., Horsman M.R. Cancer stem cell overexpression of nicotinamide N-methyltransferase enhances cellular radiation resistance. *Radiotherapy and Oncology*. 2011; 99 (3): 373–378. DOI: 10.1016/j.radonc.2011.05.086.
32. Murillo-Garzon V., Kypta R. WNT signalling in prostate cancer. *Nature Reviews Urology*. 2017; 14 (11): 683–696. DOI: 10.1038/nrrol.2017.144.
33. Jia X., Li X., Xu Y., Zhang S., Mou W., Liu Y., Liu Y., Lv D., Liu C.H., Tan X., Xiang R., Li N. SOX2 promotes tumorigenesis and increases the anti-apoptotic property of human prostate cancer cell. *Journal of Molecular Cell Biology*. 2011; 3 (4): 230–238. DOI: 10.1093/jmcb/mjr002.
34. Prasad S., Gupta S.C., Tyagi A.K. Reactive oxygen species (ROS) and cancer: Role of antioxidative nutraceuticals. *Cancer Letters*. 2017; 387: 95–105. DOI: 10.1016/j.canlet.2016.03.042.
35. Wang H., Jiang H., Van De Gucht M., De Ridder M. Hypoxic radioresistance: can ROS be the key to overcome it? *Cancers*. 2019; 11 (1): 112. DOI: 10.3390/cancers11010112.
36. Chaiswing L., Weiss H.L., Jayswal R.D., Clair D.K.S., Kyprianou N. Profiles of radioresistance mechanisms in prostate

- cancer. *Critical Reviews in Oncogenesis*. 2018; 23 (1–2): 39–67. DOI: 10.1615/CritRevOncog.2018025946.
37. Ding S., Li C., Cheng N., Cui X., Xu X., Zhou G. Redox regulation in cancer stem cells. *Oxidative Medicine and Cellular Longevity*. 2015; 2015: 750798. DOI: 10.1155/2015/750798.
 38. Ryoo I.G., Lee S.H., Kwak M.K. Redox modulating NRF2: a potential mediator of cancer stem cell resistance. *Oxidative Medicine and Cellular Longevity*. 2016; 2016: 2428153. DOI: 10.1155/2016/2428153.
 39. Albadari N., Deng S., Li W. The transcriptional factors HIF-1 and HIF-2 and their novel inhibitors in cancer therapy. *Expert Opinion on Drug Discovery*. 2019; 14 (7): 667–682. DOI: 10.1080/17460441.2019.1613370.
 40. Lee S.Y., Jeong E.K., Ju M.K., Jeon H.M., Kim M.Y., Kim C.H., Park H.G., Han S.I., Kang H.S. Induction of metastasis, cancer stem cell phenotype, and oncogenic metabolism in cancer cells by ionizing radiation. *Molecular Cancer*. 2017; 16 (1): 10. DOI: 10.1186/s12943-016-0577-4.
 41. Stark T.W., Hensley P.J., Spear A., Pu H., Strup S.S., Kyprianou N. Predictive value of epithelial-mesenchymal-transition (EMT) signature and PARP-1 in prostate cancer radioresistance. *The Prostate*. 2017; 77 (16): 1583–1591. DOI: 10.1002/pros.23435.
 42. Cai Z., Cao Y., Luo Y., Hu H., Ling H. Signalling mechanism(s) of epithelial-mesenchymal transition and cancer stem cells in tumour therapeutic resistance. *Clinica Chimica Acta*. 2018; 483: 156–163. DOI: 10.1016/j.cca.2018.04.033.
 43. Zhou Y.C., Liu J.Y., Li J., Zhang J., Xu Y.Q., Zhang H.W., Qiu L.B., Ding G.R., Su X.M., Mei-Shi, Guo G.Z. Ionizing radiation promotes migration and invasion of cancer cells through transforming growth factor-beta-mediated epithelial-mesenchymal transition. *International Journal of Radiation, Oncology, Biology, Physics*. 2011; 81 (5): 1530–1537. DOI: 10.1016/j.ijrobp.2011.06.1956.
 44. Wang S.S., Jiang J., Liang X.H., Tang Y.L. Links between cancer stem cells and epithelial-mesenchymal transition. *Oncotargets and Therapy*. 2015; 8: 2973–2980. DOI: 10.2147/OTT.S91863.
 45. Xu R., Won J.Y., Kim C.H., Kim D.E., Yim H. Roles of the phosphorylation of transcriptional factors in epithelial-mesenchymal transition. *Journal of Oncology*. 2019; 2019: 5810465. DOI: 10.1155/2019/5810465.
 46. Mahabir R., Tanino M., Elmansuri A., Wang L., Kimura T., Itoh T., Ohba Y., Nishihara H., Shirato H., Tsuda M., Tanaka S. Sustained elevation of Snail promotes glial-mesenchymal transition after irradiation in malignant glioma. *Neuro-Oncology*. 2014; 16 (5): 671–685. DOI: 10.1093/neuonc/not239.
 47. Celià-Terrassa T., Meca-Cortés Ó., Mateo F., De Paz A.M., Rubio N., Arnal-Estapé A., Ell B.J., Bermudo R., Díaz A., Guerra-Rebollo M., Lozano J.J., Estarás C., Ulloa C., Álvarez-Simón D., Milà J., Vilella R., Paciucci R., Martínez-Balbás M., García de Herreros A., Gomis R.R., Kang Y., Blanco J., Fernández P.L., Thomson T.M. Epithelial-mesenchymal transition can suppress major attributes of human epithelial tumor-initiating cells. *The Journal of Clinical Investigation*. 2012; 122 (5): 1849–1868. DOI: 10.1172/JCI59218.
 48. Wellner U., Schubert J., Burk U.C., Schmalhofer O., Zhu F., Sonntag A., Waldvogel B., Vannier C., Darling D., Hausen A., Brunton V.G., Morton J., Sansom O., Schüler J., Stemmler M.P., Herzberger C., Hopt U., Keck T., Brabletz S., Brabletz T. The EMT-activator ZEB1 promotes tumorigenicity by repressing stemness-inhibiting microRNAs. *Nature Cell Biology*. 2009; 11 (12): 1487–1495. DOI: 10.1038/ncb1998.
 49. Yang M.H., Hsu D.S.S., Wang H.W., Wang H.J., Lan H.Y., Yang W.H., Huang C.H., Kao S.Y., Tzeng C.H., Tai S.K., Chang S.Y., Lee O.K.S., Wu K.J. Bmi1 is essential in Twist1-induced epithelial-mesenchymal transition. *Nature Cell Biology*. 2010; 12 (10): 982–992. DOI: 10.1038/ncb2099.
 50. Kumar D., Shankar S., Srivastava R.K. Rottlerin induces autophagy and apoptosis in prostate cancer stem cells via PI3K/Akt/mTOR signaling pathway. *Cancer Letters*. 2014; 343 (2): 179–189. DOI: 10.1016/j.canlet.2013.10.003.
 51. Cristofani R., Marelli M.M., Cicardi M.E., Fontana F., Marzagalli M., Limonta P., Poletti A., Moretti R.M. Dual role of autophagy on docetaxel-sensitivity in prostate cancer cells. *Cell Death & Disease*. 2018; 9 (9): 889. DOI: 10.1038/s41419-018-0866-5.
 52. Lei Y., Zhang D., Yu J., Dong H., Zhang J., Yang S. Targeting autophagy in cancer stem cells as an anticancer therapy. *Cancer Letters*. 2017; 393: 33–39. DOI: 10.1016/j.canlet.2017.02.012.
 53. Paglin S., Hollister T., Delohery T., Hackett N., McMahon M., Sphicas E., Domingo D., Yahalom J. A novel response of cancer cells to radiation involves autophagy and formation of acidic vesicles. *Cancer Research*. 2001; 61 (2): 439–444.
 54. Yao B., Liu B., Shi L., Li X., Ren C., Cai M., Wang W., Li J., Sun Y., Wu Y., Wen J. PAFR selectively mediates radioresistance and irradiation-induced autophagy suppression in prostate cancer cells. *Oncotarget*. 2017; 8 (8): 13846–13854. DOI: 10.18632/oncotarget.14647.
 55. Zhang D., Tang D.G., Rycak K. Cancer stem cells: Regulation programs, immunological properties and immunotherapy. *Seminars in Cancer Biology*. 2018; 52 (Pt2): 94–106. DOI: 10.1016/j.semcancer.2018.05.001.
 56. Peitzsch C., Perrin R., Hill R.P., Dubrovskaya A., Kurth I. Hypoxia as a biomarker for radioresistant cancer stem cells. *International Journal of Radiation Biology*. 2014; 90 (8): 636–652. DOI: 10.3109/09553002.2014.916841.
 57. Luo Y., Li M., Zuo X., Basourakos S.P., Zhang J., Zhao J., Han Y., Lin Y., Wang Y., Jiang Y., Lan L. β -catenin nuclear translocation induced by HIF-1 α overexpression leads to the radioresistance of prostate cancer. *International Journal of Oncology*. 2018; 52 (6): 1827–1840. DOI: 10.3892/ijo.2018.4368.
 58. Lyssiotis C.A., Kimmelman A.C. Metabolic interactions in the tumor microenvironment. *Trends in Cell Biology*. 2017; 27 (11): 863–875. DOI: 10.1016/j.tcb.2017.06.003.
 59. Arnold C.R., Mangesius J., Skvortsova I.I., Ganswindt U. The role of cancer stem cells in radiation resistance. *Frontiers in Oncology*. 2020; 10: 164. DOI: 10.3389/fonc.2020.00164.
 60. Kanwal R., Shukla S., Walker E., Gupta S. Acquisition of tumorigenic potential and therapeutic resistance in CD133+ subpopulation of prostate cancer cells exhibiting stem-cell like characteristics. *Cancer Letters*. 2018; 430: 25–33. DOI: 10.1016/j.canlet.2018.05.014.
 61. Epel B., Maggio M.C., Barth E.D., Miller R.C., Pelizzari C.A., Krzykawska-Serda M., Sundramoorthy S.V., Aydogan B.,

- Weichselbaum R.R., Tormyshev V.M., Halpern H.J. Oxygen-guided radiation therapy. *International Journal of Radiation, Oncology, Biology, Physics*. 2019; 103 (4): 977–984. DOI: 10.1016/j.ijrobp.2018.10.041.
62. Jarosz-Biej M., Smolarczyk R., Cichoń T., Kułach, N. Tumor microenvironment as a “game changer” in cancer radiotherapy. *International Journal of Molecular Sciences*. 2019; 20 (13): 3212. DOI: 10.3390/ijms20133212.
 63. Matsui W.H. Cancer stem cell signaling pathways. *Medicine*. 2016; 95 (1-1): S8–S19. DOI: 10.1097/MD.00000000000004765.
 64. Alberti C. Prostate cancer: radioresistance molecular target-related markers and foreseeable modalities of radiosensitization. *European Review for Medical and Pharmacological Sciences*. 2014; 18 (16): 2275–2282.
 65. Chang L., Ni J., Beretov J., Wasinger V.C., Hao J., Bucci J., Malouf D., Gillatt D., Graham P.H., Li Y. Identification of protein biomarkers and signaling pathways associated with prostate cancer radioresistance using label-free LC-MS/MS proteomic approach. *Scientific Reports*. 2017; 7: 41834. DOI: 10.1038/srep41834.
 66. Seifert M., Peitzsch C., Gorodetska I., Börner C., Klink B., Dubrovskaya A. Network-based analysis of prostate cancer cell lines reveals novel marker gene candidates associated with radioresistance and patient relapse. *PLoS Computational Biology*. 2019; 15 (11): e1007460. DOI: 10.1371/journal.pcbi.1007460.
 67. Young A., Berry R., Holloway A.F., Blackburn N.B., Dickinson J.L., Skala M., Phillips J.L., Brettingham-Moore K.H. RNA-seq profiling of a radiation resistant and radiation sensitive prostate cancer cell line highlights opposing regulation of DNA repair and targets for radiosensitization. *BMC Cancer*. 2014; 14: 808. DOI: 10.1186/1471-2407-14-808.
 68. Ni J., Cozzi P., Beretov J., Duan W., Bucci J., Graham P., Li Y. Epithelial cell adhesion molecule (EpCAM) is involved in prostate cancer chemotherapy/radiotherapy response in vivo. *BMC Cancer*. 2018; 18 (1): 1092. DOI: 10.1186/s12885-018-5010-5.
 69. Zhao S.G., Chang S.L., Spratt D.E., Erho N., Yu M., Ashab H.A.D., Alshalalfa M., Speers C., Tomlins S.A., Davicioni E., Dicker A.P., Carroll P.R., Cooperberg M.R., Freedland S.J., Karnes R.J., Ross A.E., Schaeffer E.M., Den R.B., Nguyen P.L., Feng F.Y. Development and validation of a 24-gene predictor of response to postoperative radiotherapy in prostate cancer: a matched, retrospective analysis. *The Lancet Oncology*. 2016; 17 (11): 1612–1620. DOI: 10.1016/S1470-2045(16)30491-0.
 70. Lieberman H.B., Rai A.J., Friedman R.A., Hopkins K.M., Broustas C.G. Prostate cancer: unmet clinical needs and RAD9 as a candidate biomarker for patient management. *Translational Cancer Research*. 2018; 7 (6): S651–S661. DOI: 10.21037/tcr.2018.01.21.
 71. Murata K., Saga R., Monzen S., Tsuruga E., Hasegawa K., Hosokawa Y. Understanding the mechanism underlying the acquisition of radioresistance in human prostate cancer cells. *Oncology Letters*. 2019; 17 (6): 5830–5838. DOI: 10.3892/ol.2019.10219.
 72. Stuoopelytė K., Daniūnaitė K., Jankevičius F., Jarmalaitė S. Detection of miRNAs in urine of prostate cancer patients. *Medicina*. 2016; 52 (2): 116–124. DOI: 10.1016/j.medic.2016.02.007.
 73. Kanwal R., Plaga A.R., Liu X., Shukla G.C., Gupta S. MicroRNAs in prostate cancer: Functional role as biomarkers. *Cancer Letters*. 2017; 407: 9–20. DOI: 10.1016/j.canlet.2017.08.011.
 74. McDermott N., Meunier A., Wong S., Buchete V., Marignol L. Profiling of a panel of radioresistant prostate cancer cells identifies deregulation of key miRNAs. *Clinical and Translational Radiation Oncology*. 2017; 2: 63–68. DOI: 10.1016/j.ctro.2017.01.005.
 75. Rane J.K., Scaravilli M., Ylipää A., Pellacani D., Mann V.M., Simms M.S., Nykter M., Collins A.T., Visakorpi T., Maitland N.J. MicroRNA expression profile of primary prostate cancer stem cells as a source of biomarkers and therapeutic targets. *European Urology*. 2015; 67 (1): 7–10. DOI: 10.1016/j.eururo.2014.09.005.
 76. Hoey C., Ray J., Jeon J., Huang X., Taeb S., Ylanko J., Andrews D.W., Boutros P.C., Liu S.K. miRNA-106a and prostate cancer radioresistance: a novel role for LITAF in ATM regulation. *Molecular Oncology*. 2018; 12 (8): 1324–1341. DOI: 10.1002/1878-0261.12328.
 77. Wang W., Liu M., Guan Y., Wu Q. Hypoxia-responsive mir-301a and mir-301b promote radioresistance of prostate cancer cells via downregulating NDRG2. *Medical Science Monitor: International Medical Journal of Experimental and Clinical Research*. 2016; 22: 2126–2132. DOI: 10.12659/msm.896832.
 78. Razdan A., de Souza P., Roberts T.L. Role of microRNAs in treatment response in prostate cancer. *Current Cancer Drug Targets*. 2018; 18 (10): 929–944. DOI: 10.2174/1568009618666180315160125.
 79. Liao H., Xiao Y., Hu Y., Xiao Y., Yin Z., Liu L. microRNA-32 induces radioresistance by targeting DAB2IP and regulating autophagy in prostate cancer cells. *Oncology Letters*. 2015; 10 (4): 2055–2062. DOI: 10.3892/ol.2015.3551.
 80. Huang X., Taeb S., Jahangiri S., Emmenegger U., Tran E., Bruce J., Mesci A., Korpela E., Vesprini D., Wong C.S., Bristow R.G., Liu F.F., Liu S.K. miRNA-95 mediates radioresistance in tumors by targeting the sphingolipid phosphatase SGPP1. *Cancer Research*. 2013; 73 (23): 6972–6986. DOI: 10.1158/0008-5472.CAN-13-1657.
 81. Huang X., Taeb S., Jahangiri S., Korpela E., Cadonic I., Yu N., Krylov S.N., Fokas E., Boutros P.C., Liu S.K. miR-620 promotes tumor radioresistance by targeting 15-hydroxyprostaglandin dehydrogenase (HPGD). *Oncotarget*. 2015; 6 (26): 22439–22451. DOI: 10.18632/oncotarget.4210.
 82. Kutilin D.S., Zin'kovich M.S., Gusareva M.A., Faenson A.V., Karnaukhova E.A., Rozenko L.Ya., Fatkina N.B., Udalenkova I.A., Vasileva E.O., Gappoeva M.A. Gene copy number variation as a factor of radiation resistance of prostate tumor cells. *Modern Problems of Science and Education*. 2020; 4[^] 82. URL: <http://science-education.ru/ru/article/view?id=29866> (entry date: 08.07.2020) (in Russ.). DOI: 10.17513/spno.29866.

Authors contribution

Omelchuk E.P. – drafting of the manuscript. Kutilin D.S. – conception, critical revision of the manuscript for important intellectual content. Dimitriadi S.N., Gusareva M.A. – critical revision of the manuscript for important intellectual content. Timoshkina N.N. – critical revision of the manuscript for important intellectual content, final approval of the manuscript for publication.

Authors information

Omelchuk Ekaterina P., Junior Researcher, Laboratory of Molecular Oncology, National Medical Research Center for Oncology, Rostov-on-Don, Russian Federation. ORCID 0000-0003-0786-9684.

Kutilin Denis S., Cand. Sci. (Biology), Leading Researcher, Laboratory of Molecular Oncology, National Medical Research Center for Oncology, Rostov-on-Don, Russian Federation. ORCID 0000-0002-8942-3733.

Dimitriadi Sergej N., Dr. Sci. (Med.), Senior Researcher, Oncourology Department, National Medical Research Center for Oncology, Rostov-on-Don, Russian Federation. ORCID 0000-0002-2565-1518.

Gusareva Marina A., Cand. Sci. (Med.), Radiologist, Head of the Department of Radiation Therapy, National Medical Research Center for Oncology, Rostov-on-Don, Russian Federation. ORCID 0000-0002-9426-9662.

Timoshkina Nataliya N., Cand. Sci. (Biology), Head of the Laboratory of Molecular Oncology, National Medical Research Center for Oncology, Rostov-on-Don, Russian Federation. ORCID 0000-0001-6358-7361.

(✉) **Omelchuk Ekaterina P.**, e-mail: ekaterina.omelchuck@yandex.ru

Received 23.06.2020

Accepted 29.09.2020

Genetics of familial amyotrophic lateral sclerosis

Savinova A.V.¹, Shnayder N. A.^{1,2}, Nasyrova R.F.^{1,3}

¹ V.M. Bekhterev National Research Medical Center for Psychiatry and Neurology (V.M. Bekhterev NRMС PN) 3, Bekhterev Str., Saint Petersburg, 192019, Russian Federation

² V.F. Voyno-Yasenetsky Krasnoyarsk State Medical University (V.F. Voyno-Yasenetsky KrasSMU) 1, Partizana Zheleznyaka Str., Krasnoyarsk, 660022, Russian Federation

³ Kazan Federal University (KFU) 18, Kremlyovskaya Str., Kazan, 420008, Russian Federation

ABSTRACT

Aim. To analyze results of the studies covering modern scientific views on the genetics of familial amyotrophic lateral sclerosis (FALS).

Materials and methods. We searched for full-text publications containing the key words “amyotrophic lateral sclerosis”, “FALS”, and “genetics” in the literature for the past 10 years in both Russian and English in eLibrary, PubMed, Web of Science, and OMIM databases. In addition, the review includes earlier publications of historical interest.

This review summarizes all existing information on four most widespread genes associated with FALS: *SOD1*, *TARDBP*, *FUS*, and *C9ORF72*. The review also describes the functions of these genes and possible pathogenetic mechanisms of motor neuron death in amyotrophic lateral sclerosis (ALS), such as mitochondrial dysfunction, oxidative stress, glutamate excitotoxicity, damage to axonal transport components, and pathological neurofilament aggregation.

Conclusion. As modern methods of molecular genetic diagnostics evolve, our knowledge about multifactorial FALS genetics expands. This information should be taken into consideration in clinical practice of neurologists. Information about the genes associated with ALS and understanding of particular pathogenetic mechanisms of the disease play a key role in the development of effective therapeutic strategies.

Key words: amyotrophic lateral sclerosis, ALS, familial ALS, genetics, *SOD*, *FUS*, *TARDBP*, *C9ORF72*.

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

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

For citation: Savinova A.V., Shnayder N.A., Nasyrova R.F. Genetics of familial amyotrophic lateral sclerosis. *Bulletin of Siberian Medicine*. 2021; 20 (3): 193–202. <https://doi.org/10.20538/1682-0363-2021-3-193-202>.

Генетика семейных форм бокового амиотрофического склероза

Савинова А.В.¹, Шнайдер Н.А.^{1,2}, Насырова Р.Ф.^{1,3}

¹ Национальный медицинский исследовательский центр психиатрии и неврологии (НМИЦ ПН) имени В.М. Бехтерева
Россия, 192019, г. Санкт-Петербург, ул. Бехтерева, 3

² Красноярский государственный медицинский университет (КрасГМУ) имени профессора В.Ф. Войно-Ясенецкого

Россия, 660022, г. Красноярск, ул. Партизана Железняка, 1

³ Казанский федеральный университет (КФУ)

Россия, 420008, г. Казань, ул. Кремлевская, 18

РЕЗЮМЕ

Анализируются результаты исследований, отражающих современное представление о генетике семейных форм бокового амиотрофического склероза (сБАС).

Проведен поиск полнотекстовых публикаций на русском и английском языках за последнее десятилетие в базах данных eLibrary, PubMed, Web of Science, OMIM, используя ключевые слова «боковой амиотрофический склероз» (БАС), «сБАС», «генетика». Кроме того, в обзор включены более ранние публикации, имеющие исторический интерес.

Представлены современные данные, накопленные по четырем самым распространенным генам возникновения сБАС: *SOD1*, *TARDBP*, *FUS* и *C9ORF72*. Рассмотрена функция этих генов, а также возможные патогенетические механизмы гибели мотонейронов при БАС: митохондриальная дисфункция, глутаматная эксайтотоксичность, окислительный стресс, поражение компонентов системы аксонального транспорта, патологическая агрегация нейрофиламентов.

По мере развития современных методов молекулярно-генетической диагностики расширяются знания в понимании генетики семейных мультифакторных форм БАС, что важно учитывать в клинической практике врачей-неврологов. Выявление генов, ответственных за возникновение БАС, а также понимание конкретных патогенетических механизмов развития заболевания играют ключевую роль в разработке эффективных терапевтических стратегий.

Ключевые слова: боковой амиотрофический склероз, БАС, семейный БАС, генетика, *SOD*, *FUS*, *TARDBP*, *C9ORF72*.

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

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

Для цитирования: Савинова А.В., Шнайдер Н.А., Насырова Р.Ф. Генетика семейных форм бокового амиотрофического склероза. *Бюллетень сибирской медицины*. 2021; 20 (3): 193–202. <https://doi.org/10.20538/1682-0363-2021-3-193-202>.

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease characterized by a loss of both central and peripheral motor neurons [1].

ALS occurs in 1.47–2.43 cases per 100 thousand people [2, 3]. The loss of neurons in ALS patients usually manifests itself in adulthood through such clinical symptoms as a loss of fine motor skills in limbs, intermittent claudication, and slurred speech. Over time, ALS symptoms progress and manifest themselves through limb paresis, muscular atrophy, fasciculations, and weight loss. In the end, this condition leads to death from respiratory failure within 32 months on average after the first manifestations.

ALS is considered to be a multifactorial disease. The exact etiology is currently unknown. However, scientists have described different pathogenetic mechanisms of motor neuron death in ALS: genetic factors [4, 5]; disruption in metabolism and ribonucleic acid (RNA) processing [6, 7]; glutamate excitotoxicity [8, 9]; oxidative stress [10]; mitochondrial dysfunction [11–13]; damaged axonal transport components [14, 15]; pathological neurofilament aggregation [16–18]; toxic effects of glial, dendritic, and antigen-presenting cells [19]. Even though ALS is mostly sporadic, 10% of cases are familial (heritable), which have both autosomal dominant (AD) and autosomal recessive (AR) inheritance patterns [20, 21].

Genetic etiology of ALS was first described in 1993 [22]. Further analysis of *SOD1* and neighboring

genes responsible for the development of the disease showed multiple pathogenic mutations that are specific for familial ALS (FALS) cases. Further connection analysis and sequencing of the ALS candidate genes led to the identification of genes associated with AD, AR, and X-linked inheritance of the disease [23]. Next generation sequencing expanded researchers' capabili-

ties and significantly increased the identification of genes associated with ALS.

To date, more than 25 ALS loci and 4 loci of ALS combined with frontotemporal dementia (FTD) have been identified [24]. In most loci, specific genes causing the disease have been identified (Table).

Table

Genetic heterogeneity of ALS				
Locus	Associated genes	Chromosomal location	Inheritance	OMIM
ALS 1	<i>SOD1</i>	21q22.11	AD (AR)	105400
ALS 2	<i>ALS2</i>	2q33.1	AR	
ALS 3	Unknown	18q21	AD	606640
ALS 4	<i>SETX</i>	9q34.13	AD	602433
ALS 5	<i>SPG11</i>	15q21.1	AR	602099
ALS 6	<i>FUS</i>	16p11.2	AD (AR)	608030
ALS 7	Unknown	20p13	AD	608031
ALS 8	<i>VAPB</i>	20q13.32	AD	608627
ALS 9	<i>ANG</i>	14q11.2	AD	611895
ALS 10	<i>TARDBP</i>	1p36.22	AD	612069
ALS 11	<i>FIG4</i>	6q21	AD	612577
ALS 12	<i>OPTN</i>	10p13	AD (AR)	602432
ALS 13	<i>ATXN2</i>	12q24.12	AD	183090
ALS 14	<i>VCP</i>	9p13.3	AD	613954
ALS 15	<i>UBQLN2</i>	Xp11.21	X-linked	300857
ALS 16	<i>SIGMAR1</i>	9p13.3	AD (AR)	614373
ALS 17	<i>CHMP2B</i>	3p11.2	AD	614696
ALS 18	<i>PFN1</i>	17p13.2	AD	614808
ALS 19	<i>ERBB4</i>	2q34	AD	615515
ALS 20	<i>hnRNPA1</i>	12q13.13	AD	615426
ALS 21	<i>MATR3</i>	5q31.2	AD	606070
ALS 22	<i>TUBA4A</i>	2q35	AD	616208
ALS 23	<i>ANXA11</i>	10q22.3	AD	617839
ALS 24	<i>NEK1</i>	4q33	AD	617892
ALS 25	<i>KIF5A</i>	12q13.3	AD	617921
ALS-FTD 1	<i>C9ORF72</i>	9p21.2	AD	105550
ALS-FTD 2	<i>CHCHD10</i>	22q11.23	AD	615911
ALS-FTD 3	<i>SQSTM1</i>	5q35.3	AD	616437
ALS-FTD 4	<i>TBK1</i>	12q14.2	AD	616439

Note: ALS – amyotrophic lateral sclerosis; ALS-FTD – ALS combined with frontotemporal dementia; OMIM – Online Mendelian Inheritance in Man; AD – autosomal dominant; AR – autosomal recessive.

This review summarizes the data from the studies of the four most common monogenic ALS forms caused by mutations in the *SOD1*, *TARDBP*, *FUS*, and *C9ORF72* genes. Functions, contribution to the pathogenesis of ALS, and significance of these mechanisms for the development of new approaches to therapy were described for each gene in detail.

LOCUS ALS 1 (THE *SOD1* GENE)

The *SOD1* gene is located on the chromosome 21q22.11. This gene encodes superoxide dismutase

1, which is the main cytoplasmic antioxidant enzyme that binds copper and zinc and forms an extremely stable homodimer. SOD1 dimers are found in cytosol and intermembrane space of mitochondria. They provide an important antioxidant defense mechanism by catalyzing the production of oxygen and hydrogen peroxide from superoxide compounds, which are produced in cellular respiration [25].

SOD1 mutation was first described by D.R. Rosen et al. in 1993 and was the first identified genetic cause of FALS [22]. Most *SOD1* mutations are characterized

by AD inheritance [26]. However, cases of AR inheritance were also described [27, 28]. The rate of *SOD1* mutations varies from 12 to 23.5% in FALS patients and from 5 to 10% in sporadic ALS cases [29, 30]. Currently, more than 100 mutations of the *SOD1* gene have been described. The majority of them are point mutations. These point mutations are spread throughout the gene and do not provide functional or structural clues to better understanding of the FALS genesis. The reason for it is that the phenotype, duration, and severity of the disease can vary significantly depending on the sites involved. D90A is the most common variant of a missense mutation worldwide. Patients with A4V, H43R, L84V, G85R N86S, and G93A variants demonstrate rapid progression of the disease and a shorter life expectancy, while patients with G93C, D90A, and H46R variants usually have a longer life expectancy [31]. These mutations are accompanied by destabilization of the mutant protein molecule, which can be a trigger for disease development [32, 33].

Initially, mutations in the *SOD1* gene were considered to be linked to a decrease in the enzyme activity. This led to the assumption that the pathogenesis of ALS is associated with a loss of dismutase enzyme activity [22, 34]. However, a later study by D.W. Cleveland et al. (1995) showed that superoxide dismutase 1 (*SOD1*) activity does not correlate with the severity of the disease. This indicates that a toxic mechanism of the enzyme activity increase may be involved in the ALS pathogenesis [35]. Conformational and functional changes in *SOD1* induced by mutations exert their toxic effects through a number of mechanisms. These include excitotoxicity, oxidative stress, mitochondrial dysfunction, and prion-like propagation of mutant *SOD1* (mt*SOD1*) [36]. mt*SOD1* increases the levels of oxidative compounds (hydroxyl peroxide radicals [37–39], peroxynitrite [40]), which are toxic to cells. Besides, excessive enzyme activity of mt*SOD1* is associated with the release of free zinc ions [41]. In addition, mt*SOD1* bind to other proteins [42], for example, heat shock proteins, and disrupt their apoptotic activity [43, 44]. In general, redox regulation [45], mediated by excessive formation of toxic *SOD1* aggregates [46], changes.

As in other neurodegenerative diseases associated with protein aggregation, the question of whether mutant forms of the protein are responsible for toxicity is being discussed. K. Forsberg et al. (2019) discovered granular *SOD1*-immunoreactive inclusions in motor neurons of FALS patients without pathogenic mutations in the *SOD1* gene. This may indicate that

wrong conformation of *SOD1* may be a consequence of a pathogenetic cascade associated with mutations in other genes (*C9ORF72HRE*, *FUS*, *KIF5A*, *NEK1*, *VAPB*, and *ALSIN*). Therefore, the authors suggest that new anti-*SOD1* antibodies may be a promising therapeutic strategy in FALS [47].

LOCUS ALS 6 (GENE *FUS*)

The *FUS* gene is located on the chromosome 16p11.2. It was first identified as an oncogene in liposarcoma. The *FUS* gene encodes the ubiquitous 526 amino acid protein belonging to the FET family of RNA-binding proteins. Under normal physiological conditions, the *FUS* protein is localized mainly in the nucleus, but it can also pass into the cytoplasm while participating in nucleocytoplasmic transport [48]. *FUS* shares many physiological roles with TDP-43 (encoded by the *TARDBP* gene) such as transcription, pre-mRNA splicing, RNA transport, and translation regulation [49]. Even though they have much in common, TDP-43 and *FUS* regulate different processes associated with RNA [50, 51].

Currently, more than 50 different mutations in the *FUS* gene have been identified in FALS patients, and they are mainly characterized by AD inheritance. While most of these mutations are missense mutations, there are also rare cases of deletions, insertions, and nonsense mutations [52]. These mutations eventually lead to redistribution of *FUS* into the cytoplasm [53]. Other pathogenic variants of *FUS* increase the propensity of the protein to form aggregates. This reveals different pathomechanisms of the FALS genesis [54].

Y. Kino et al. (2015) proposed that FALS occurs following a decrease in the *FUS* protein function. Pathological redistribution of *FUS* into the cytoplasm makes it incapable of performing its functions in the nucleus [55].

In contrast to the theory of insufficient functional activity of *FUS*, scientists have put forward theories of the FALS genesis associated with an excessive toxic increase in the protein function. J.C. Mitchell et al. (2013) created a line of transgenic mice with excessive expression of *FUS*. As a result, this line of mice developed an aggressive ALS phenotype, in which the accumulation of cytoplasmic *FUS* was detected [56].

The question of whether neurotoxicity is a consequence of primary accumulation of *FUS* aggregates or a secondary increase in *FUS* in the cytoplasm after its redistribution is not fully explored. There is some evidence that toxicity can be caused directly by *FUS* aggregates. The accumulation of cytoplasmic *FUS* and

an aggressive phenotype were observed in the transgenic mouse models that express aggregate-prone FUS variants which lack the ability to recognize and bind RNA [57, 58]. There is also evidence that soluble cytoplasmic FUS may be toxic. Some studies on animal models of FALS (on transgenic rats in particular) demonstrated an increase in cytoplasmic FUS without aggregate formation [59–61]. S. Hennig et al. (2015) proposed that FUS aggregation can be a compensatory mechanism that protects cells from a toxic increase in soluble cytoplasmic FUS. The authors believe that the tendency of FUS to aggregate is not a pathological, but a normal cell function [62]. Impaired cellular function can also be a direct consequence of exposure to pathogenic FUS variants, which cause splicing defects, DNA damage, and disruption of FUS autoregulation [63, 64].

LOCUS ALS 10 (GENE *TARDBP*)

The *TARDBP* gene is located on the chromosome 1p36.22. It encodes TDP-43, a 414 amino acid DNA/RNA-binding protein. TDP-43 is usually localized in the nucleus, but it has both a nuclear localization signal and a nuclear export signal. This gives TDP-43 the ability to move freely between the nucleus and the cytoplasm [65]. TDP-43 regulates gene expression and participates in several stages of RNA processing: pre-mRNA splicing, regulation of mRNA stability, mRNA transport, translation, and regulation of non-coding RNA regions [66, 67].

Histological studies of the spinal cord specimens showed that neuronal ubiquitinated cytoplasmic inclusions were present in the majority of ALS patients [68]. However, in 2006, there was a change in the understanding of the FALS pathogenesis, when TDP-43 was discovered to be the main component of ubiquitinated protein aggregates found in FALS patients [69, 70]. Further histological studies confirmed that TDP-43 is present in cytoplasmic aggregates in most ALS patients, including sporadic cases without pathogenic variants in the *TARDBP* gene and in patients with hexanucleotide repeat expansions in *C9ORF72* [71, 72]. TDP-43 aggregation in ubiquitin-positive cytoplasmic inclusions of neurons in the brain and spinal cord is currently considered a distinctive pathological feature of ALS.

To date, at least 48 different *TARDBP* gene mutations have been associated with ALS development [53]. Most of these mutations are missense mutations located in the glycine-rich region at the carboxyl terminus of the transcript. The carboxy-terminal region

interacts with other heterogeneous ribonucleoproteins and participates in the regulation of pre-mRNA splicing [73].

The cytoplasmic accumulation of TDP-43 is accompanied by redistribution of TDP-43 and its decrease in the nucleus. Due to this feature, a loss of normal TDP-43 function in the nucleus and / or a toxic increase in the protein function are proposed to be ALS development mechanisms.

Various animal models were created to test the hypothesis on function loss. In the absence of TDP-43, mice are not viable, which demonstrates that TDP-43 is vital for embryonic development [74]. Induced *TDP-43* knockout in adult mice is also lethal [75]. The mice that were heterozygous for *TARDBP* deletion showed motor deficits, but no degeneration of motor neurons and no decrease in the level of the TDP-43 protein were observed [74].

Most of the evidence for the increased function hypothesis comes from overexpression models. A neurodegeneration model was reproduced in the line of rodents with overexpression of TDP-43 of both wild and mutant types [76–78].

Both lack and overexpression of TDP-43 cause the disease. This emphasizes the importance of strictly controlled regulation of this protein. A. Koyama et al. (2016) discovered that a decrease in TDP-43 in the nucleus disrupts autoregulation and activates continuous synthesis of TDP-43 [79]. TDP-43 homeostasis is crucial for normal cellular function. An excess of TDP-43 in the cytoplasm can lead to formation of inclusions and cellular dysfunction, while nuclear depletion can cause dysregulation of mRNA metabolism [80, 81].

In addition to abnormal distribution and aggregation of TDP-43 in ALS, there is evidence of post-translational modifications associated with abnormal TDP-43. These modifications include ubiquitination, proteolytic cleavage, and phosphorylation [82]. The timing of these post-translational modifications and the role that each of them plays in the onset of the disease remain unclear.

LOCUS ALS-FTD 1 (GENE *C9ORF72*)

The *C9ORF72* gene is located on the chromosome 9p21.2. M. DeJesus-Hernandez et al. (2011) discovered that expansion of GGGGCC (G4C2) hexanucleotide repeats in the non-coding regions of the *C9ORF72* gene is one of the most common causes of ALS and FTD [6]. It is these expansions of hexanucleotide repeats that were found in 34% of FALS cases and 5% of sporadic ALS cases [4]. It is worth

mentioning that G4C2 repeats are more common in European populations than in Asian ones. Despite the fact that healthy people also have G4C2 hexanucleotide repeats, their number does not exceed 20, while in FALS patients, their number reaches hundreds and thousands [6].

Although the role of the *C9ORF72* gene in FALS development has already been established, the cellular function of the protein encoded by this gene has not yet been fully understood. Recent studies indicate that this gene participates in the regulation of vesicular transport [83]. C9ORF72 proteins co-localized with Rab proteins take part in autophagy and endocytosis. A.J. Waite et al. (2014) found out that in patients with ALS and hexanucleotide repeat expansion, the levels of the C9ORF72 protein and mRNA are decreased [84]. However, a loss of function in this gene alone cannot cause ALS symptoms independently [85].

Disruption of RNA processing plays a key role in the ALS development. Expansions of G4C2 repeats in the *C9ORF72* gene are located in the intron between the first two exons. Processing of such transcripts creates isoforms that contain one or both exons as well as a fragment of the expansion of G4C2 repeats [86].

Other possible processing disruptions include abortive transcription, impaired splicing of the intron containing G4C2 repeats, and nuclear aggregation [87]. Some of the transcripts that contain G4C2 repeats undergo RAN translation. This leads to the formation of pathological dipeptides that form inclusions in the central nervous system (CNS). It can also contribute to neurodegeneration [88]. Nuclear structures that disable RNA-binding proteins can be formed from RNA transcripts containing G4C2 repeats. These structures also affect RNA expression and splicing [89].

P. Fratta et al. (2012) point out that guanine-rich repeats are prone to formation of such a secondary DNA structure as a G-quadruplex. This promotes formation of pathological bonds with various proteins, which also inhibits transcription [90].

It is currently unknown which of the above-described mechanisms has the greatest influence on the ALS progression.

CONCLUSION

The studies analyzed above show a significant contribution of the mutations in the genes in question to the FALS pathogenesis. Further studies of the molecular genetic mechanisms behind the formation of these mutations will expand the understanding of the genetic mechanisms in the development of famil-

ial and multifactorial forms of ALS. This is important for further development of diagnostic approaches and pathogenetic therapeutic strategies in real clinical practice.

REFERENCES

1. Rowland L.P. Amyotrophic lateral sclerosis. *Curr. Opin. Neurol.* 1994; 7 (4): 310–315. DOI: 10.1097/00019052-199408000-00006.
2. Chiò A., Logroscino G., Traynor B.J., Collins J., Simeone J.C., Goldstein L.A., White L.A. Global epidemiology of amyotrophic lateral sclerosis: a systematic review of the published literature. *Neuroepidemiology.* 2013; 41 (2): 118–130. DOI: 10.1159/000351153.
3. Ingre C., Roos P.M., Piehl F. Risk factors for amyotrophic lateral sclerosis. *Clin. Epidemiol.* 2015; 7: 181–193. DOI: 10.2147/CLEP.S37505.
4. Zou Z.Y., Zhou Z.R., Che C.H., Liu C.Y., He R.L., Huang H.P. Genetic epidemiology of amyotrophic lateral sclerosis: a systematic review and meta-analysis. *J. Neurol. Neurosurg. Psychiatry.* 2017; 88 (7): 540–549. DOI: 10.1136/jnnp-2016-315018.
5. Veldink J.H. ALS genetic epidemiology ‘How simplex is the genetic epidemiology of ALS?’ *J. Neurol. Neurosurg. Psychiatry.* 2017; 88 (7): 537. DOI: 10.1136/jnnp-2016-315469.
6. DeJesus-Hernandez M., Mackenzie I.R., Boeve B.F., Boxer A.L., Baker M., Rutherford N.J., Nicholson A.M., Finch N.A., Flynn H., Adamson J., Kouri N., Wojtas A., Sengdy P., Hsiung G.Y., Karydas A., Seeley W.W., Josephs K.A., Coppola G., Geschwind D.H., Wszolek Z.K., Feldman H., Knopman D.S., Petersen R.C., Miller B.L., Dickson D.W., Boylan K.B., Graff-Radford N.R., Rademakers R. Expanded GGGGCC hexanucleotide repeat in noncoding region of *C9ORF72* causes chromosome 9p-linked FTD and ALS. *Neuron.* 2011; 72 (2): 245–256. DOI: 10.1016/j.neuron.2011.09.011.
7. Chen K.W., Chen J.A. Functional roles of long non-coding RNAs in motor neuron development and disease. *J. Biomed. Sci.* 2020; 27 (1): 38. DOI: 10.1186/s12929-020-00628-z.
8. Shaw P.J. Molecular and cellular pathways of neurodegeneration in motor neurone disease. *J. Neurol. Neurosurg. Psychiatry.* 2005; 76 (8): 1046–1057. DOI: 10.1136/jnnp.2004.048652.
9. Shaw P.J., Forrest V., Ince P.G., Richardson J.P., Wastell H.J. CSF and plasma amino acid levels in motor neuron disease: elevation of CSF glutamate in a subset of patients. *Neurodegeneration.* 1995; 4 (2): 209–216. DOI: 10.1006/neur.1995.0026.
10. Shaw P.J., Ince P.G., Falkous G., Mantle D. Oxidative damage to protein in sporadic motor neuron disease spinal cord. *Ann. Neurol.* 1995; 38 (4): 691–695. DOI: 10.1002/ana.410380424.
11. Manfredi G., Xu Z. Mitochondrial dysfunction and its role in motor neuron degeneration in ALS. *Mitochondrion.* 2005; 5 (2): 77–87. DOI: 10.1016/j.mito.2005.01.002.
12. Shi P., Gal J., Kwinter D.M., Liu X., Zhu H. Mitochondrial dysfunction in amyotrophic lateral sclerosis. *Biochim. Biophys. Acta.* 2010; 1802 (1): 45–51. DOI: 10.1016/j.bba-dis.2009.08.012.
13. Kodavati M., Wang H., Hegde M.L. Altered mitochondrial dynamics in motor neuron disease: An emerging perspective. *Cells.* 2020; 9 (4): 1065. DOI: 10.3390/cells9041065

14. De Vos K.J., Grierson A.J., Ackerley S., Miller C.C. Role of axonal transport in neurodegenerative diseases. *Ann. Rev. Neurosci.* 2008; 31: 151–173. DOI: 10.1146/annurev.neuro.31.061307.090711.
15. De Vos K.J., Chapman A.L., Tennant M.E., Manser C., Tudor E.L., Lau K.F., Brownlee J., Ackerley S., Shaw P.J., McLoughlin D.M., Shaw C.E., Leigh P.N., Miller C.C.J., Grierson A.J. Familial amyotrophic lateral sclerosis-linked SOD1 mutants perturb fast axonal transport to reduce axonal mitochondria content. *Hum. Mol. Genet.* 2007; 16 (22): 2720–2728. DOI: 10.1093/hmg/ddm226.
16. Brownlee J., Ackerley S., Grierson A.J., Jacobsen N.J., Shea K., Anderton B.H., Leigh P.N., Shaw C.E., Miller C.C. Charcot-Marie-Tooth disease neurofilament mutations disrupt neurofilament assembly and axonal transport. *Hum. Mol. Genet.* 2002; 11 (23): 2837–2844. DOI: 10.1093/hmg/11.23.2837.
17. Perrone Capano C., Pernas-Alonso R., di Porzio U. Neurofilament homeostasis and motoneuron degeneration. *Bioessays.* 2001; 23 (1): 24–33. DOI: 10.1002/1521-1878 (200101)23:1<24::AID-BIES1004>3.0.CO;2-H.
18. Xu Z., Henderson R.D., David M., McCombe P.A. Neurofilaments as Biomarkers for Amyotrophic Lateral Sclerosis: A Systematic Review and Meta-Analysis. *PLoS One.* 2016; 11 (10): e0164625. DOI: 10.1371/journal.pone.0164625.
19. Henkel J.S., Engelhardt J.I., Siklós L., Simpson E.P., Kim S.H., Pan T., Goodman J.C., Siddique T., Beers D.R., Appel S.H. Presence of dendritic cells, MCP-1, and activated microglia/macrophages in amyotrophic lateral sclerosis spinal cord tissue. *Ann. Neurol.* 2004; 55 (2): 221–235. DOI: 10.1002/ana.10805.
20. Siddique T., Deng H.X. Genetics of amyotrophic lateral sclerosis. *Hum. Mol. Genet.* 1996; 5: 1465–1470. DOI: 10.1093/hmg/5.supplement_1.1465.
21. Abramychева N.Yu., Lysogorskaya E.V., Shpilukova Yu.S., Vetchinova A.S., Zakharova M.N., Illarionovskiy S.N. Molecular structure of amyotrophic lateral sclerosis in Russian population. *Neuromuscular Diseases.* 2016; 6 (4): 21–27 (in Russ.). DOI: 10.17650/2222-8721-2016-6-4-21-27.
22. Rosen D.R., Siddique T., Patterson D., Figlewicz D.A., Sapp P., Hentati A., Donaldson D., Goto J., O'Regan J.P., Deng H.X. Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. *Nature.* 1993; 362 (6415): 59–62. DOI: 10.1038/362059a0.
23. Fahed A.C., McDonough B., Gouvion C.M., Newell K.L., Dure L.S., Bebin M., Bick A.G., Seidman J.G., Harter D.H., Seidman C.E. UBQLN2 mutation causing heterogeneous X-linked dominant neurodegeneration. *Ann. Neurol.* 2014; 75 (5): 793–798. DOI: 10.1002/ana.24164.
24. Kunst C.B. Complex genetics of amyotrophic lateral sclerosis. *Am. J. Hum. Genet.* 2004; 75 (6): 933–947. DOI: 10.1086/426001.
25. Keller G.A., Warner T.G., Steimer K.S., Hallewell R.A. Cu,Zn superoxide dismutase is a peroxisomal enzyme in human fibroblasts and hepatoma cells. *Proc. Natl. Acad. Sci. USA.* 1991; 88 (16): 7381–7385. DOI: 10.1073/pnas.88.16.7381.
26. Andersen P.M., Nilsson P., Ala-Hurula V., Keränen M.L., Tarvainen I., Haltia T., Nilsson L., Binzer M., Forsgren L., Marklund S.L. Amyotrophic lateral sclerosis associated with homozygosity for an Asp90Ala mutation in Cu,Zn-superoxide dismutase. *Nat. Genet.* 1995; 10 (1): 61–66. DOI: 10.1038/ng0595-61.
27. Hand C.K., Mayeux-Portas V., Khoris J., Briolotti V., Clavelou P., Camu W., Rouleau G.A. Compound heterozygous D90A and D96N SOD1 mutations in a recessive amyotrophic lateral sclerosis family. *Ann Neurol.* 2001; 49 (2): 267–271. DOI: 10.1002/1531-8249(20010201)49:2<267::aid-ana51>3.0.co;2-d.
28. Al-Chalabi A., Andersen P.M., Chioza B., Shaw C., Sham P.C., Robberecht W., Matthijs G., Camu W., Marklund S.L., Forsgren L., Rouleau G., Laing N.G., Hulse P.V., Siddique T., Leigh P.N., Powell J.F. Recessive amyotrophic lateral sclerosis families with the D90A SOD1 mutation share a common founder: evidence for a linked protective factor. *Hum Mol Genet.* 1998; 7 (13): 2045–2050. DOI: 10.1093/hmg/7.13.2045.
29. Marangi G., Traynor B.J. Genetic causes of amyotrophic lateral sclerosis: new genetic analysis methodologies entailing new opportunities and challenges. *Brain Res.* 2015; 1607: 75–93. DOI: 10.1016/j.brainres.2014.10.009.
30. Lysogorskaya E.V., Abramychева N.Yu., Zakharova M.N., Stepanova M.S., Moroz A.A., Rossokhin A.V., Illarionovskiy S.N. Genetic studies of Russian patients with amyotrophic lateral sclerosis. *Amyotroph. Lateral. Scler. Frontotemporal. Degener.* 2015; 17 (1-2): 135–141. DOI: 10.3109/21678421.2015.1107100.
31. Yamashita S., Ando Y. Genotype-phenotype relationship in hereditary amyotrophic lateral sclerosis. *Transl. Neurodegener.* 2015; 4: 13. DOI: 10.1186/s40035-015-0036-y.
32. Lindberg M.J., Tibell L., Oliveberg M. Common denominator of Cu/Zn superoxide dismutase mutants associated with amyotrophic lateral sclerosis: decreased stability of the apo state. *Proc. Natl. Acad. Sci. USA.* 2002; 99 (26): 16607–16612. DOI: 10.1073/pnas.262527099.
33. Lysogorskaya E.V., Abramychева N.Yu., Zakharova M.N., Illarionovskiy S.N. Frequency of mutations in the SOD1 gene in Russian patients with amyotrophic lateral sclerosis. *Medical Genetics.* 2013; 12 (4): 32–37 (in Russ.). DOI: 10.1234/XXXX-XXXX-2013-4-32-37.
34. Deng H.X., Hentati A., Tainer J.A., Iqbal Z., Cayabyab A., Hung W.Y., Getzoff E.D., Hu P., Herzfeldt B., Roos R.P. et al. Amyotrophic lateral sclerosis and structural defects in Cu,Zn superoxide dismutase. *Science.* 1993; 261 (5124): 1047–1051. DOI: 10.1126/science.8351519.
35. Cleveland D.W., Laing N., Hulse P.V., Brown R.H. Jr. Toxic mutants in Charcot's sclerosis. *Nature.* 1995; 378 (6555): 342–343. DOI: 10.1038/378342a0.
36. Hayashi Y., Homma K., Ichijo H. SOD1 in neurotoxicity and its controversial roles in SOD1 mutation-negative ALS. *Adv. Biol. Regul.* 2016; 60: 95–104. DOI: 10.1016/j.jbior.2015.10.006.
37. Wiedau-Pazos M., Goto J.J., Rabizadeh S., Gralla E.B., Roe J.A., Lee M.K., Valentine J.S., Bredesen D.E. Altered reactivity of superoxide dismutase in familial amyotrophic lateral sclerosis. *Science.* 1996; 271 (5248): 515–518. DOI: 10.1126/science.271.5248.515.
38. Yim M.B., Kang J.H., Yim H.S., Kwak H.S., Chock P.B., Stadtman E.R. A gain-of-function of an amyotrophic lateral

- sclerosis-associated Cu,Zn-superoxide dismutase mutant: An enhancement of free radical formation due to a decrease in Km for hydrogen peroxide. *Proc. Natl. Acad. Sci. USA*. 1996; 93 (12): 5709–5714. DOI: 10.1073/pnas.93.12.5709.
39. Van Landeghem G.F., Tabatabaie P., Beckman G., Beckman L., Andersen P.M. Manganese-containing superoxide dismutase signal sequence polymorphism associated with sporadic motor neuron disease. *Eur. J. Neurol*. 1999; 6 (6): 639–644. DOI: 10.1046/j.1468-1331.1999.660639.x.
 40. Beckman J.S., Carson M., Smith C.D., Koppenol W.H. ALS, SOD and peroxynitrite. *Nature*. 1993; 364 (6438): 584. DOI: 10.1038/364584a0.
 41. Wong P.C., Pardo C.A., Borchelt D.R., Lee M.K., Copeland N.G., Jenkins N.A., Sisodia S.S., Cleveland D.W., Price D.L. An adverse property of a familial ALS-linked SOD1 mutation causes motor neuron disease characterized by vacuolar degeneration of mitochondria. *Neuron*. 1995; 14 (6): 1105–1116. DOI: 10.1016/0896-6273(95)90259-7.
 42. Kunst C.B., Mezey E., Brownstein M.J., Patterson D. Mutations in SOD1 associated with amyotrophic lateral sclerosis cause novel protein interactions. *Nat. Genet*. 1997; 15 (1): 91–94. DOI: 10.1038/ng0197-91.
 43. Shinder G.A., Lacourse M.C., Minotti S., Durham H.D. Mutant Cu/Zn-superoxide dismutase proteins have altered solubility and interact with heat shock/stress proteins in models of amyotrophic lateral sclerosis. *J. Biol. Chem*. 2001; 276 (16): 12791–12796. DOI: 10.1074/jbc.M010759200.
 44. Okado-Matsumoto A., Fridovich I. Amyotrophic lateral sclerosis: a proposed mechanism. *Proc. Natl. Acad. Sci. USA*. 2002; 99 (13): 9010–9014. DOI: 10.1073/pnas.132260399.
 45. Ferri A., Gabbianelli R., Casciati A., Celsi F., Rotilio G., Carri M.T. Oxidative inactivation of calcineurin by Cu,Zn superoxide dismutase G93A, a mutant typical of familial amyotrophic lateral sclerosis. *J. Neurochem*. 2001; 79 (3): 531–538. DOI: 10.1046/j.1471-4159.2001.00558.x.
 46. Bruijn L.I., Houseweart M.K., Kato S., Anderson K.L., Anderson S.D., Ohama E., Reaume A.G., Scott R.W., Cleveland D.W. Aggregation and motor neuron toxicity of an ALS-linked SOD1 mutant independent from wild-type SOD1. *Science*. 1998; 281 (5384): 1851–1854. DOI: 10.1126/science.281.5384.1851.
 47. Forsberg K., Graffmo K., Pakkenberg B., Weber M., Nielsen M., Marklund S., Brännström T., Andersen P.M. Misfolded SOD1 inclusions in patients with mutations in C9orf72 and other ALS/FTD-associated genes. *J. Neurol. Neurosurg. Psychiatry*. 2019; 90 (8): 861–869. DOI: 10.1136/jnnp-2018-319386.
 48. Zinszner H., Sok J., Immanuel D., Yin Y., Ron D. TLS (FUS) binds RNA in vivo and engages in nucleo-cytoplasmic shuttling. *J. Cell Sci*. 1997; 110 (15): 1741–1750.
 49. Ratti A., Buratti E. Physiological functions and pathobiology of TDP-43 and FUS/TLS proteins. *J. Neurochem*. 2016; 138(1): 95–111. DOI: 10.1111/jnc.13625.
 50. Lagier-Tourenne C., Polymenidou M., Hutt K.R., Vu A.Q., Baughn M., Huelga S.C., Clutario K.M., Ling S.C., Liang T.Y., Mazur C., Wanciewicz E., Kim A.S., Watt A., Freier S., Hicks G.G., Donohue J.P., Shiue L., Bennett C.F., Ravits J., Cleveland D.W., Yeo G.W. Divergent roles of ALS-linked proteins FUS/TLS and TDP-43 intersect in processing long pre-mRNAs. *Nat. Neurosci*. 2012; 15 (11): 1488–1497. DOI:10.1038/nn.3230.
 51. Colombrita C., Onesto E., Buratti E., de la Grange P., Gumina V., Baralle F.E., Silani V., Ratti A. From transcriptomic to protein level changes in TDP-43 and FUS loss-of-function cell models. *Biochim. Biophys. Acta*. 2015; 1849 (12): 1398–1410. DOI: 10.1016/j.bbaggm.2015.10.015.
 52. Lattante S., Rouleau G.A., Kabashi E. TARDBP and FUS mutations associated with amyotrophic lateral sclerosis: summary and update. *Hum. Mutat*. 2013; 34 (6): 812–826. DOI: 10.1002/humu.22319.
 53. Vance C., Scotter E.L., Nishimura A.L., Troakes C., Mitchell J.C., Kathe C., Urwin H., Manser C., Miller C.C., Hortobágyi T., Dragunow M., Rogelj B., Shaw C.E. ALS mutant FUS disrupts nuclear localization and sequesters wild-type FUS within cytoplasmic stress granules. *Hum. Mol. Genet*. 2013; 22 (13): 2676–2688. DOI: 10.1093/hmg/ddt117.
 54. Nomura T., Watanabe S., Kaneko K., Yamanaka K., Nukina N., Furukawa Y. Intranuclear aggregation of mutant FUS/TLS as a molecular pathomechanism of amyotrophic lateral sclerosis. *J. Biol. Chem*. 2014; 289 (2): 1192–1202. DOI: 10.1074/jbc.M113.516492.
 55. Kino Y., Washizu C., Kurosawa M., Yamada M., Miyazaki H., Akagi T., Hashikawa T., Doi H., Takumi T., Hicks G.G., Hattori N., Shimogori T., Nukina N. FUS/TLS deficiency causes behavioral and pathological abnormalities distinct from amyotrophic lateral sclerosis. *Acta Neuropathol. Commun*. 2015; 3: 24. DOI: 10.1186/s40478-015-0202-6.
 56. Mitchell J.C., McGoldrick P., Vance C., Hortobágyi T., Sreedharan J., Rogelj B., Tudor E.L., Smith B.N., Klasen C., Miller C.C., Cooper J.D., Greensmith L., Shaw C.E. Overexpression of human wild-type FUS causes progressive motor neuron degeneration in an age- and dose-dependent fashion. *Acta Neuropathol*. 2013; 125 (2): 273–288. DOI: 10.1007/s00401-012-1043-z.
 57. Shelkova T.A., Peters O.M., Deykin A.V., Connor-Robson N., Robinson H., Ustyugov A.A., Bachurin S.O., Ermolkevich T.G., Goldman I.L., Sadchikova E.R., Kovrazhkina E.A., Skvortsova V.I., Ling S.C., Da Cruz S., Parone P.A., Buchman V.L., Ninkina N.N. Fused in sarcoma (FUS) protein lacking nuclear localization signal (NLS) and major RNA binding motifs triggers proteinopathy and severe motor phenotype in transgenic mice. *J. Biol. Chem*. 2013; 288 (35): 25266–25274. DOI: 10.1074/jbc.M113.492017.
 58. Robinson H.K., Deykin A.V., Bronovitsky E.V., Ovchinnikov R.K., Ustyugov A.A., Shelkova T.A., Kukharsky M.S., Ermolkevich T.G., Goldman I.L., Sadchikova E.R., Kovrazhkina E.A., Bachurin S.O., Buchman V.L., Ninkina N.N. Early lethality and neuronal proteinopathy in mice expressing cytoplasm-targeted FUS that lacks the RNA recognition motif. *Amyotroph. Lateral Scler. Frontotemporal. Degener*. 2015; 16 (5-6): 402–409. DOI: 10.3109/21678421.2015.1040994.
 59. Huang C., Zhou H., Tong J., Chen H., Liu Y.J., Wang D., Wei X., Xia X.G. FUS transgenic rats develop the phenotypes of amyotrophic lateral sclerosis and frontotemporal lobar degeneration. *PLoS Genet*. 2011; 7 (3): e1002011. DOI: 10.1371/journal.pgen.1002011.

60. Seckic-Zahirovic J., Sendscheid O., El Oussini H., Jambeau M., Sun Y., Mersmann S., Wagner M., Dieterlé S., Sinniger J., Dirrig-Grosch S., Drenner K., Birling M.C., Qiu J., Zhou Y., Li H., Fu X.D., Rouaux C., Shelkovnikova T., Witting A., Ludolph A.C., Kiefer F., Storkebaum E., Lagier-Tourenne C., Dupuis L. Toxic gain of function from mutant FUS protein is crucial to trigger cell autonomous motor neuron loss. *EMBO J.* 2016; 35 (10): 1077–1097. DOI: 10.15252/embj.201592559.
61. Sharma A., Lyashchenko A.K., Lu L., Nasrabady S.E., Elmaleh M., Mendelsohn M., Nemes A., Tapia J.C., Mentis G.Z., Shneider N.A. ALS-associated mutant FUS induces selective motor neuron degeneration through toxic gain of function. *Nat. Commun.* 2016; 7: 10465. DOI: 10.1038/ncomms10465.
62. Hennig S., Kong G., Mannen T., Sadowska A., Kobelke S., Blythe A., Knott G.J., Iyer K.S., Ho D., Newcombe E.A., Hosoki K., Goshima N., Kawaguchi T., Hatters D., Trinkle-Mulcahy L., Hirose T., Bond C.S., Fox A.H. Prion-like domains in RNA binding proteins are essential for building subnuclear paraspeckles. *J. Cell Biol.* 2015; 210 (4): 529–539. DOI: 10.1083/jcb.201504117.
63. Zhou Y., Liu S., Liu G., Oztürk A., Hicks G.G. ALS-associated FUS mutations result in compromised FUS alternative splicing and autoregulation. *PLoS Genet.* 2013; 9 (10): e1003895. DOI: 10.1371/journal.pgen.1003895.
64. Qiu H., Lee S., Shang Y., Wang W.Y., Au K.F., Kamiya S., Barmada S.J., Finkbeiner S., Lui H., Carlton C.E., Tang A.A., Oldham M.C., Wang H., Shorter J., Filiano A.J., Roberson E.D., Tourtellotte W.G., Chen B., Tsai L.H., Huang E.J. ALS-associated mutation FUS-R521C causes DNA damage and RNA splicing defects. *J. Clin. Invest.* 2014; 124 (3): 981–999. DOI: 10.1172/JCI72723.
65. Ayala Y.M., Zago P., D'Ambrogio A., Xu Y.F., Petrucelli L., Buratti E., Baralle F.E. Structural determinants of the cellular localization and shuttling of TDP-43. *J. Cell Sci.* 2008; 121 (22): 3778–3785. DOI: 10.1242/jcs.038950.
66. Buratti E., Baralle F.E. The multiple roles of TDP-43 in pre-mRNA processing and gene expression regulation. *RNA Biol.* 2010; 7 (4): 420–429. DOI: 10.4161/rna.7.4.12205.
67. Tollervey J.R., Curk T., Rogelj B., Briese M., Cereda M., Kayikci M., König J., Hortobágyi T., Nishimura A.L., Zupunski V., Patani R., Chandran S., Rot G., Zupan B., Shaw C.E., Ule J. Characterizing the RNA targets and position-dependent splicing regulation by TDP-43. *Nat. Neurosci.* 2011; 14 (4): 452–458. DOI: 10.1038/nn.2778.
68. Leigh P.N., Whitwell H., Garofalo O., Buller J., Swash M., Martin J.E., Gallo J.M., Weller R.O., Anderton B.H. Ubiquitin-immunoreactive intraneuronal inclusions in amyotrophic lateral sclerosis. Morphology, distribution, and specificity. *Brain.* 1991; 114 (2): 775–788. DOI: 10.1093/brain/114.2.775.
69. Arai T., Hasegawa M., Akiyama H., Ikeda K., Nonaka T., Mori H., Mann D., Tsuchiya K., Yoshida M., Hashizume Y., Oda T. TDP-43 is a component of ubiquitin-positive tau-negative inclusions in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Biochem. Biophys. Res. Commun.* 2006; 351 (3): 602–611. DOI: 10.1016/j.bbrc.2006.10.093.
70. Neumann M., Sampathu D.M., Kwong L.K., Truax A.C., Micsenyi M.C., Chou T.T., Bruce J., Schuck T., Grossman M., Clark C.M., McCluskey L.F., Miller B.L., Masliah E., Mackenzie I.R., Feldman H., Feiden W., Kretschmar H.A., Trojanowski J.Q., Lee V.M. Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Science.* 2006; 314 (5796): 130–133. DOI: 10.1126/science.1134108.
71. Giordana M.T., Piccinini M., Grifoni S., De Marco G., Vercellino M., Magistrello M., Pellerino A., Buccinnà B., Lupino E., Rinaudo M.T. TDP-43 redistribution is an early event in sporadic amyotrophic lateral sclerosis. *Brain Pathol.* 2010; 20 (2): 351–360. DOI: 10.1111/j.1750-3639.2009.00284.x.
72. Schipper L.J., Raaphorst J., Aronica E., Baas F., de Haan R., de Visser M., Troost D. Prevalence of brain and spinal cord inclusions, including dipeptide repeat proteins, in patients with the C9ORF72 hexanucleotide repeat expansion: a systematic neuropathological review. *Neuropathol. Appl. Neurobiol.* 2016; 42 (6): 547–560. DOI: 10.1111/nan.12284.
73. Buratti E., Brindisi A., Giombi M., Tisminetzky S., Ayala Y.M., Baralle F.E. TDP-43 binds heterogeneous nuclear ribonucleoprotein A/B through its C-terminal tail: an important region for the inhibition of cystic fibrosis transmembrane conductance regulator exon 9 splicing. *J. Biol. Chem.* 2005; 280 (45): 37572–37584. DOI: 10.1074/jbc.M505557200.
74. Kraemer B.C., Schuck T., Wheeler J.M., Robinson L.C., Trojanowski J.Q., Lee V.M., Schellenberg G.D. Loss of murine TDP-43 disrupts motor function and plays an essential role in embryogenesis. *Acta Neuropathol.* 2010; 119 (4): 409–419. DOI: 10.1007/s00401-010-0659-0.
75. Chiang P.M., Ling J., Jeong Y.H., Price D.L., Aja S.M., Wong P.C. Deletion of TDP-43 down-regulates Tbc1d1, a gene linked to obesity, and alters body fat metabolism. *Proc. Natl. Acad. Sci. USA.* 2010; 107 (37): 16320–16324. DOI: 10.1073/pnas.1002176107.
76. Ash P.E., Zhang Y.J., Roberts C.M., Saldi T., Hutter H., Buratti E., Petrucelli L., Link C.D. Neurotoxic effects of TDP-43 overexpression in *C. elegans*. *Hum. Mol. Genet.* 2010; 19 (16): 3206–3218. DOI: 10.1093/hmg/ddq230.
77. Kabashi E., Lin L., Tradewell M.L., Dion P.A., Bercier V., Bourgouin P., Rochefort D., Bel Hadj S., Durham H.D., Vande Velde C., Rouleau G.A., Drapeau P. Gain and loss of function of ALS-related mutations of TARDBP (TDP-43) cause motor deficits *in vivo*. *Hum. Mol. Genet.* 2010; 19 (4): 671–683. DOI: 10.1093/hmg/ddp534.
78. Xu Y.F., Zhang Y.J., Lin W.L., Cao X., Stetler C., Dickson D.W., Lewis J., Petrucelli L. Expression of mutant TDP-43 induces neuronal dysfunction in transgenic mice. *Mol. Neurodegener.* 2011; 6: 73. DOI: 10.1186/1750-1326-6-73.
79. Koyama A., Sugai A., Kato T., Ishihara T., Shiga A., Toyoshima Y., Koyama M., Konno T., Hirokawa S., Yokoseki A., Nishizawa M., Kakita A., Takahashi H., Onodera O. Increased cytoplasmic TARDBP mRNA in affected spinal motor neurons in ALS caused by abnormal autoregulation of TDP-43. *Nucleic. Acids Res.* 2016; 44 (12): 5820–5836. DOI: 10.1093/nar/gkw499.
80. Highley J.R., Kirby J., Jansweijer J.A., Webb P.S., Hewamadduma C.A., Heath P.R., Higginbottom A., Raman R., Ferraiuolo L., Cooper-Knock J., McDermott C.J., Wharton S.B., Shaw P.J., Ince P.G. Loss of nuclear TDP-43 in amyotrophic lateral sclerosis (ALS) causes altered expression of splicing

- machinery and widespread dysregulation of RNA splicing in motor neurones. *Neuropathol. Appl. Neurobiol.* 2014; 40 (6): 670–685. DOI: 10.1111/nan.12148.
81. Klim J.R., Williams L.A., Limone F., Guerra San Juan I., Davis-Dusenbery B.N., Mordes D.A., Burberry A., Steinbaugh M.J., Gamage K.K., Kirchner R., Moccia R., Cassel S.H., Chen K., Wainger B.J., Woolf C.J., Eggan K. ALS-implicated protein TDP-43 sustains levels of STMN2, a mediator of motor neuron growth and repair. *Nat. Neurosci.* 2019; 22 (2): 167–179. DOI: 10.1038/s41593-018-0300-4.
 82. Buratti E. TDP-43 post-translational modifications in health and disease. *Expert. Opin. Ther. Targets.* 2018; 22 (3): 279–293. DOI: 10.1080/14728222.2018.1439923.
 83. Farg M.A., Sundaramoorthy V., Sultana J.M., Yang S., Atkinson R.A., Levina V., Halloran M.A., Gleeson P.A., Blair I.P., Soo K.Y., King A.E., Atkin J.D. C9ORF72, implicated in amyotrophic lateral sclerosis and frontotemporal dementia, regulates endosomal trafficking. *Hum. Mol. Genet.* 2014; 23 (13): 3579–3595. DOI: 10.1093/hmg/ddu068.
 84. Waite A.J., Bäumer D., East S., Neal J., Morris H.R., Ansorge O., Blake D.J. Reduced C9orf72 protein levels in frontal cortex of amyotrophic lateral sclerosis and frontotemporal degeneration brain with the C9ORF72 hexanucleotide repeat expansion. *Neurobiol. Aging.* 2014; 35 (7): 1779.e5–1779.e13. DOI: 10.1016/j.neurobiolaging.2014.01.016.
 85. Koppers M., Blokhuis A.M., Westeneng H.J., Terpstra M.L., Zundel C.A., Vieira de Sá R., Schellevis R.D., Waite A.J., Blake D.J., Veldink J.H., van den Berg L.H., Pasterkamp R.J. C9orf72 ablation in mice does not cause motor neuron degeneration or motor deficits. *Ann. Neurol.* 2015; 78 (3): 426–438. DOI: 10.1002/ana.24453.
 86. Sareen D., O'Rourke J.G., Meera P., Muhammad A.K., Grant S., Simpkinson M., Bell S., Carmona S., Ornelas L., Sahabian A., Gendron T., Petrucelli L., Baughn M., Ravits J., Harms M.B., Rigo F., Bennett C.F., Otis T.S., Svendsen C.N., Baloh R.H. Targeting RNA foci in iPSC-derived motor neurons from ALS patients with a C9ORF72 repeat expansion. *Sci. Transl. Med.* 2013; 5 (208): 208ra149. DOI: 10.1126/scitranslmed.3007529.
 87. Barker H.V., Niblock M., Lee Y.B., Shaw C.E., Gallo J.M.. RNA Misprocessing in C9orf72-Linked Neurodegeneration. *Front. Cell Neurosci.* 2017; 11: 195. DOI: 10.3389/fnecel.2017.00195.
 88. Ash P.E., Bieniek K.F., Gendron T.F., Caulfield T., Lin W.L., DeJesus-Hernandez M., van Blitterswijk M.M., Jansen-West K., Paul J.W., Rademakers R., Boylan K.B., Dickson D.W., Petrucelli L. Unconventional translation of C9ORF72 GGGGCC expansion generates insoluble polypeptides specific to c9FTD/ALS. *Neuron.* 2013; 77 (4): 639–646. DOI: 10.1016/j.neuron.2013.02.004.
 89. Todd T.W., Petrucelli L. Insights into the pathogenic mechanisms of Chromosome 9 open reading frame 72 (C9orf72) repeat expansions. *J. Neurochem.* 2016; 138(1): 145–162. DOI: 10.1111/jnc.13623.
 90. Fratta P., Mizielinska S., Nicoll A.J., Zloh M., Fisher E.M., Parkinson G., Isaacs A.M. C9orf72 hexanucleotide repeat associated with amyotrophic lateral sclerosis and frontotemporal dementia forms RNA G-quadruplexes. *Sci. Rep.* 2012; 2: 1016. DOI: 10.1038/srep01016.

Authors information

Savinova Alina V., Neurologist, Clinical Resident, V.M. Bekhterev NRMC PN, Saint Petersburg, Russian Federation. ORCID 0000-0002-7036-5326.

Shnayder Natalia A., Dr. Sci. (Med.), Professor, Leading Researcher, Department of Personalized Psychiatry and Neurology, V.M. Bekhterev NRMC PN, Saint Petersburg; Neurologist, Neurological Center of Epileptology, Neurogenetics, and Brain Research, University Clinic, V.F. Voyno-Yasenetsky KrasSMU, Krasnoyarsk, Russian Federation. ORCID 0000-0002-2840-837X.

Nasyrova Regina F., Dr. Sci. (Med.), Principal Researcher, Head of the Department of Personalized Psychiatry and Neurology, V.M. Bekhterev NRMC PN, Saint Petersburg; Principal Researcher, Research Laboratory OpenLab “Gene and Cell Technologies”, Institute of Fundamental Medicine and Biology (IFMB), KFU, Kazan, Russian Federation. ORCID 0000-0003-1874-9434.

(✉) **Nasyrova Regina F.**, e-mail: nreginaf77@gmail.com

Received 03.03.2020

Accepted 29.09.2020

MicroRNA: the role in the pathophysiology of atrial fibrillation and potential use as a biomarker

Chaulin A.M., Duplyakov D.V.

*Samara State Medical University
89, Chapaevskaya Str., Samara, 443099, Russian Federation*

ABSTRACT

The aim of the study was to analyze medical literature on the role of microRNA in the pathophysiology of atrial fibrillation and the possibilities of using microRNAs as biomarkers.

The analysis of modern medical literature was carried out using the PubMed – NCBI database.

Atrial fibrillation (AF) is a common and serious cardiovascular disease. The pathophysiological mechanisms underlying the development of atrial fibrillation are not entirely clear. In addition, there are no optimal biomarkers for early detection and assessment of the prognosis for patients with atrial fibrillation. Recently, the attention of researchers has been directed to the molecules of microRNA. There is a lot of evidence that they are involved in the pathogenesis of neurological, oncological, and cardiovascular diseases. This review examines the role of microRNAs in the pathophysiology of atrial fibrillation. The possibility of using microRNA as a biomarker for the diagnosis and prediction of atrial fibrillation is also discussed.

MicroRNAs play a crucial role in the pathophysiology of atrial fibrillation, regulating the mechanisms of atrial remodeling, such as electrical remodeling, structural remodeling, remodeling of the autonomic nervous system, and impaired regulation of calcium levels. The stability of microRNAs and the possibility to study them in various biological fluids and tissues, including blood, make these molecules a promising diagnostic biomarker for various cardiovascular diseases. The presented data clearly indicate that AF is associated with changes in the expression level of various microRNAs, which can be quantified using a polymerase chain reaction. Further research is required to assess the role of microRNAs as biomarkers for atrial fibrillation, in particular to establish precise reference limits.

Key words: microRNA, atrial fibrillation, pathophysiology, biomarker, laboratory diagnostics.

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

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

For citation: Chaulin A.M., Duplyakov D.V. MicroRNA: the role in the pathophysiology of atrial fibrillation and potential use as a biomarker. *Bulletin of Siberian Medicine*. 2021; 20 (3): 203–212. <https://doi.org/10.20538/1682-0363-2021-3-203-212>.

МикроРНК: роль в патофизиологии фибрилляции предсердий и возможности использования в качестве биомаркера

Чаулин А.М., Дупляков Д.В.

Самарский государственный медицинский университет
Россия, 443099, г. Самара, ул. Чапаевская, 89

РЕЗЮМЕ

Проведен анализ современной медицинской литературы по базе данных PubMed – NCBI. Фибрилляция предсердий является широко распространенным и серьезным сердечно-сосудистым заболеванием. Патофизиологические механизмы, лежащие в основе развития фибрилляции предсердий, не совсем ясны. Кроме того, отсутствуют оптимальные биомаркеры для раннего выявления и оценки прогноза пациентов с фибрилляцией предсердий.

В последнее время внимание исследователей привлекли молекулы микрорибонуклеиновой кислоты (микроРНК). Накоплено немало данных, согласно которым они участвуют в патогенезе неврологических, онкологических и сердечно-сосудистых заболеваний. Рассмотрена роль микроРНК в патофизиологии фибрилляции предсердий. Также обсуждается возможность использования микроРНК в качестве биомаркеров для диагностики и прогнозирования фибрилляции предсердий.

Ключевые слова: микроРНК, фибрилляция предсердий, патофизиология, биомаркер, лабораторная диагностика.

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

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

Для цитирования: Чаулин А.М., Дупляков Д.В. МикроРНК: роль в патофизиологии фибрилляции предсердий и возможности использования в качестве биомаркера. *Бюллетень сибирской медицины*. 2021; 20 (3): 203–212. <https://doi.org/10.20538/1682-0363-2021-3-203-212>.

INTRODUCTION

Atrial fibrillation (AF) is the most common and persistent type of cardiac arrhythmia. The most common risk factors for AF are heart failure, diabetes, hypertension, hyperthyroidism, obesity, gender, and the presence of structural heart diseases [1].

Many risk factors for AF, including genetic, molecular, and environmental ones, contribute to the development of other cardiovascular diseases (CVD) [2]. According to the latest data, there are more than 33 million people with AF worldwide. It is worth noting that the incidence is characterized by gender peculiarities – in men, the frequency of AF is 3 times higher than in women [3].

The simplest and most commonly used method for diagnosing AF is electrocardiography (ECG), but this method has a significant drawback that consists in short duration of recording of the electrical activity of the heart, which limits the diagnosis in asymptomatic pa-

tients [4]. Existing laboratory biomarkers of myocardial damage (cardiac troponins, natriuretic peptides, creatine phosphokinase (CPK), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), etc.) are of little importance for predicting the course of AF [5–7]. The increase in cardiac troponins in AF occurs due to damage to cardiomyocytes, which is provoked by a decrease in the blood supply to the myocardium. It is believed that insufficient blood supply to the myocardium in AF occurs following shortening of the myocardial relaxation phase (diastole) [7]. According to some data, troponins correlate well with the severity of arrhythmia and can be used as prognostic markers [8], while according to other data, they are ineffective [9]. Therefore, there is a need to search for new and more reliable biomarkers for early diagnosis of AF.

Recently, due to the discovery of new regulatory molecules, the genetic component has been considered as a significant risk factor for the development of AF [10, 11]. MicroRNAs (miRNAs) were first dis-

covered in 1993 by a group of researchers under the guidance of R. Lee in a free-living nematode *Caenorhabditis elegans* [12]. MiRNAs are single-stranded ribonucleic acids that do not encode protein and consist of about 22 nucleotides [13]. According to a number of studies, miRNAs play a vital role in various developmental processes in animals and humans, including growth, proliferation, differentiation, and cell metabolism [14]. In mammals, about 2,200 different miRNAs have been found, that regulate about one third of protein-encoding genes [15]. It was reported that changes in miRNA expression are associated with various pathological conditions, such as neurological diseases, autoimmune disorders, cardiovascular diseases, and cancer. For example, a change in the character of miRNA expression can promote transformation of healthy cells into malignant ones [16].

The process of cardiac remodeling was associated with changes in the expression of miRNA in the heart tissue and blood, while miRNA levels had prognostic and diagnostic value [17–19]. It was also reported that modulation of miRNA expression decreases or increases susceptibility to the development of AF in vivo. Therefore, miRNAs can be a potential target for the effects of therapeutic agents used to treat AF [20]. Understanding the pathophysiological mechanisms regulated by miRNAs is essential for improving diagnostic and treatment strategies in AF.

ROLE OF MIRNA IN THE PATHOPHYSIOLOGY OF AF

According to recent data, miRNAs play a crucial role in the pathophysiology of AF, regulating the mechanisms of atrial remodeling. The decrease and increase in the miRNA expression are genetically programmed and, under normal conditions, contribute to the development of the cardiovascular system in humans and animals. At the same time, changes in the level of miRNA expression in circulating blood and tissues can also occur in pathological conditions associated with the development of various CVDs, including AF, which lead to myocardial remodeling.

To date, due to numerous experimental and clinical studies, information on the role of miRNAs in the pathogenesis of AF has been accumulated. The following key pathogenetic mechanisms in the initiation and progression of AF can be distinguished, in the regulation of which miRNAs are involved: electrical remodeling, structural remodeling, remodeling of the cardiac autonomic nervous system, and impaired intracellular homeostasis of calcium ions (Ca^{2+}). The

major part of this review article is devoted to consistent discussion of the role of miRNAs in each of these mechanisms.

MiRNA-mediated electrical remodeling in AF

Electrical remodeling of cardiomyocytes is the most common change associated with AF. Electrical remodeling occurs due to a decrease in the L-type calcium channel conductance (ICaL) and an increase in the inward rectifier potassium channel conductance (IK1). Changes in the electrical properties of Ca^{2+} -dependent potassium channels and connexin 40 and connexin 43 gap junction ion channels also induce electrical remodeling associated with AF [21]. Several different miRNAs are involved in the process of electrical remodeling: miRNA-1, miRNA-328, and miRNA-499, each of which exhibits several specific properties.

MiRNA-1 is abundantly expressed in cardiac and skeletal muscles and plays an important role in the embryogenesis (development) of muscle tissue. It was found that miRNA-1 promotes differentiation and proliferation of myoblasts [22]. Changes in the expression of miRNA-1 affect the electrophysiology of the heart, increasing the risk of developing cardiac arrhythmia. Thus, a study of atrial tachycardia on a rabbit model showed that overexpression of miRNA-1 shortens the atrial effective refractory period induced by tachycardia and increases the inward rectifier potassium channel current (IKs) by reducing the expression of *KCNE1* and *KCNB2* genes encoding potassium channel subunits. Knockdown (inactivation) of miRNA-1, on the contrary, weakens the suppression of *KCNE1* and *KCNB2* genes, shortens the atrial effective refractory period, and increases IKs. Thus, it was found that *KCNE1* and *KCNB2* are target genes for miRNA-1. It was also suggested that targeted suppression of these potassium channel genes enhances the duration and incidence of AF. Therefore, the study demonstrated the crucial role of miRNA-1 in electrical remodeling and its clinical significance as a potential therapeutic target for AF [23]. Another study examined the expression of cardiac muscle-specific miRNAs, including miRNA-1, in the right atrium of postoperative patients. It was found that after coronary artery bypass grafting, the expression of miRNA-1 in the myocardial tissue of the right atrium increased and it contributed to postoperative apoptosis of cardiomyocytes and played an essential role in the development of postoperative AF. Postoperative AF is a serious complication of cardiac surgery which

is associated with poor prognosis. However, there were no differences in the level of miRNA-1 in the blood plasma of patients with postoperative AF and patients with normal sinus rhythm or without AF in the medical history [24]. Some studies have shown that miRNA-1 expression is lower in elderly patients with AF than in younger patients with normal sinus rhythm. A decrease in the expression of miRNA-1 leads to the activation of transcription of *HCN2* and *HCN4* – genes encoding cyclic nucleotide-gated channels activated by hyperpolarization. Therefore, it was found that with age the expression of miRNA-1 decreases; while the expression of *HCN2* and *HCN4*, on the contrary, increases, and these alterations contribute to electrophysiological changes, increasing the likelihood of AF development [25]. In patients with persistent AF, a decrease in the expression of miRNA-1, an increase in the expression of Kir2.1 potassium channel subunits, and a corresponding increase in IK1 conductance were observed. This leads to a slowdown in cardiac conduction and increases the risk of AF induction [26]. A number of studies confirm the relationship between the expression of miRNA-1 and the emergence of arrhythmia [25–28]. In particular, miRNA-1 was shown to modulate electrical remodeling of the heart by decreasing the concentration of intracellular calcium ions, which ultimately reduce the expression of *CACNB2* [27]. In addition, down-regulation of Ca²⁺ + regulatory proteins, such as calmodulin (CaM), protein phosphatase 2A (PP2A), Na⁺ / Ca²⁺ + exchanger (NCX), and phospholamban (PLN), contributes to the pathogenesis of AF by shortening the atrial effective refractory period [28].

MiRNA-328 is also involved in atrial remodeling. In a study by Y. Lu et al., it was shown that the expression level of miRNA-328 in the atrial myocardium in animals and patients with AF was several times higher than in the controls [29]. Activation of miRNA-328 decreases the expression of the *CACNA1C* and *CACNB1* genes encoding the L-type calcium channel subunits, which leads to a decrease in ICaL and, as a consequence, reduces the action potential duration, that predisposes to the development of AF [29, 30]. Another study reported that the level of miRNA-328 in the blood plasma of patients with AF was higher than in the controls. It is worth noting that the expression level of miRNA-328 in patients with AF also differed depending on the site of blood collection. Thus, the level of miRNA-328 in the blood plasma collected from the left atrial appendage was higher than that in the peripheral blood and blood obtained from the pul-

monary vein [31]. The authors of this work suggested that the localized expression of miRNA-328 in the left atrium may be involved in the process of cardiac remodeling in patients with AF.

MiRNA-499. A comparative study of patients with permanent AF and patients with normal sinus rhythm showed that activation of miRNA-499 significantly suppressed the expression of cardiac SK3 (small conductance calcium-activated potassium channel 3) by targeting the *KCNN3* gene [32]. It was reported that miRNA-499 lowers the expression of the *CACNB2* gene encoding the L-type calcium channel subunit, which contributes to the development of AF [33]. The expression of miRNA-499 in patients with AF with rapid ventricular rate was 2.3 times higher than in AF patients with slow ventricular response and in patients from the control group [34].

MiRNA-mediated structural remodeling in AF

MiRNAs that are involved in structural remodeling regulate protein-encoding genes responsible for the formation of the extracellular matrix and promote atrial fibrosis by regulating pro- and antifibrotic signaling pathways [35]. These miRNAs are mainly involved in reducing the conduction rate and increasing the re-entrant activity [36]. The following miRNAs are involved in the structural remodeling of the heart in AF: miRNA-21, miRNA-29, miRNA-126, miRNA-150, and miRNA-483.

MiRNA-21. The expression of miRNA-21 is increased in cardiomyocytes in patients with persistent AF [37]. This causes a decrease in the expression of *CACNA1C* and *CACNB2* genes, encoding two subunits of voltage-gated calcium channels, which leads to a decrease in ICaL [37]. Elevated expression of miRNA-21 in fibroblasts increases the risk of cardiac fibrosis and associated AF. According to O. Adam et al., elevated expression of miRNA-21 in the myocardium of rats and humans with AF increases the activity of mitogen-activated protein kinase / extracellular signal-regulated kinases (MAPK / ERK signaling pathway), which promotes atrial remodeling and fibrosis [38]. Administration of a miRNA-21 antagonist in vivo potentially reduces atrial fibrosis and the risk of AF [39]. In addition, increased expression of miRNA-21 promotes atrial remodeling in an experimental rat model by activating phosphoinositide 3-kinase (PI3K), which leads to inhibition of *PTEN* gene expression [40]. Therefore, suppression of signaling pathways associated with miRNA-21 may be a new approach to AF therapy [39, 40].

MiRNA-29 plays a crucial role in the remodeling of extracellular matrix proteins [41] by regulating the *COL1A1*, *COL3A1*, and *FBN1* genes encoding collagen 1A1, collagen 3A1, and fibrillin. The level of miRNA-29 in the plasma of patients with AF and patients simultaneously suffering from chronic heart failure and AF is significantly lower than in the control group. In mice, adenovirus-mediated inhibition of miRNA-29 markedly increases the expression of *COL1A1* miRNA and collagen content in the cardiac tissue, which indicates a potential role of miRNA-29 in fibrotic remodeling [42].

MiRNA-126 is involved in the angiogenesis by regulating the expression of epidermal growth factor-like domain 7 (EGFL7). It was shown that miRNA-126 is abundantly expressed in the human heart and detected in significant amounts in the blood serum [43]. The level of miRNA-126 in AF patients is noticeably lower than in the controls. In addition, the level of miRNA-126 in patients with heart failure and AF is significantly lower than in patients suffering only from heart failure or in patients only with AF. The expression of miRNA-126 positively correlated with the left ventricular ejection fraction ($r = 0.374$, $p < 0.01$) and negatively correlated with the level of the N-terminal brain natriuretic propeptide ($r = -0.783$, $p < 0.01$). This indicates a relationship between the level of miRNA-126 in the blood serum and the severity of the disease [44].

MiRNA-150 plays an essential role in the pathogenesis of AF. The expression of miRNA-150 was low in platelets and serum of patients with chronic systolic heart failure with or without AF. The expression level of miRNA-150 in platelets of patients with AF was 3.2 times lower than in platelets of the controls. Likewise, the serum miRNA-150 level in patients with AF was 1.5 times lower than in patients with heart failure, but without AF. In addition, serum miRNA-150 levels closely correlated with platelet miRNA-150 levels ($r = 0.65$, $p = 0.0087$). The researchers suggested that a lower level of miRNA-150 in platelets may promote their activation and contribute to a prothrombotic state in AF [45]. A prospective study investigated the expression of miRNA-150 in blood plasma and atrial tissue of patients with and without AF and in patients undergoing cardiac ablation. Plasma miRNA-150 levels in AF patients were 2 times lower than in the controls. In addition, the plasma miRNA-150 level in patients with paroxysmal atrial fibrillation was lower than in patients with persistent AF. The expression level of miRNA-150 one month after AF ablation was

3 times higher than before the surgery [46]. According to Z. Liu et al., a decrease in the miRNA-150 level in patients with AF was associated with an increase in the expression of inflammatory cytokines and mediators, such as interleukin (*IL-6*), *IL-18*, tumor necrosis factor alpha (TNF- α), transforming growth factor beta (TGF- β), and C-reactive protein (CRP). In patients with AF, miRNA-150 levels closely correlated with CRP ($r = 0.77$) [18].

MiRNA-483. The level of circulating miRNA-483 in the blood may be a potential biomarker of the risk of AF in postoperative patients [47]. It was found that activation of transcription of the *IGF2* gene encoding the insulin-like growth factor is the result of miRNA-483 overexpression. This, in turn, enhances the expression of proinflammatory mediators by regulating pathways mediated by *IL-6* and nuclear factor kappa B (NF- κ B). In patients with higher miRNA-483 expression in the serum prior to coronary artery bypass grafting, the risk of postoperative AF was higher [47]. This suggests that miRNA-483 may be a potential biomarker for predicting early development of postoperative AF.

MiRNA-mediated remodeling of the cardiac autonomic nervous system in AF

It is known that changes in the electrical activity of the heart are closely related to the stimulation of the vagus nerve and release of acetylcholine, which leads to shortened duration of the action potential, contributing to the progression of AF [48]. Modulation of the activity of the autonomic nervous system is a very valuable and promising strategy in the treatment of atrial arrhythmia. It was shown that at least two miRNAs, miRNA-30 and miRNA-206, are involved in the remodeling of the cardiac autonomic nervous system in AF.

MiRNA-30. Dysregulation of the cardiac autonomic nervous system mediated by miRNA-30 plays a fundamental role in the initiation and maintenance of AF by increasing the current through G-protein-gated inwardly rectifying potassium channels (IKACH), which decreases the duration of the action potential [49]. An increase in the miRNA-30 expression caused inhibition of the *KCNJ3* gene expression and a decrease in acetylcholine-activated K^+ current (IK⁺, ACh) in patients with persistent AF [50].

MiRNA-206. In vitro and in vivo experiments have shown that overexpression of miRNA-206 in the autonomic ganglia suppresses the expression of the *SOD1* gene encoding superoxide dismutase, the enzyme of

the antioxidant defense, which leads to an increase in the formation of reactive oxygen species and shortening of the effective refractory period in the atria [51]. This study confirmed the relationship between the overproduction of reactive oxygen species and increased predisposition to AF. It was also reported that an increase in the expression of miRNA-206 in an experimental dog model suppressed the *GCHI* gene encoding GTP cyclohydrolase I, a key enzyme in the de novo synthesis of tetrahydrobiopterin, which is a coenzyme for nitric oxide synthesis. A decrease in the levels of tetrahydrobiopterin and nitric oxide in the atria was accompanied by reduction of the effective refractory period and contributed to the development of AF. On the contrary, overexpression of the *GCHI* gene prevented the development of AF [52]. Therefore, in this study, it was shown that miRNA-206-mediated inhibition of the *GCHI* gene promotes the onset of AF. Further investigation of this mechanism is a promising direction for development of therapy.

MiRNA-MEDIATED IMPAIRMENT OF INTRACELLULAR HOMEOSTASIS OF Ca^{2+} IONS IN AF

Changes in miRNA expression in the heart affect the homeostasis of Ca^{2+} ions in cardiomyocytes, leading to delayed afterdepolarization [53]. Delayed afterdepolarization is the result of increased release of diastolic Ca^{2+} from the sarcoplasmic reticulum through

the ryanodine receptor 2 (RyR2) and promotes the release of Ca^{2+} through the sodium-calcium exchanger (NCX) [54]. Impaired regulation of intracellular homeostasis of Ca^{2+} ions is mediated by several miRNAs, such as miRNA-106 and miRNA-208.

MiRNA-106. In an experimental study, it was shown that miRNA-106 suppresses the translation (biosynthesis) of RyR2, while a decrease in the expression of miRNA-106, observed in the atrial myocardium during AF, activates the expression of RyR2, which increases the release of Ca^{2+} from the sarcoplasmic reticulum, contributing to the pathogenesis of AF. Thus, in mice with knocked out miRNA-106, a spontaneous increase in Ca^{2+} release through RyR2 was observed, which caused an increased incidence of ectopic atrial rhythm and AF [55].

MiRNA-208. It was shown that miRNA-208 plays an important role in the regulation of calcium levels. An increase in the expression of miRNA-208 in atrial myocytes obtained from patients with AF is associated with a decrease in the expression of Ca-ATPase (SERCA2a) in the sarcoplasmic reticulum, which is necessary for the transport of Ca^{2+} ions from the cytosol to the sarcoplasmic reticulum. In addition, increased levels of miRNA-208 reduce the expression of L-type calcium channel subunits (CACNA1C and CACNB2) [56].

The above stated data on the role of miRNAs in the pathophysiology of AF and the possibility of their use as biomarkers are summarized in the Table.

Table

The role of miRNAs in the pathophysiology of AF and the possibility of their use as biomarkers				
Pathophysiological mechanism	miRNA	Targets and effects	Association between increased / decreased levels of miRNA with AF	Reference
Electrical remodeling	miRNA-1	Decrease in the expression of <i>KCNE1</i> and <i>KCNB2</i> , <i>HCN2</i> , <i>HCN4</i>	Both increase and decrease in miRNA-1 levels may be associated with AF.	[23–26]
	miRNA-328	Decrease in the expression of <i>CACNA1C</i> and <i>CACNB1</i>	Elevated miRNA-328 levels are associated with AF	[29–31]
	miRNA-499	Decrease in the expression of <i>KCNJ3</i> and <i>CACNB2</i>	Increased miRNA-499 levels are associated with AF	[32–34]
Structural remodeling	miRNA-21	Decrease in the expression of <i>CACNA1C</i> , <i>CACNB2</i> , <i>PTEN</i>	Increased miRNA-21 levels are associated with AF	[37–40]
	miRNA-29	Decrease in the expression of <i>COL1A1</i> , <i>COL3A1</i> , <i>FBN1</i>	Decreased miRNA-29 levels are associated with AF	[41, 42]
	miRNA-126	Decrease in the expression of <i>EGFL7</i>	Decreased miRNA-126 levels are associated with AF	[43, 44]
	miRNA-150	Decrease in the expression of <i>IL-6</i> , <i>IL-18</i> , <i>TNFα</i> , <i>TGFβ</i> , <i>CRP</i>	Decreased miRNA-150 levels are associated with AF	[18, 45]
	miRNA-483	Increase in the expression of <i>IGF2</i>	Increased miRNA-483 levels are associated with AF	[47]
Remodeling of the autonomic nervous system	miRNA-30	Decrease in the expression of <i>KCNJ3</i>	Increased miRNA-30 levels are associated with AF	[50]
	miRNA-206	Decrease in the expression of <i>SOD1</i> and <i>GCHI</i>	Increased miRNA-206 levels are associated with AF	[51, 52]
Impaired regulation of calcium levels	miRNA-106	Decrease in the expression of <i>RyR2</i>	Decreased miRNA-106 levels are associated with AF	[55]
	miRNA-208	Decrease in the expression of <i>SERCA2a</i> , <i>CACNA1C</i> , <i>CACNB2</i>	Increased miRNA-208 levels are associated with AF	[56]

MiRNAs AS BIOMARKERS OF AF

The expression of miRNA in tissues and blood can be used as a biomarker in various diseases. According to the National Institutes of Health (NIH) and the World Health Organization (WHO), a biomarker is a biological molecule or its products that can be measured in blood, tissue, or other body fluids as an indicator of normal and abnormal body functioning or that can predict morbidity. Currently, there are no suitable biomarkers for primary diagnosis of AF. The use of natriuretic peptides and cardiac troponins, which are significant in a number of cardiovascular diseases (myocardial infarction, heart failure, etc.), is ineffective in the diagnosis and prognosis of AF [6]. At the same time, high stability, sensitivity, specificity, and prognostic properties of circulating miRNAs make them attractive biomarkers for early diagnosis of numerous diseases, including AF [57].

MiRNAs are very stable under extreme conditions, such as changes in acidity (pH) and high or low temperatures, and can withstand multiple freeze-thaw cycles. MiRNAs can be easily detected with high specificity and sensitivity in serum and plasma using a polymerase chain reaction [58]. Since miRNAs are bound to high-density lipoproteins or incorporated into microvesicles, exosomes, and apoptotic bodies, they are resistant to RNase activity. However, exogenous miRNAs are rapidly degraded by RNase in the plasma. The stability of miRNAs and the possibility of their detection in many biological fluids make them promising biomarkers for many CVDs, including AF [59, 60].

Numerous studies have shown that certain types of miRNAs responsible for gene regulation and heart remodeling, in particular miRNA-1 [24–28], miRNA-328 [29–31], miRNA-499 [32–34], miRNA-21 [37–40], miRNA-150 [45, 46], and a number of others (Table), may become a new class of diagnostic and prognostic biomarkers for AF.

CONCLUSION

By influencing the electrical and structural remodeling of the atria, remodeling of the autonomic nervous system of the heart and impaired homeostasis of intracellular calcium, miRNAs create the prerequisites for the emergence of atrial fibrillation (AF). Stability of miRNAs and a possibility to study them in various biological fluids and tissues, including blood, make them promising diagnostic biomarkers for AF. Further studies are required to assess the role of miRNAs as new biomarkers of AF development.

REFERENCES

1. Lee S.H., Park S.J., Byeon K., On Y.K., Kim J.S., Shin D.G., Cho J.G., Kim Y.N., Kim Y.H., KORAF Investigators. Risk factors between patients with lone and non-lone atrial fibrillation. *J. Korean Med Sci.* 2013; 28 (8): 1174–1180. DOI: 10.3346/jkms.2013.28.8.1174.
2. Pellman J., Sheikh F. Atrial fibrillation: mechanisms, therapeutics, and future directions. *Compr. Physiol.* 2015; 5 (2): 649–665. DOI: 10.1002/cphy.c140047.
3. Ko D., Rahman F., Martins M.A., Hylek E.M., Ellinor P.T., Schnabel R.B., Benjamin E.J., Christophersen I.E. Atrial fibrillation in women: treatment. *Nat. Rev. Cardiol.* 2017; 14 (2): 113–124. DOI: 10.1038/nrcardio.2016.171.
4. Turagam M.K., Mirza M., Werner P.H., Sra J., Kress D.C., Tajik A.J., Jahangir A. Circulating Biomarkers Predictive of Post-operative Atrial Fibrillation. *Cardiol Rev.* 2016; 24 (2): 76–87. DOI: 10.1097/CRD.0000000000000059.
5. Chaubin A.M., Karslyan L.S., Grigoryeva E.V., Nurbaltayeva D.A., Duplyakov D.V. Clinical and diagnostic value of cardiac markers in human biological fluids. *Cardiology.* 2019; 59 (11): 66–75 (in Russ.). DOI: 10.18087/cardio.2019.11.n414.
6. Chaubin A.M., Duplyakov D.V. Increased cardiac troponins, not associated with acute coronary syndrome. Part 1. *Cardiology: News, Opinions, Training.* 2019; 7 (2): 13–23 (in Russ.). DOI: 10.24411/2309-1908-2019-12002.
7. Chaubin A.M., Duplyakov D.V. Increased cardiac troponins, not associated with acute coronary syndrome. Part 2. *Cardiology: News, Opinions, Training.* 2019; 7 (2): 24–35 (in Russ.). DOI: 10.24411/2309-1908-2019-12003.
8. Gonzalez-Del-Hoyo M., Cediell G., Carrasquer A., Bonet G., Vasquez-Nunez K., Boque C., Ali S., Bardaji A. Prognostic implications of troponin I elevation in emergency department patients with tachyarrhythmia. *Clin. Cardiol.* 2019; 42 (5): 546–552. DOI: 10.1002/clc.23175.
9. Zellweger M.J., Schaer B.A., Cron T.A., Pfisterer M.E., Oswald S. Elevated troponin levels in the absence of coronary artery disease after supraventricular tachycardia. *Swiss Med. Wkly.* 2003; 133 (31–32): 439–441.
10. Fox C.S., Parise H., D'Agostino R.B. Sr., Lloyd-Jones D.M., Vasan R.S., Wang T.J., Levy D., Wolf P.A., Benjamin E.J. Parental atrial fibrillation as a risk factor for atrial fibrillation in offspring. *JAMA.* 2004; 291 (23): 2851–2855. DOI: 10.1001/jama.291.23.2851.
11. Low S.K., Takahashi A., Ebana Y., Ozaki K., Christophersen I.E., Ellinor P.T., AFGEn Consortium, Ogishima S., Yamamoto M., Satoh M., Sasaki M., Yamaji T., Iwasaki M., Tsugane S., Tanaka K., Naito M., Wakai K., Tanaka H., Furukawa T., Kubo M., Ito K., Kamatani Y., Tanaka T. Identification of six new genetic loci associated with atrial fibrillation in the Japanese population. *Nat. Genet.* 2017; 49 (6): 953–958. DOI: 10.1038/ng.3842.
12. Lee R.C., Feinbaum R.L., Ambros V. The *C. elegans* heterochronic gene *lin-4* encodes small RNAs with antisense complementarity to *lin-14*. *Cell.* 1993; 75 (5): 843–854. DOI: 10.1016/0092-8674(93)90529-y.
13. Kozomara A., Griffiths-Jones S. miRBase: annotating high confidence microRNAs using deep sequencing data. *Nucleic Acids Res.* 2014; 42: 68–73. DOI: 10.1093/nar/gkt1181.

14. MacFarlane L.A., Murphy P.R. MicroRNA: biogenesis, function and role in cancer. *Curr Genomics*. 2010; 11 (7): 537–561. DOI: 10.2174/138920210793175895.
15. Ardekani A.M., Naeini M.M. The role of microRNAs in human diseases. *Avicenna J. Med Biotechnol*. 2010; 2(4): 161–179.
16. Ha T.Y. MicroRNAs in Human Diseases: From Cancer to Cardiovascular Disease. *Immune Netw*. 2011; 11 (3): 135–154. DOI: 10.4110/in.2011.11.3.135.
17. Van den Berg N.W.E., Kawasaki M., Berger W.R., Neefs J., Meulendijks E., Tijssen A.J., de Groot J.R. MicroRNAs in Atrial Fibrillation: from Expression Signatures to Functional Implications. *Cardiovasc. Drugs Ther*. 2017; 31 (3): 345–365. DOI: 10.1007/s10557-017-6736-z.
18. Liu Z., Zhou C., Liu Y., Wang S., Ye P., Miao X., Xia J. The expression levels of plasma microRNAs in atrial fibrillation patients. *PLoS One*. 2012; 7 (9): e44906. DOI: 10.1371/journal.pone.0044906.
19. Hodgkinson C.P., Kang M.H., Dal-Pra S., Mirotsoy M., Dzau V.J. MicroRNAs and cardiac regeneration. *Circ. Res*. 2015; 116 (10): 1700–1711. DOI: 10.1161/CIRCRESA-HA.116.304377.
20. Neudecker V., Brodsky K.S., Kreth S., Ginde A.A., Eltzschig H.K. Emerging roles for microRNAs in perioperative medicine. *Anesthesiology*. 2016; 124 (2): 489–506. DOI: 10.1097/ALN.0000000000000969.
21. Chaldoupi S.M., Loh P., Hauer R.N., de Bakker J.M., van Rijen H.V. The role of connexin40 in atrial fibrillation. *Cardiovasc. Res*. 2009; 84 (1): 15–23. DOI: 10.1093/cvr/cvp203.
22. Chen J.F., Mandel E.M., Thomson J.M., Wu Q., Callis T.E., Hammond S.M., Conlon F.L., Wang D.Z. The role of microRNA-1 and microRNA-133 in skeletal muscle proliferation and differentiation. *Nat. Genet*. 2006; 38(2): 8228–8833. DOI: 10.1038/ng1725.
23. Jia X., Zheng S., Xie X., Zhang Y., Wang W., Wang Z., Zhang Y., Wang J., Gao M., Hou Y. MicroRNA-1 accelerates the shortening of atrial effective refractory period by regulating KCNE1 and KCNB2 expression: an atrial tachypacing rabbit model. *PLoS One*. 2013; 8 (12): e85639. DOI: 10.1371/journal.pone.0085639.
24. Tsoporis J.N., Fazio A., Rizos I.K., Izhar S., Proteau G., Salpeas V., Rigopoulos A., Sakadakis E., Toumpoulis I.K., Parker T.G. Increased right atrial appendage apoptosis is associated with differential regulation of candidate MicroRNAs 1 and 133A in patients who developed atrial fibrillation after cardiac surgery. *J. Mol. Cell Cardiol*. 2018; 121: 25–32. DOI: 10.1016/j.yjmcc.2018.06.005.
25. Li Y.D., Hong Y.F., Yusufuaji Y., Tang B.P., Zhou X.H., Xu G.J., Li J.X., Sun L., Zhang J.H., Xin Q., Xiong J., Ji Y.T., Zhang Y. Altered expression of hyperpolarization-activated cyclic nucleotide-gated channels and microRNA-1 and -133 in patients with age-associated atrial fibrillation. *Mol. Med. Rep*. 2015; 12 (3): 3243–3248. DOI: 10.3892/mmr.2015.3831.
26. Girmatsion Z., Biliczki P., Bonauer A., Wimmer-Greinecker G., Scherer M., Moritz A., Bukowska A., Goette A., Nattel S., Hohnloser S.H., Ehrlich J.R. Changes in microRNA-1 expression and IK1 up-regulation in human atrial fibrillation. *Heart Rhythm*. 2009; 6 (12): 1802–1809. DOI: 10.1016/j.hrthm.2009.08.035.
27. Lu Y., Hou S., Huang D., Luo X., Zhang J., Chen J., Xu W. Expression profile analysis of circulating microRNAs and their effects on ion channels in Chinese atrial fibrillation patients. *Int. J. Clin. Exp. Med*. 2015; 8 (1): 845–853.
28. Santulli G., Iaccarino G., De Luca N., Trimarco B., Condorelli G. Atrial fibrillation and microRNAs. *Front. Physiol*. 2014; 5: 15. DOI: 10.3389/fphys.2014.00015.
29. Lu Y., Zhang Y., Wang N., Pan Z., Gao X., Zhang F., Zhang Y., Shan H., Luo X., Bai Y., Sun L., Song W., Xu C., Wang Z., Yang B. MicroRNA-328 contributes to adverse electrical remodeling in atrial fibrillation. *Circulation*. 2010; 122 (23): 2378–2387. DOI: 10.1161/CIRCULATIONA-HA.110.958967.
30. Kim G.H. MicroRNA regulation of cardiac conduction and arrhythmias. *Transl. Res*. 2013; 161 (5): 381–392. DOI: 10.1016/j.trsl.2012.12.004.
31. Soeki T., Matsuura T., Bando S., Tobiume T., Uematsu E., Ise T., Kusunose K., Yamaguchi K., Yagi S., Fukuda D., Yamada H., Wakatsuki T., Shimabukuro M., Sata M. Relationship between local production of microRNA-328 and atrial substrate remodeling in atrial fibrillation. *J. Cardiol*. 2016; 68 (6): 472–477. DOI: 10.1016/j.jjcc.2015.12.007.
32. Ling T.Y., Wang X.L., Chai Q., Lau T.W., Koestler C.M., Park S.J., Daly R.C., Greason K.L., Jen J., Wu L.Q., Shen W.F., Shen W.K., Cha Y.M., Lee H.C. Regulation of the SK3 channel by microRNA-499—potential role in atrial fibrillation. *Heart Rhythm*. 2013; 10 (7): 1001–1009. DOI: 10.1016/j.hrthm.2013.03.005.
33. Ling T.Y., Wang X.L., Chai Q., Lu T., Stulak J.M., Joyce L.D., Daly R.C., Greason K.L., Wu L.Q., Shen W.K., Cha Y.M., Lee H.C. Regulation of cardiac CACNB2 by microRNA-499: Potential role in atrial fibrillation. *BBA Clin*. 2017; 7: 78–84. DOI: 10.1016/j.bbacli.2017.02.002.
34. Da Silva A.M.G., de Araujo J.N.G., de Oliveira K.M., Novaes A.E.M., Lopes M.B., de Sousa J.C.V., Filho A.A.A., Luchessi A.D., de Rezende A.A., Hirata M.H., Silbiger V.N. Circulating miRNAs in acute new-onset atrial fibrillation and their target mRNA network. *J. Cardiovasc. Electrophysiol*. 2018; 29 (8): 1159–1166. DOI: 10.1111/jce.13612.
35. Xu J., Cui G., Esmailian F., Plunkett M., Marelli D., Ardehali A., Odum J., Laks H., Sen L. Atrial extracellular matrix remodeling and the maintenance of atrial fibrillation. *Circulation*. 2004; 109 (3): 363–368. DOI: 10.1161/01.CIR.0000109495.02213.52.
36. Liu H., Chen G.X., Liang M.Y., Qin H., Rong J., Yao J.P., Wu Z.K. Atrial fibrillation alters the microRNA expression profiles of the left atria of patients with mitral stenosis. *BMC Cardiovasc. Disord*. 2014; 14: 10. DOI: 10.1186/1471-2261-14-10.
37. Barana A., Matamoros M., Dolz-Gaiton P., Pérez-Hernández M., Amoros I., Nunez M., Sacristán S., Pedraz Á., Pinto Á., Fernández-Avilés F., Tamargo J., Delpón E., Caballero R. Chronic atrial fibrillation increases microRNA-21 in human atrial myocytes decreasing L-type calcium current. *Circ. Arrhythm. Electrophysiol*. 2014; 7 (5): 861–868. DOI: 10.1161/CIRCEP.114.001709.
38. Adam O., Lohfelme B., Thum T., Gupta S.K., Puhl S.L., Schaffers H.J., Böhm M., Laufs U. Role of miR-21 in the pathogen-

- esis of atrial fibrosis. *Basic. Res. Cardiol.* 2012; 107 (5): 278. DOI: 10.1007/s00395-012-0278-0.
39. Thum T., Gross C., Fiedler J., Fischer T., Kissler S., Bussen M., Galuppo P., Just S., Rottbauer W., Frantz S., Castoldi M., Soutschek J., Koteliensky V., Rosenwald A., Basson M.A., Licht J.D., Pena J.T., Rouhanifard S.H., Muckenthaler M.U., Tuschl T., Martin G.R., Bauersachs J., Engelhardt S. MicroRNA-21 contributes to myocardial disease by stimulating MAP kinase signalling in fibroblasts. *Nature.* 2008; 456 (7224): 980–984. DOI: 10.1038/nature07511
 40. Zhang K., Zhao L., Ma Z., Wang W., Li X., Zhang Y., Yuan M., Liang X., Li G. Doxycycline attenuates atrial remodeling by interfering with microRNA-21 and downstream phosphatase and tensin homolog (PTEN)/phosphoinositide 3-kinase (PI3K) signaling pathway. *Med. Sci. Monit.* 2018; 24: 5580–5587. DOI: 10.12659/MSM.909800.
 41. Kriegel A.J., Liu Y., Fang Y., Ding X., Liang M. The miR-29 family: genomics, cell biology, and relevance to renal and cardiovascular injury. *Physiol. Genomics.* 2012; 44 (4): 237–244. DOI: 10.1152/physiolgenomics.00141.2011.
 42. Dawson K., Wakili R., Ordog B., Clauss S., Chen Y., Iwasaki Y., Voigt N., Qi X.Y., Sinner M.F., Dobrev D., Kääh S., Nattel S. MicroRNA29: a mechanistic contributor and potential biomarker in atrial fibrillation. *Circulation.* 2013; 127 (14): 1466–1475. DOI: 10.1161/CIRCULATIONA-HA.112.001207.
 43. Ishizaki T., Tamiya T., Taniguchi K., Morita R., Kato R., Okamoto F., Saeiki K., Nomura M., Nojima Y., Yoshimura A. miR126 positively regulates mast cell proliferation and cytokine production through suppressing Spred1. *Genes. Cells.* 2011; 16 (7): 803–814. DOI: 10.1111/j.1365-2443.2011.01529.x.
 44. Wei X.J., Han M., Yang F.Y., Wei G.C., Liang Z.G., Yao H., Ji C.W., Xie R.S., Gong C.L., Tian Y. Biological significance of miR-126 expression in atrial fibrillation and heart failure. *Braz. J. Med. Biol. Res.* 2015; 48 (11): 983–989. DOI: 10.1590/1414-431X20154590.
 45. Goren Y., Meiri E., Hogan C., Mitchell H., Lebanony D., Salman N., Schliamser J.E., Amir O. Relation of reduced expression of MiR-150 in platelets to atrial fibrillation in patients with chronic systolic heart failure. *Am. J. Cardiol.* 2014; 113 (6): 976–981. DOI: 10.1016/j.amjcard.2013.11.060.
 46. McManus D.D., Tanriverdi K., Lin H., Esa N., Kinno M., Mandapati D., Tam S., Okike O.N., Ellinor P.T., Keaney J.F. Jr., Donahue J.K., Benjamin E.J., Freedman J.E. Plasma microRNAs are associated with atrial fibrillation and change after catheter ablation (the miRhythm study). *Heart Rhythm.* 2015; 12 (1): 3–10. DOI: 10.1016/j.hrthm.2014.09.050.
 47. Harling L., Lambert J., Ashrafian H., Darzi A., Gooderham N.J., Athanasiou T. Elevated serum microRNA 483-5p levels may predict patients at risk of post-operative atrial fibrillation. *Eur. J. Cardiothorac. Surg.* 2017; 51 (1): 73–78. DOI: 10.1093/ejcts/ezw245.
 48. Rao M., Hu J., Zhang Y., Gao F., Zhang F., Yang Z., Zhang X., Hou Y. Time-dependent cervical vagus nerve stimulation and frequency-dependent right atrial pacing mediates induction of atrial fibrillation. *Anatol. J. Cardiol.* 2018; 20 (4): 206–212. DOI: 10.14744/AnatolJCardiol.2018.73558.
 49. Shen M.J., Choi E.K., Tan A.Y., Lin S.F., Fishbein M.C., Chen L.S., Chen P.S. Neural mechanisms of atrial arrhythmias. *Nat. Rev. Cardiol.* 2011; 9 (1): 30–39. DOI: 10.1038/nrcardio.2011.139.
 50. Morishima M., Iwata E., Nakada C., Tsukamoto Y., Takanari H., Miyamoto S., Moriyama M., Ono K. Atrial fibrillation-mediated upregulation of miR-30d regulates myocardial electrical remodeling of the g-protein-gated K(+) channel, IK.ACh. *Circ. J.* 2016; 80 (6): 1346–1355. DOI: 10.1253/circj.CJ-15-1276.
 51. Zhang Y., Zheng S., Geng Y., Xue J., Wang Z., Xie X., Wang J., Zhang S., Hou Y. MicroRNA profiling of atrial fibrillation in canines: miR-206 modulates intrinsic cardiac autonomic nerve remodeling by regulating SOD1. *PLoS One.* 2015; 10 (3): e0122674. DOI: 10.1371/journal.pone.0122674.
 52. Wei J., Zhang Y., Li Z., Wang X., Chen L., Du J., Liu J., Liu J., Hou Y. GCH1 attenuates cardiac autonomic nervous remodeling in canines with atrial-tachypacing via tetrahydrobiopterin pathway regulated by microRNA-206. *Pacing Clin. Electrophysiol.* 2018; 41 (5): 459–471. DOI: 10.1111/pace.13289.
 53. Harada M., Luo X., Murohara T., Yang B., Dobrev D., Nattel S. MicroRNA regulation and cardiac calcium signaling: role in cardiac disease and therapeutic potential. *Circ. Res.* 2014; 114 (4): 689–705. DOI: 10.1161/CIRCRESA-HA.114.301798.
 54. Denham N.C., Pearman C.M., Caldwell J.L., Madders G.W.P., Eisner D.A., Trafford A.W., Dibb K.M. Calcium in the pathophysiology of atrial fibrillation and heart failure. *Front. Physiol.* 2018; 9: 1380. DOI: 10.3389/fphys.2018.01380.
 55. Chiang D.Y., Kongchan N., Beavers D.L., Alsina K.M., Voigt N., Neilson J.R., Jakob H., Martin J.F., Dobrev D., Wehrens X.H., Li N. Loss of microRNA-106b-25 cluster promotes atrial fibrillation by enhancing ryanodine receptor type-2 expression and calcium release. *Circ. Arrhythm. Electrophysiol.* 2014; 7(6): 1214–1222. DOI: 10.1161/CIRCEP.114.001973.
 56. Canon S., Caballero R., Herranz-Martínez A., Perez-Hernández M., Lopez B., Atienza F., Jalife J., Hove-Madsen L., Delpón E., Bernad A. miR-208b upregulation interferes with calcium handling in HL-1 atrial myocytes: Implications in human chronic atrial fibrillation. *J. Mol. Cell Cardiol.* 2016; 99: 162–173. DOI: 10.1016/j.yjmcc.2016.08.012.
 57. D'Alessandra Y., Devanna P., Limana F., Straino S., Di Carlo A., Brambilla P.G., Rubino M., Carena M.C., Spazzafumo L., De Simone M., Micheli B., Biglioli P., Achilli F., Martelli F., Maggiolini S., Marenzi G., Pompilio G., Capogrossi M.C. Circulating microRNAs are new and sensitive biomarkers of myocardial infarction. *Eur. Heart J.* 2010; 31 (22): 2765–2773. DOI: 10.1093/eurheartj/ehq167.
 58. Widera C., Gupta S.K., Lorenzen J.M., Bang C., Bauersachs J., Bethmann K., Kempf T., Wollert K.C., Thum T. Diagnostic and prognostic impact of six circulating microRNAs in acute coronary syndrome. *J. Mol. Cell Cardiol.* 2011; 51 (5): 872–875. DOI: 10.1016/j.yjmcc.2011.07.011.
 59. Zampetaki A., Willeit P., Drozdov I., Kiechl S., Mayr M. Profiling of circulating microRNAs: from single biomarkers to re-wired networks. *Cardiovasc. Res.* 2012; 93 (4): 555–562. DOI: 10.1093/cvr/cvr266.

60. Natsume Y., Oaku K., Takahashi K., Nakamura W., Oono A., Hamada S., Yamazoe M., Ihara K., Sasaki T., Goya M., Hirao K., Furukawa T., Sasano T. Combined analysis of hu-

man and experimental murine samples identified novel circulating microRNAs as biomarkers for atrial fibrillation. *Circ. J.* 2018; 82 (4): 965–973. DOI: 10.1253/circj.CJ-17-1194.

Authors information

Chaulin Aleksey M., Post-Graduate Student, Assistant, Department of Histology and Embryology, Samara State Medical University, Samara, Russian Federation. ORCID 0000-0002-2712-0227.

Duplyakov Dmitry V., Dr. Sci. (Med.), Professor, Department of Cardiology and Cardiovascular Surgery, Samara State Medical University, Samara, Russian Federation. ORCID 0000-0002-6453-2976.

(✉) **Chaulin Aleksey M.**, e-mail: alekseymichailovich22976@gmail.com

Received 26.05.2020

Accepted 29.09.2020

УДК 616.12-008.313.3-073.97-71-08
<https://doi.org/10.20538/1682-0363-2021-3-213-218>

The first experience of using non-invasive real-time mapping in an electrophysiology laboratory for treatment of ventricular arrhythmia

Artyukhina E.A., Dedukh E.V., Yashkov M.V., Revishvili A.Sh.

*A.V. Vishnevsky National Medical Research Center of Surgery
27, Bolshaya Serpukhovskaya Str., Moscow, 117997, Russian Federation*

ABSTRACT

A clinical case of treatment for ventricular arrhythmia from the right ventricular outflow tract using non-invasive real-time electrophysiological mapping and the “Astrocard” navigation system (“Meditek”, Russia) is presented. This clinical case demonstrates the accuracy of non-invasive real-time mapping for the diagnosis and treatment of ventricular arrhythmias.

Key words: non-invasive multichannel cardiac mapping, electrophysiological mapping, ventricular arrhythmias, radiofrequency ablation.

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

Source of financing. The study was carried out within the Russian Science Foundation grant (Project No. 19-15-00406).

For citation: Artyukhina E.A., Dedukh E.V., Yashkov M.V., Revishvili A.Sh. The first experience of using non-invasive real-time mapping in an electrophysiology laboratory for treatment of ventricular arrhythmia. *Bulletin of Siberian Medicine*. 2021; 20 (3): 213–218. <https://doi.org/10.20538/1682-0363-2021-3-213-218>.

Первый опыт использования неинвазивного картирования в режиме реального времени в условиях электрофизиологической лаборатории для лечения желудочковой аритмии

Артюхина Е.А., Дедух Е.В., Яшков М.В., Ревিশвили А.Ш.

*Национальный медицинский исследовательский центр (НМИЦ) хирургии им. А.В. Вишневского
Россия, 117997, г. Москва, Большая Серпуховская ул., 27*

РЕЗЮМЕ

Представлен клинический случай лечения желудочковой аритмии из выводного отдела правого желудочка с использованием неинвазивного электрофизиологического картирования в режиме реального времени и отечественной навигационной системы «Астрокард» (АО «Медитек», Россия). Данное клиническое наблюдение демонстрирует точность неинвазивного картирования в режиме реального времени для диагностики и лечения желудочковых нарушений ритма сердца.

✉ Dedukh Elizaveta V., e-mail: dedukh.elizaveta@mail.ru

Ключевые слова: неинвазивное многоканальное картирование сердца, электрофизиологическое картирование, желудочковые аритмии, радиочастотная абляция.

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

Источник финансирования. Работа выполнена в рамках гранта Российского научного фонда (проект № 19-15-00406).

Для цитирования: Артюхина Е.А., Дедух Е.В., Яшков М.В., Ревিশвили А.Ш. Первый опыт использования неинвазивного картирования в режиме реального времени в условиях электрофизиологической лаборатории для лечения желудочковой аритмии. *Бюллетень сибирской медицины*. 2021; 20 (3): 213–218. <https://doi.org/10.20538/1682-0363-2021-3-213-218>.

INTRODUCTION

Radiofrequency catheter ablation is the main method of interventional treatment for premature ventricular contractions (PVCs). It improves the patient's quality of life and prevents the development of cardiomyopathies and heart failure [1]. High-density mapping and ablation catheters with contact force monitoring help operators in mapping and eliminating PVCs, but the effectiveness and safety of such procedures still depend on the localization of the arrhythmia focus [2]. Also, the mapping process complicates the occurrence of rapid, unstable, and polymorphic ventricular tachycardia (VT) and sometimes makes it impossible.

Diagnostic algorithms using electrocardiography (ECG) can identify the area of origin of ventricular arrhythmias, but their spatial localization is not accurate enough due to various factors (the patient's obesity, peculiarities of the heart location in the chest, and abnormalities in the placement of ECG leads) [3].

A more accurate method for diagnosing PVCs is non-invasive mapping. Although the effectiveness of non-invasive mapping in the topical diagnosis of PVCs localized in the right ventricular outflow tract (RVOT) is known and previously reported [4, 5], the technique described in this clinical case was used to diagnose ventricular arrhythmias for the first time. This technique makes it possible to carry out real-time mapping of the right and left ventricles (intraoperatively) and identify the PVC localization by its absolute location.

The aim of this clinical case was to test the method by comparing the localization of PVC on the map obtained via non-invasive real-time mapping and the localization of the focus on the 3D navigation mapping system during catheter ablation.

CLINICAL CASE

Patient A., 64 years old, complains of irregular heartbeat and fatigue.

Medical history. From a young age, irregular heartbeat has been noted. During the routine medical examination, PVCs were detected on the ECG. In recent months, attacks have become more frequent, and increased fatigue has appeared.

Preoperative examination. ECG – sinus rhythm, frequent PVCs with morphology of the left bundle branch block (Fig.1).

24-hour Holter monitoring. Basic rhythm – sinus rhythm, the average frequency is 81 bpm. Single PVCs – 28,937; bigeminy – 58; paired PVCs – 42; unstable ventricular tachycardia (VT) – 9. The longest VT consisted of 26 complexes.

Echocardiography. Right ventricle – inflow tract: 28 mm. The Simpson's ejection fraction (EF) – 47%. The thickness of the left ventricular myocardium was normal. There were no local contractility disorders. Moderate mitral and slight tricuspid regurgitation. Tachyarrhythmia (extrasystole). There were no blood discharges. During the study, an attack of tachycardia with a heart rate of 127–133 beats per minute occurred, as a result of which the Simpson's EF decreased to 38%, and the area of mitral regurgitation increased.

Coronary angiography. Balanced type of coronary blood supply. No hemodynamically significant stenoses of the coronary arteries were revealed.

Non-invasive multichannel mapping. Registration of surface electrograms from 224 surface electrodes from the patient's torso, followed by multispiral computed tomography (MSCT) in the ECG synchronization mode with contrasting of the ventricles. Further, in the electrophysiological laboratory, the patient was diagnosed with unstable ventricular tachycardia; the data were registered in the AMICARD 01K complex. Data processing and analysis took place intraoperatively (Fig. 2).

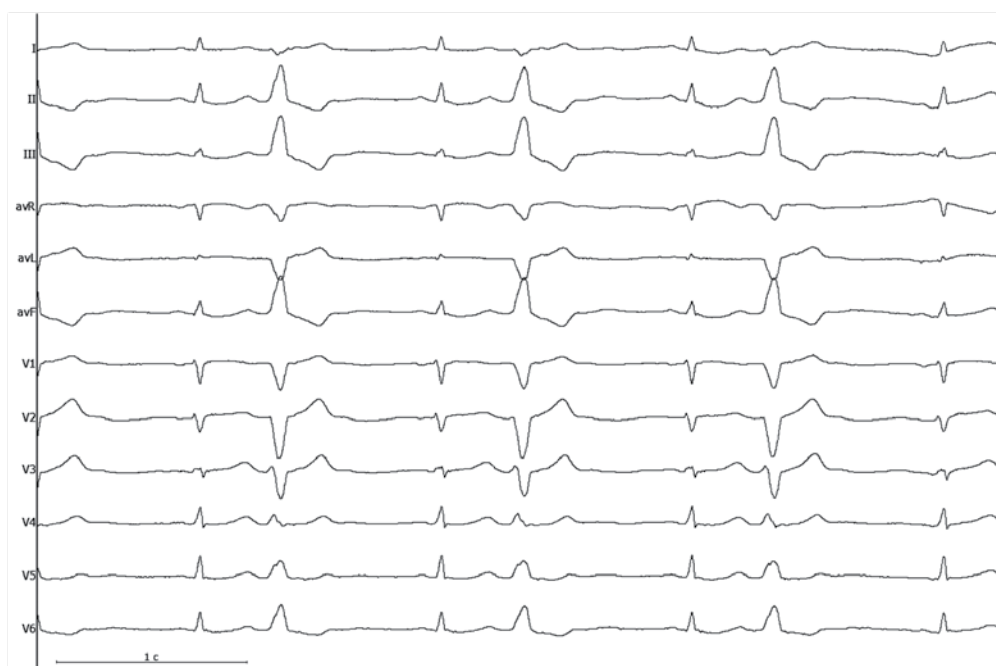


Fig. 1. 12-channel ECG, sinus rhythm with ventricular bigeminy



Fig. 2. The process of PVC mapping and treatment in the electrophysiological laboratory: *a* – fluoroscopic control and data from the “Astrocard” navigation system (“Meditek”, Russia), *b* – the AMICARD 01K hardware and software complex (“Amikard”, Russia)

When analyzing the obtained 3D model of the heart ventricles, the area of early activation was detected on the endocardial surface in RVOT. The result of non-invasive real-time mapping was an isochronous map of

the heart ventricles (Fig. 3), which was imported into the navigation mapping system. This made it possible to more accurately compare the localization of the early activation site during ventricular tachycardia.



Fig.3. Results of real-time multichannel mapping performed intraoperatively in the electrophysiological laboratory: a map of the early activation site (red) in RVOT

INTRAOPERATIVE MAPPING AND RADIOFREQUENCY ABLATION

In the electrophysiological laboratory, the right femoral and left subclavian veins were punctured, through which the electrode was positioned into the coronary sinus. Frequent PVCs and episodes of unstable VT with the spread of electrical activity from RVOT were recorded.

The anatomical reconstruction of the right ventricle was performed using the domestic “Astrocard” navigation system, where the data of non-invasive mapping were imported. A right ventricle activation map was constructed.

Endocardial mapping data were constantly compared with non-invasive mapping data by visualizing the right ventricle in appropriate projections (Fig. 4).

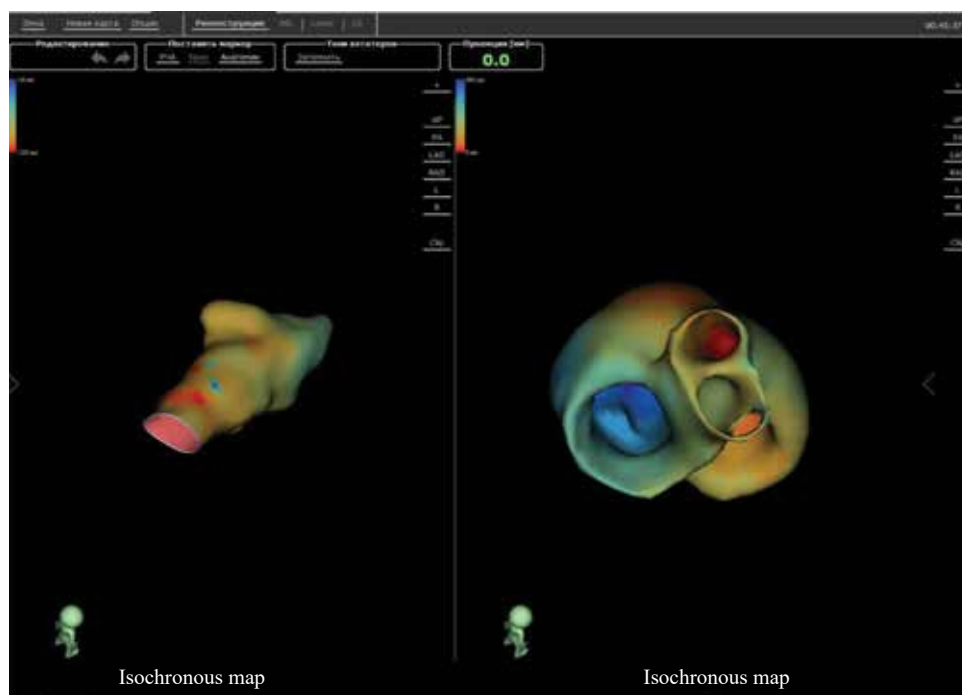


Fig. 4. Endocardial mapping of the right ventricle in the electrophysiological laboratory: the result of non-invasive mapping was imported into the navigation system (presented on the right side of the screen)

When performing imaging in the propagation of excitation mode, the early activation zone was in the RVOT area, which completely coincided with the results of non-invasive mapping. In this area, radiofrequency exposure was performed. After the exposure, complete elimination of ventricular activity was achieved.

The total duration of the exposure was 7 minutes. The parameters of radiofrequency exposure using an irrigated ablation electrode were 35 W. The absence of PVC after the exposure was registered within 20 minutes. Arrhythmias were not induced by stimulation methods. The follow-up period was 3 months, arrhythmias were not recorded.

Despite the fact that this clinical case describes the typical and most common localization of PVCs, the technique used in this clinical case was used for the first time. It will be able to demonstrate the possibilities of real-time mapping for the diagnosis of ventricular arrhythmias, which will be an extremely important step in mapping rapid and polymorphic VT to study the mechanism of their occurrence and successful interventional treatment.

DISCUSSION

The two most common systems for non-invasive cardiac mapping are CardioInsight Technologies Inc., Cleveland, Ohio [6, 7] and EP Solutions SA, Yverdon-les-Bains, Switzerland [8–10].

In a study by S. Jamil-Copley et al., 24 patients with PVCs localized in the right ventricular outflow tract (18 patients) and the left ventricular outflow tract (6 patients) were examined [6]. All patients included in the study underwent non-invasive mapping at the pre-operative stage. The accuracy of determining the PVCs localization according to the non-invasive mapping data in comparison with the invasive mapping data was 96%.

E. Wissner et al. showed in their study that in 18 (86%) of 21 patients with PVC / VT, the correct ventricular segment was diagnosed as a result of non-invasive mapping by single extrasystoles [11].

In fact, the non-invasive mapping technique has the potential to accurately diagnose the epicardial or endocardial origin and the chamber of origin of PVCs. This clinical case compared the PVC localization obtained following the analysis of the ECG and the data of invasive endocardial mapping, as well as the data of non-invasive real-time mapping. In previous studies, epicardial breakouts obtained using both methods resulted in very large mean absolute errors exceeding 70 mm [12].

To date, the electrophysiological nature of polymorphic VT and ventricular fibrillation (VF) is still

not fully understood. This is prevented by the patient's hemodynamic instability during the development of these arrhythmias, which requires immediate cardioversion. Currently, the main method for studying the mechanisms of arrhythmias is endocardial electroanatomical mapping of the heart. Arrhythmia mapping in order to study its mechanism is possible only with a large number of points that record the local activity of the heart during arrhythmia. This is possible only with periodic and stable arrhythmias, in which stable hemodynamics is maintained.

VF and polymorphic VT cause dramatic depression of central hemodynamics. They can spontaneously stop or transform into other, more dangerous arrhythmias. This makes it impossible to perform endocardial electroanatomical mapping. Therefore, an extremely important task for studying the mechanisms and interventional treatment of life-threatening ventricular arrhythmias is the development of a method that allows for VT mapping with minimal recording of myocardial electrical activity during arrhythmias.

The non-invasive real-time mapping technique described in this case will overcome many of these limitations.

This technique can provide important information about the basic mechanism of arrhythmia using a single ectopic beat, which will be recorded in the electrophysiological laboratory, where, based on these data, it is possible to perform interventional treatment of ventricular arrhythmia.

CONCLUSION

The key findings from this study:

1) The new method of non-invasive real-time mapping worked successfully and identified the PVC localization in RVOT.

2) High accuracy of non-invasive real-time mapping was demonstrated in comparison with invasive mapping. This made it possible to effectively eliminate PVCs by catheter ablation.

3) From a theoretical and mathematical points of view, this technology has the potential to determine the transmural distribution of sites of origin (epicardial or endocardial origin).

Although this study provides only preliminary results, it will help to map life-threatening, rapid and polymorphic VTs that impair hemodynamic stability.

Non-invasive real-time mapping further enhances the benefits of the non-invasive mapping system and expands clinical applicability of the method in treatment of life-threatening ventricular arrhythmias.

REFERENCES

1. Zhong L., Lee Y.H., Huang X.M., Asirvatham S.J., Shen W.K., Friedman P.A., Hodge D.O., Slusser J.P., Song Z.Y., Packer D.L., Cha Y.M. Relative efficacy of catheter ablation vs antiarrhythmic drugs in treating premature ventricular contractions: a single-center retrospective study. *Heart Rhythm*. 2014; 11 (2):187–193. DOI: 10.1016/j.hrthm.2013.10.033.
2. Komatsu Y., Nogami A., Shinoda Y., Masuda K., Machino T., Kuroki K., Yamasaki H., Sekiguchi Y., Aonuma K. Idiopathic ventricular arrhythmias originating from the vicinity of the communicating vein of cardiac venous systems at the left ventricular summit. *Circ. Arrhythm. Electrophysiol.* 2018; 11 (1): e005386. DOI: 10.1161/CIRCEP.117.005386.
3. Anter E., Frankel D.S., Marchlinski F.E., Dixit S. Effect of electrocardiographic lead placement on localization of outflow tract tachycardias. *Heart Rhythm*. 2012; 9 (5): 697–703. DOI: 10.1016/j.hrthm.2011.12.007.
4. Inaba T., Nakazawa Y., Yoshida K., Kato Y., Hattori A., Kimura T., Hoshi T., Ishizu T., Seo Y., Sato A., Sekiguchi Y., Nogami A., Watanabe S., Horigome H., Kawakami Y., Aonuma K. Routine clinical heart examinations using SQUID magnetocardiography at university of tsukuba hospital. *Supercond. Sci. Technol.* 2017; 30 (11). DOI: 10.1088/1361-6668/aa8c26.
5. Ito Y., Shiga K., Yoshida K., Ogata K., Kandori A., Inaba T., Nakazawa Y., Sekiguchi Y., Tada H., Sekihara K., Aonuma K. Development of a magnetocardiography-based algorithm for discrimination between ventricular arrhythmias originating from the right ventricular outflow tract and those originating from the aortic sinus cusp: a pilot study. *Heart Rhythm*. 2014; 11 (9): 1605–1612. DOI: 10.1016/j.hrthm.2014.05.032.
6. Jamil-Copley S., Bokan R., Kojodjojo P., Qureshi N., Ko-Wing M., Hayat S., Kyriacou A., Sandler B., Sohaib A., Wright I., Davies D.W., Whinnett Z., Peters S.N., Kanagaratnam P., Lim P.B. Noninvasive electrocardiographic mapping to guide ablation of outflow tract ventricular arrhythmias. *Heart Rhythm*. 2014; 11 (4): 587–594. DOI: 10.1016/j.hrthm.2014.01.013.
7. Cakulev I., Sahadevan J., Arruda M., Goldstein R.N., Hong M., Intini A., Mackall J.A., Stambler B.S., Ramanathan C., Jia P., Strom M., Waldo A.L. Confirmation of novel noninvasive high-density electrocardiographic mapping with electrophysiology study: implications for therapy. *Circ. Arrhythm. Electrophysiol.* 2013; 6 (1): 68–75. DOI: 10.1161/CIRCEP.112.975813.
8. Revishvili A.S., Wissner E., Lebedev D.S., Lemes C., Deiss S., Metzner A., Kalinin V.V., Sopov O.V., Labartkava E.Z., Kalinin A.V., Chmelevsky M., Zubarev S.V., Chaykovskaya M.K., Tsiklauri M.G., Kuck K.H. Validation of the mapping accuracy of a novel non-invasive epicardial and endocardial electrophysiology system. *Europace*. 2015; 17 (8): 1282–1288. DOI: 10.1093/europace/euu339.
9. Wissner E., Saguner A.M., Metzner A., Chmelevsky M., Tsyganov A., Deiss S., Maurer T., Kuck K.H. Radiofrequency ablation of premature ventricular contractions originating from the aortomitral continuity localized by use of a novel noninvasive epicardial and endocardial electrophysiology system. *Heart Rhythm Case Rep.* 2016; 2 (3): 255–257. DOI: 10.1016/j.hrcr.2016.02.004.
10. Wang Y., Cuculich P.S., Zhang J., Desouza K.A., Vijayakumar R., Chen J., Faddis M.N., Lindsay B.D., Smith T.W., Rudy Y. Noninvasive electroanatomic mapping of human ventricular arrhythmias with electrocardiographic imaging. *Sci. Transl. Med.* 2011; 3 (98): 98ra84. DOI: 10.1126/scitranslmed.3002152.
11. Wissner E., Revishvili A., Metzner A., Tsyganov A., Kalinin V., Lemes C., Saguner A.M., Maurer T., Deiss S., Sopov O., Labartkava E., Chmelevsky M., Kuck K.H. Noninvasive epicardial and endocardial mapping of premature ventricular contractions. *Europace*. 2017; 19 (5): 843–849. DOI: 10.1093/europace/euw103.
12. Duchateau J., Sacher F., Pambrun T., Derval N., Chamorro-Servent J., Denis A., Ploux S., Hocini M., Jaïs P., Bernus O., Haïssaguerre M., Dubois R. Performance and limitations of noninvasive cardiac activation mapping. *Heart Rhythm*. 2019; 16 (3): 435–442. DOI: 10.1016/j.hrthm.2018.10.010.

Authors information

Artyukhina Elena A., Dr. Sci. (Med.), Head of the Department of Electrophysiological X-ray Endovascular Methods for the Diagnosis and Treatment of Arrhythmias, A.V. Vishnevsky National Medical Research Center of Surgery, Moscow, Russian Federation. ORCID 0000-0001-7065-0250.

Dedukh Elizaveta V., Post-Graduate Student, A.V. Vishnevsky National Medical Research Center of Surgery, Moscow, Russian Federation. ORCID 0000-0002-4799-7456.

Yashkov Maxim V., Post-Graduate Student, A.V. Vishnevsky National Medical Research Center of Surgery, Moscow, Russian Federation. ORCID 0000-0003-3997-8252.

Revishvili Amiran Sh., Dr. Sci. (Med.), Professor, Academician of the Russian Academy of Sciences, Director of the A.V. Vishnevsky National Medical Research Center of Surgery, Moscow, Russian Federation. ORCID 0000-0003-1791-9163.

(✉) **Dedukh Elizaveta V.**, e-mail: dedukh.elizaveta@mail.ru

Received 25.11.2020

Accepted 29.09.2020

A clinical case of lung damage in granulomatosis with polyangiitis

**Kraposhina A.Yu.^{1,2}, Demko I.V.^{1,2}, Sobko E.A.^{1,2}, Gordeeva N.V.^{1,2}, Soloveva I.A.^{1,2},
Matveeva I.V.², Kazmerchuk O.V.¹, Katser A.B.¹, Abramov Yu.I.¹, Malchik N.V.¹**

¹ V.F. Voino-Yasenetsky Krasnoyarsk State Medical University
1, Partizana Zheleznyaka Str., Krasnoyarsk, 660022, Russian Federation

² Krasnoyarsk Regional Clinical Hospital
3A, Partizana Zheleznyaka Str., Krasnoyarsk, 660022, Russian Federation

ABSTRACT

The article presents a case of granulomatosis with polyangiitis, which is difficult for clinical diagnosis. Recently, the incidence of systemic vasculitis has increased, and given the similarity of clinical symptoms with other pathologies, a doctor of any specialty should carry out differential diagnosis. Granulomatosis with polyangiitis is characterized mainly by damage to the upper respiratory tract, lungs, and kidneys. The main method for diagnosing granulomatosis with polyangiitis is biopsy with histological examination, which allows to detect inflammation in the affected tissues. A feature of granulomatosis with polyangiitis is detection of antineutrophil cytoplasmic antibodies in the blood serum. However, these antibodies are recorded only in 80% of patients and are not detected in the remission phase, which makes diagnosis much more difficult.

Key words: granulomatosis with polyangiitis, lung damage, system vasculitis.

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

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

For citation: Kraposhina A.Yu., Demko I.V., Sobko E.A., Gordeeva N.V., Soloveva I.A., Matveeva I.V., Kazmerchuk O.V., Katser A.B., Abramov Yu.I., Malchik N.V. A clinical case of lung damage in granulomatosis with polyangiitis. *Bulletin of Siberian Medicine*. 2021; 20 (3): 219–224. <https://doi.org/10.20538/1682-0363-2021-3-219-224>.

Клинический случай: поражение легких при гранулематозе с полиангиитом

**Крапошина А.Ю.^{1,2}, Демко И.В.^{1,2}, Собко Е.А.^{1,2}, Гордеева Н.В.^{1,2}, Соловьева И.А.^{1,2},
Матвеева И.В.², Казмерчук О.В.¹, Кацер А.Б.¹, Абрамов Ю.И.¹, Мальчик Н.В.¹**

¹ Красноярский государственный медицинский университет (КрасГМУ) имени профессора В.Ф. Войно-Ясенецкого
Россия, 660022, г. Красноярск, ул. Партизана Железняка, 1

² Краевая клиническая больница (ККБ)
Россия, 660022, г. Красноярск, ул. Партизана Железняка, 3а

РЕЗЮМЕ

Представлен сложный для клинической диагностики случай заболевания гранулематозом с полиангиитом. В последнее время увеличилась заболеваемость системными васкулитами, а учитывая схожесть

клинических симптомов с другими патологиями, врачу любой специальности следует проводить дифференциальную диагностику. Гранулематоз с полиангиитом характеризуется преимущественно поражением верхних дыхательных путей, легких и почек. Ключевым методом диагностики гранулематоза с полиангиитом является биопсия с гистологическим исследованием, позволяющим выявить воспалительный процесс в пораженных тканях. Особенностью гранулематоза с полиангиитом является регистрация антинейтрофильных цитоплазматических антител в сыворотке крови, однако данные антитела регистрируются лишь у 80% больных и не выявляются в фазе ремиссии, что значительно затрудняет диагностику.

Ключевые слова: гранулематоз с полиангиитом, поражение легких, системные васкулиты.

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

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

Для цитирования: Крапошина А.Ю., Демко И.В., Собко Е.А., Гордеева Н.В., Соловьева И.А., Матвеева И.В., Казмерчук О.В., Кацер А.Б., Абрамов Ю.И., Мальчик Н.В. Клинический случай: поражение легких при гранулематозе с полиангиитом. *Бюллетень сибирской медицины*. 2021; 20 (3): 219–224. <https://doi.org/10.20538/1682-0363-2021-3-219-224>.

INTRODUCTION

In recent years, there has been an increase in the incidence of systemic vasculitis. Lesions are characterized by non-specific symptoms in the onset and similarity of clinical symptoms with other pathologies, which makes differential diagnosis difficult, despite continuously developing diagnostic methods in rheumatology. One of the types of systemic vasculitis is granulomatosis with polyangiitis (Wegener's granulomatosis) – giant cell necrotizing granulomatous vasculitis of small caliber vessels associated with the production of antineutrophil cytoplasmic antibodies (ANCA) and characterized by a damage predominantly to the upper respiratory tract, lungs, and kidneys [1–3]. The disease is multi-organ in nature and is characterized by a variety of symptoms. The onset of the disease most often occurs at the age of over 40 years; its incidence is 1.5 times higher in the European population [2].

Despite many years of study, the etiology of granulomatosis with polyangiitis remains unknown. However, most often, the manifestation of the disease is preceded by infections caused by *Staphylococcus aureus*, pneumocystis, as well as acute respiratory viral infection (ARVI), vaccination, and antibiotic prophylaxis, which trigger the formation of autoantigens [4]. The autoimmune process activates a cascade of cellular reactions that reproduce polymorphic-cellular granulomatous inflammation, which leads to the activation of macrophages, antigen-presenting cells, and

then effector memory T cells and plasmacytes capable of producing not only IL-6, IL-10, TNF- α , but also ANCA [5].

During these processes, polymorphonuclear leukocytes penetrate into the bloodstream, disrupt the vascular permeability, and produce lysosomal enzymes, which results in necrosis of the vessel wall and its occlusion. Most often, the first foci that reflect autoimmune granulomatous inflammation are the upper respiratory tract, lungs, and kidneys. Biopsy with histological examination is considered to be the key method for diagnosing granulomatosis with polyangiitis, since only these results can confirm the nature of the inflammatory process in the affected tissues. Undoubtedly, the hallmark of Wegener's granulomatosis is detection of ANCA in the blood serum, but it should be taken into account that the studied antibodies are usually recorded only in 80% of patients and cannot be detected in remission [6].

We present a case of granulomatosis with polyangiitis that is difficult for clinical diagnosis.

CLINICAL CASE

Patient K, female, 40 years old, was admitted to the Department of Otorhinolaryngology at the Krasnoyarsk Regional Clinical Hospital in July 2019 with complaints of severe headaches, pain behind the ear, purulent drainage from the right ear, periodic rise in the body temperature to 37.5–37.8° C, and balance problems (staggering) when walking.

From the anamnesis: acute onset of the disease (2 weeks ago) – earache and purulent drainage from the right ear occurred. The patient went to a private clinic to see an ENT doctor, where she was prescribed with antibacterial and antifungal treatment, which turned out to be ineffective. The patient was sent to the outpatient clinic of the Regional Clinical Hospital.

Upon admission: clear consciousness, moderate severity of illness (SOI), the skin was clean and of normal moisture, the musculoskeletal system was without abnormalities. *Status localis*: the nasal septum was deviated. The inferior turbinates were edematous. Upon percussion of the right mastoid process, sharp tenderness was noted. The right external auditory canal was filled with purulent drainage; the left external auditory canal was free. The tympanic membrane was not visualized. Otoscopy: in the right external auditory canal, there was profusely purulent discharge; after sanitation, in the projection of the tympanic membrane, there was pink tissue with a bumpy surface (polyp?), when touched with the instrument, it was tender but did not bleed. Vesicular respiration was observed. Respiratory rate (RR) was 14 per min. The heart tones were clear. The blood pressure was 110 / 70 mm Hg., the heart rate – 56 beats per minute. The abdomen was soft and painless and participated in the act of breathing. Urination was free and painless. A preliminary diagnosis was made: acute suppurative otitis media, right ear, perforation stage; acute mastoiditis, right side. Meningitis?

The blood test showed relative leukocytosis ($9.16 \times 10^9 / l$), hypochromic anemia (erythropenia $3.55 \times 10^{12} / l$, HB – 113 g / l), an increase in C-reactive protein (132.5 mg / l) and fibrinogen (5.1 g / l), as well as an increase in the erythrocyte sedimentation rate (ESR) (61 mm / h). To exclude otogenic neuroinfection, a cerebrospinal fluid (CSF) analysis was conducted, which did not reveal meningitis. A CT scan of the temporal bones showed data corresponding to right-sided acute otitis media. Chest X-ray showed that the lungs were without visible infiltrative darkening. The pleural cavities were free, pleurodiaphragmatic adhesions on the right side were detected. Endoscopic electrosurgical removal of the middle ear tumor was performed. The diagnosis after the surgery was the following: chronic purulent epimesotympanitis with a polyp in the right external auditory canal and symptoms of otitis externa, subtotal tympanic membrane defect. The surgical material was taken for histopathological examination, which registered squamous cell papilloma with diffuse chronic inflammation.

At the department, the patient received antibacterial (ceftazidime) and anti-inflammatory therapy. Despite the treatment, the patient complained of severe cough, deterioration of the condition, and a rise in the body temperature to 38.8 °C. To exclude pneumonia, chest X-ray was performed, which revealed a peripheral formation in the lower lobe of the right lung. The patient underwent chest multi-slice spiral computed tomography (MSCT) – the findings may correspond to those in destructive pneumonia, which should be differentiated from an abscess; bilateral pleural effusion. Blood tests revealed leukocytosis ($10.9 \times 10^9 / l$), neutrophilia (74.3%), increased hypochromic anemia (107 g / l), and accelerated ESR (58 mm / h).

Having been examined by a thoracic surgeon, the patient was diagnosed with lung abscess with pneumonia in the lower lobe of the right lung. Antibacterial therapy (cefoperazone / sulbactam 2g 3 times a day) was prescribed. Sputum culture for acid-fast mycobacteria (AFB) was negative. Tracheobronchoscopy showed mild right-sided diffuse bronchitis with mild mucus hypersecretion.

Ultrasound of the pleural cavities revealed effusion ($V = 80 \text{ cm}^3$). Transthoracic puncture was performed, during which 200.0 ml of clear fluid of light straw color was obtained. A cytological examination of the pleural fluid identified red blood cells, eosinophilic substance, a pronounced number of neutrophilic leukocytes at the stage of degenerative changes, numerous fragments of destroyed cellular elements, and signs of dystrophic changes.

After the instrumental studies, it was decided to transfer the patient to the Department of Thoracic Surgery for further treatment and change the conservative treatment strategy – levofloxacin, meropenem, amikacin, and blood transfusion (erythromass) No. 6 were prescribed. Repeated chest MSCT showed negative dynamics due to an increase in the area of previously identified changes. The appearance of a rounded formation with a diameter of up to 12 mm was also noted nearby. There was a lucid area in the center, and a ground-glass opacity was determined around. The result may correspond to an abscess, with traces of pleural effusion on both sides. At the medial surface on the right, there was encysted pleurisy. In the blood test, signs of inflammation and hypochromic anemia were also detected. The acid-fast bacillus (AFB) test was negative.

Establishing a final diagnosis was difficult. Differential diagnosis was carried out with the following diseases: peripheral tumor in the lower lobe of the right

lung with decay, infiltrative pulmonary tuberculosis at the stage of decay, and lung abscess. Repeated chest MSCT showed negative dynamics due to an increase in the area of previously identified changes. The appearance of a rounded formation with a diameter of up to 12 mm was also noted nearby. There was a lucid area in the center, and a ground-glass opacity was determined around. The findings corresponded to an abscess, with traces of pleural effusion on both sides. At the medial surface on the right, there was encysted pleurisy.

It was decided to perform a right-sided thoracotomy and a lower lobectomy. During the surgical intervention, a palpable dense formation of about 10 cm in size was found in the basal segments of the right lung lower lobe, in the projection of which the lung was tightly attached to the diaphragm and the lateral costal surface of the chest wall. In S1, a focal formation of up to 0.5 cm was revealed. It was decided to resect the focal formation in the segment and remove the lower lobe. The main postoperative diagnosis was lung abscess. However, the pathohistological study of the surgical biopsy material showed morphological signs of granulomatous inflammation (single epithelioid cell granulomas and a large number of Langhans multinucleated giant cells).

In September 2019, the patient was transferred to the Krasnoyarsk Regional Tuberculosis Dispensary No.1 for further treatment with the following diagnosis: infiltrative tuberculosis in the lower lobe of the right lung in the phase of decay; *Mycobacterium tuberculosis* (–); exacerbation of chronic suppurative otitis media, right ear; acute suppurative otitis media, left ear; secondary (against the background of purulent epimesotimpanitis) peripheral neuritis of the facial nerve on the right side with slight paresis.

In the Krasnoyarsk Regional Tuberculosis Dispensary No.1, the patient had received anti-TB therapy for 22 days; no positive dynamics were observed. 17 sputum and ear content AFB tests were negative, and a polymerase chain reaction (PCR) was conducted twice – the results also did not reveal tuberculosis infection. It was noted that the disease progressed on the part of ENT organs: bilateral otitis media; mastoiditis on the right side, gingival fibromatosis, fibrotic changes in the nasal cavity with signs of mucosal bleeding.

In October 2019, the patient was urgently hospitalized in the ENT Department of the Regional Clinical Hospital with complaints of earaches, more on the right side, hearing loss in both ears, purulent discharge from the ears, and numbness of the right cheek and

zygomatic region. On the basis of complaints and anamnesis, the following diagnosis was made: chronic bilateral suppurative otitis media, exacerbation; acute mastoiditis, right side? An antromastoidotomy was performed on the right side: the antrum was small, slit-shaped, and expanded; in the antrum, granulations and altered mucosa with polyps were observed; single cells of the mastoid process were opened in the pathology-affected area, no purulent discharge was obtained, and the mucosa was hyperplastic. The bone was softened like a spongy bone and easily removed with a spoon. Pathohistological examination of the surgical material registered no findings for granulomatous inflammation and revealed signs of chronic inflammation of moderate intensity.

Given frequent exacerbations of chronic suppurative otitis media and the presence of an intensive destructive process in the lungs with a pronounced inflammatory response against the background of the ongoing anti-bacterial and anti-TB therapy, the doctor made a decision to hold a case conference with the involvement of a pulmonologist and a nephrologist.

During the patient's examination, leg swelling and swollen, painless axillary lymph nodes on the right side with the maximum size of no more than 2 cm were noted. On the anterior abdominal wall above the navel, there was a bluish induration covered with a crust from which purulent discharge periodically appeared. On the inner third of the right thigh and the lumbar area, indurations of 2.5–3 cm in diameter were palpated. They were painless; the skin above them was not altered. In the lungs, dry wheezing, mainly in the lower parts on the right, was noted. The data obtained, such as a lack of response to the therapy, systemic inflammatory response syndrome, respiratory syndrome, anemia, swelling, facial neuritis on the right, and lesions of the ENT organs, suggested the development of systemic vasculitis.

To confirm the diagnosis of the patient, chest MSCT was performed: two cavities with unevenly thickened walls were detected in the basal zone on the left. In the lower lobe of the right lung, rounded formations with clear, even contours, of 11.4 mm in diameter, were determined. These formations were located subpleurally. The MSCT data might correspond to Wegener's granulomatosis. Ultrasound of the pleural cavity registered fluid ($V = 100 \text{ cm}^3$) with a fibrinous component in the right pleural cavity. A pathohistological examination of the right bronchial mucosa revealed a pronounced diffuse polymorphic cellular inflammatory infiltrate among the fibrous tissue, which corresponds

to the picture of the inflammatory process. A pathomorphological examination of the gingival mucosa registered a productive granulomatous inflammation in the soft tissues of the gingival mucosa. Taking into account the anamnesis, the obtained data correspond to Wegener's granulomatosis.

It was decided to hold a repeated case conference, at which a conclusion was made. Taking into account the clinical features, such as damage to the upper and lower respiratory tract, hearing organs, and oral cavity, the data of the morphological studies, the productive granulomatous inflammation in the soft tissues of the gingival mucosa, negative AFB tests, unsuccessful anti-TB therapy, despite the negative ANCA, the following diagnosis was made: Granulomatosis with polyangiitis, high intensity, severe course, with damage to the lungs (multiple infiltrates with destruction) and hearing organs (bilateral otitis media, mastoiditis on the right side), gingival fibromatosis. Anemia, moderate severity.

After methylprednisolone (1,000 mg No. 3) and cyclophosphamide (1,000 mg No. 1) pulse therapy with subsequent oral administration of methylprednisolone (48 mg / day), positive clinical, laboratory, and radiological dynamics were observed. Follow-up chest MSCT from 14.11.2019 revealed a decrease in the amount of fluid in the right pleural cavity, as well as a decrease in the size of foci and infiltration in the upper segments of the right lung. By February 2020, the patient had received five courses cyclophosphamide pulse therapy at a dose of 1,000 mg. Currently, the patient continues to take methylprednisolone at a dose of 38 mg / day.

CONCLUSION

The presented clinical case demonstrates the complexity of differential diagnosis of systemic lesions. The diversity of the clinical course and the absence of

specific symptom complexes cause a noticeable difficulty in the diagnosis and treatment of granulomatosis with polyangiitis. Despite this, a careful comparison of clinical manifestations and laboratory and instrumental data and immunological search for markers of the disease can lead to earlier and more accurate diagnosis of systemic vasculitis. It should be noted that late diagnosis may cause development of irreversible damage to vital organs, which can lead not only to deterioration of the quality of life, but also to a decrease in the five-year survival rate.

REFERENCES

1. Baranov A.A., Alekseeva E.I. Clinical guidelines for pediatricians. Rheumatic diseases in children. M.: Pediatr, 2016: 144 (in Russ.).
2. Petty R.E., Laxer R.M., Lindsley C.B., Wedderburn L. Textbook of pediatric rheumatology; 5th ed. Elsevier Saunders, Philadelphia, 2016: 736.
3. Gordeeva N.V., Demko I.V., Mamaeva M.G., Zeleny S.V., Guseva I.S., Sobko E.A., Kraposhina A.Yu., Soloveva I.A., Chubarova S.V. Hypersensitivity pneumonitis (difficult clinical case). *Siberian Medical Review*. 2015; 5: 92–95 (in Russ.).
4. Edmondson S.J., Attaran S., Rosendahl U.P. Complete resolution of Wegener's granulomatosis lung granuloma after aortic root replacement. *Aorta (Stamford)*. 2016; 4 (1): 25–28. DOI: 10.12945/j.aorta.2016.15.009.
5. Kubaisi B., Abu Samra K., Foster C.S. Granulomatosis with polyangiitis (Wegener's disease): An updated review of ocular disease manifestations. *Intractable Rare Dis. Res.* 2016; 5 (2): 61–69. DOI: 10.5582/irdr.2016.01014.
6. Rosenbaum J.T., Choi D., Wilson D., Grossniklaus H.E., Harrington C.A., Sibley C.H., Dailey R.A., Ng J.D., Steele E.A., Czyz C.N., Foster J.A., Tse D., Alabiad C., Dubovy S., Parekh P.K., Harris G.J., Kazim M., Patel P.J., White V.A., Dolman P.J., Korn B.S., Kikkawa D.O., Edward D.P., Alkatan H.M., al-Hussain H., Yeatts R.P., Selva D., Stauffer P., Planck S.R. Orbital pseudotumor can be a localized form of granulomatosis with polyangiitis as revealed by gene expression profiling. *Exp. Mol. Pathol.* 2015; 99 (2): 271–278. DOI: 10.1016/j.yexmp.2015.07.002.

Authors contribution

Kraposhina A.Yu. – conception and design, substantiation of the manuscript, critical revision of the manuscript for important intellectual content. Demko I.V. – final approval of the manuscript for publication. Sobko E.A. – substantiation of the manuscript, critical revision of the manuscript for important intellectual content. Gordeeva N.V., Soloveva I.A., Matveeva I.V. – conception and design. Kazmerchuk O.V., Katser A.B., Abramov Yu.I., Malchik N.V. – analysis and interpretation of data.

Authors information

Kraposhina Angelina Yu., Cand. Sci. (Med.), Associate Professor, Department of Internal Diseases and Immunology, V.F. Voino-Yasenetsky Krasnoyarsk State Medical University; Pulmonologist of the Medical and Diagnostic Department, Krasnoyarsk Regional Clinical Hospital, Krasnoyarsk, Russian Federation. ORCID 0000-0001-6896-877X.

Demko Irina V., Dr. Sci. (Med.), Professor, Head of the Department of Internal Diseases and Immunology, V.F. Voino-Yasenetsky Krasnoyarsk State Medical University; Head of the Pulmonology and Allergy Center, Krasnoyarsk Regional Clinical Hospital, Krasnoyarsk, Russian Federation. ORCID 0000-0001-8982-5292.

Sobko Elena A., Dr. Sci. (Med.), Associate Professor, Professor of the Department of Internal Diseases and Immunology, V.F. Voino-Yasenetsky Krasnoyarsk State Medical University; Head of the Department of Allergy, Krasnoyarsk Regional Clinical Hospital, Krasnoyarsk, Russian Federation. ORCID 0000-0002-9377-5213.

Gordeeva Natalya V., Cand. Sci. (Med.), Associate Professor, Department of Internal Diseases and Immunology, V.F. Voino-Yasenetsky Krasnoyarsk State Medical University; Pulmonologist of the Medical and Diagnostic Department, Krasnoyarsk Regional Clinical Hospital, Krasnoyarsk, Russian Federation. ORCID 0000-0002-0586-8349.

Soloveva Irina A., Dr. Sci. (Med.), Associate Professor, Department of Internal Diseases and Immunology, V.F. Voino-Yasenetsky Krasnoyarsk State Medical University; Pulmonologist of the Medical and Diagnostic Department, Krasnoyarsk Regional Clinical Hospital, Krasnoyarsk, Russian Federation. ORCID 0000-0002-1999-9534.

Matveeva Irina V., Head of the Department of Nephrology and Rheumatology, Krasnoyarsk Regional Clinical Hospital, Krasnoyarsk, Russian Federation.

Kazmerchuk Olga V., Researcher, Department of Internal Diseases and Immunology, V.F. Voino-Yasenetsky Krasnoyarsk State Medical University, Krasnoyarsk, Russian Federation. ORCID 0000-0001-7999-4113.

Katser Anna B., Researcher, Department of Internal Diseases and Immunology, V.F. Voino-Yasenetsky Krasnoyarsk State Medical University, Krasnoyarsk, Russian Federation. ORCID 0000-0002-6649-8900.

Abramov Yuri I., Researcher, Department of Internal Diseases and Immunology, V.F. Voino-Yasenetsky Krasnoyarsk State Medical University, Krasnoyarsk, Russian Federation. ORCID 0000-0002-9937-1025.

Malchik Natalya V., Researcher, Department of Internal Diseases and Immunology, V.F. Voino-Yasenetsky Krasnoyarsk State Medical University, Krasnoyarsk, Russian Federation.

(✉) **Kraposhina Angelina Yu.**, e-mail: angelina-maria@inbox.ru

Received 03.06.2020

Accepted 29.09.2020

A clinical case of idiopathic pulmonary fibrosis against the background of comorbid pathology

Teteneva A.V.^{1,3}, Kalyuzhin V.V.¹, Chernyavskaya G.M.¹, Beshpalova I.D.¹, Chernogoryuk G.E.¹, Zavadovskaya V.D.¹, Zhogina T.V.¹, Ustyuzhanina E.A.², Kuzin E.V.², Varfolomeeva I.A.², Sedlyar O.V.², Medikova E.A.¹, Koshchavtseva Yu.I.¹, Potapov K.V.¹, Karzilov A.I.¹, Porovsky Ya.V.¹, Solovev M.M.¹

¹ *Siberian State Medical University
2, Moscow Trakt, Tomsk, 634050, Russian Federation*

² *Tomsk Regional Clinical Hospital
96, I. Chernykh Str., Tomsk, 634063, Russian Federation*

³ *Medical and Sanitary Hospital No. 2
3, Bela Kuna Str., Tomsk, 634040, Russian Federation*

ABSTRACT

Idiopathic pulmonary fibrosis (IPF) is one of the most common diseases in the group of interstitial lung diseases, which is characterized by persistent progression and poor prognosis. Over the past decade, experts have made significant progress in developing a diagnostic algorithm for IPF patients. This algorithm includes analysis of clinical, laboratory, and instrumental data, primarily the results of high-resolution computed tomography (HRCT). Precise adherence to the diagnostic algorithm and correct interpretation of HRCT data are prerequisites for IPF diagnosis.

Specialists of the Tomsk region have developed routing of patients with suspected IPF. The presented clinical case is a successful example of adhering to this algorithm. Wide implementation of modern diagnostic algorithms into diagnosis and treatment of IPF and quality improvement of imaging methods, primarily HRCT, carried out as a part of the differential diagnosis, open up prospects for early diagnosis of this pathology. A timely prescribed antifibrotic therapy (nintedanib, pirfenidone) in IPF allows to slow down pathological progression and improves the prognosis.

Key words: idiopathic pulmonary fibrosis, usual interstitial pneumonia, nintedanib, antifibrotic therapy, treatment.

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

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

For citation: Teteneva A.V., Kalyuzhin V.V., Chernyavskaya G.M., Beshpalova I.D., Chernogoryuk G.E., Zavadovskaya V.D., Zhogina T.V., Ustyuzhanina E.A., Kuzin E.V., Varfolomeeva I.A., Sedlyar O.V., Medikova E.A., Koshchavtseva Yu.I., Potapov K.V., Karzilov A.I., Porovsky Ya.V., Solovev M.M. A clinical case of idiopathic pulmonary fibrosis against the background of comorbid pathology. *Bulletin of Siberian Medicine*. 2021; 20 (3): 225–231. <https://doi.org/10.20538/1682-0363-2021-3-225-231>.

Клинический случай идиопатического легочного фиброза на фоне коморбидной патологии

Тетенева А.В.^{1,3}, Калюжин В.В.¹, Чернявская Г.М.¹, Беспалова И.Д.¹, Черногорюк Г.Э.¹, Завадовская В.Д.¹, Жогина Т.В.¹, Устюжанина Е.А.², Кузин Е.В.², Варфоломеева И.А.², Седляр О.В.², Медикова Е.А.¹, Кощавцева Ю.И.¹, Потапов К.В.¹, Карзилов А.И.¹, Поровский Я.В.¹, Соловьев М.М.¹

¹ Сибирский государственный медицинский университет (СибГМУ)
Россия, 634050, г. Томск, Московский тракт, 2

² Томская областная клиническая больница (ТОКБ)
Россия, 634063, г. Томск, ул. И. Черных, 96

³ Медико-санитарная часть № 2
Россия, 634040, г. Томск, ул. Бела Куна, 3

РЕЗЮМЕ

Идиопатический легочный фиброз (ИЛФ) относится к наиболее распространенным заболеваниям из группы интерстициальных заболеваний легких и характеризуется неуклонным прогрессированием и неблагоприятным прогнозом. В течение последнего десятилетия был достигнут значительный прогресс в разработке диагностического алгоритма для пациентов с ИЛФ, предполагающий анализ клинических, лабораторных и инструментальных данных, прежде всего, результатов компьютерной томографии высокого разрешения (КТВР). Точное следование алгоритму диагностики и правильная интерпретация данных КТВР являются необходимым условием для постановки диагноза ИЛФ.

В Томской области разработана маршрутизация больных с подозрением на ИЛФ. Примером успешного следования этому алгоритму является представленный клинический случай. Широкое внедрение в лечебно-диагностический процесс современных алгоритмов диагностики ИЛФ и повышение качества визуализационных методов, прежде всего КТВР, проводимые в рамках дифференциального диагноза, открывают перспективы ранней диагностики данного патологического процесса, а своевременно назначенная антифибротическая терапия (нинтеданиб, пирфенидон) при ИЛФ позволяет замедлить прогрессирование патологического процесса и улучшить прогноз.

Ключевые слова: идиопатический легочный фиброз, обычная интерстициальная пневмония, нинтеданиб, антифибротическая терапия, лечение.

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

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

Для цитирования: Тетенева А.В., Калюжин В.В., Чернявская Г.М., Беспалова И.Д., Черногорюк Г.Э., Завадовская В.Д., Жогина Т.В., Устюжанина Е.А., Кузин Е.В., Варфоломеева И.А., Седляр О.В., Медикова Е.А., Кощавцева Ю.И., Потапов К.В., Карзилов А.И., Поровский Я.В., Соловьев М.М. Клинический случай идиопатического легочного фиброза на фоне коморбидной патологии. *Бюллетень сибирской медицины*. 2021; 20 (3): 225–231. <https://doi.org/10.20538/1682-0363-2021-3-225-231>.

INTRODUCTION

According to current data, interstitial lung disease (ILD) makes up 20% of all lung diseases. Common features of ILD are progressive shortness of breath, restrictive lung disease, and diffuse bilateral changes in X-ray and CT examinations. The diagnosis of

ILD is still extremely unsatisfactory. Diagnostic errors account for up to 80%, and adequate specialized care is often provided to patients 1.5–2 years after the first manifestations of the disease. Such a depressing state of affairs is explained not only by the absence of pathognomonic signs (clinical, radiological, functional, laboratory and even morphological) in ILD, but

also by a lack of doctors' knowledge about this pathology and a wrong opinion about its low prevalence [1].

The most common disease in the ILD group is idiopathic pulmonary fibrosis (IPF). On average, IPF makes up 20–30% of all ILD cases. IPF is a specific form of chronic progressive idiopathic interstitial pneumonia of unknown origin. IPF occurs primarily in the elderly and affects only the lungs. It is associated with a histological and (or) radiological pattern of usual interstitial pneumonia (UIP) [2].

The most common clinical symptoms of IPF include slow progressive shortness of breath and non-productive cough present in 80–90% of patients, crepitations (Velcro crackles) during the height of inspiration in the lower parts of the lungs, which in timbre resembles the sound of hook-and-loop fasteners on clothing, drumstick fingers, and watch-glass nails (in 25–50% of patients). It should be noted that the disease can be almost asymptomatic at early stages.

IPF is characterized by a progressive, malignant course with a high risk of developing an adverse outcome. Without treatment, the average life expectancy of patients is 3 years, and the five-year survival rate is about 30% [1]. High mortality rate of patients with this pathology is explained by the peculiarities of the disease pathogenesis – predominance of fibrosis and slight inflammatory changes. The main mechanism that leads to the development of progressive pulmonary fibrosis is persistent damage to the alveolar epithelium with subsequent impairment of its regeneration. It is followed by activation of fibroblasts and myofibroblasts and excessive deposition of extracellular matrix components. These changes determine the ineffectiveness of traditional immunosuppressive therapy in IPF patients [2].

In this regard, appearance of two new antifibrotic medications for treatment of this pathology – pirfenidone and nintedanib – is essential. They were approved by the FDA in 2014 and registered in 2016–2017 in the Russian Federation. These medications have proven effective in IPF as they slow down the progression of the disease and reduction of lung volumes, improving the survival rate of patients [3, 4]. In May 2019 (SENSCIS trial, 600 patients), it was proved that nintedanib slows down the progression of the disease and the loss of the forced vital capacity (FVC) of the lungs decreases [5]. Moreover, it was revealed that nintedanib treatment slows down a decrease in FVC parameters regardless of their initial level. It gives grounds to recommend it both at the earliest stage of the disease, even before the development of clinical-

ly significant functional respiratory disorders, and in a significant decrease in the functional parameters of respiration [6, 7]. Thus, the appearance of medications that improve the prognosis of the disease determines the importance of early IPF diagnosis.

Over the past decade, significant progress has been made in the development of a diagnostic algorithm for patients with IPF, involving the analysis of clinical, laboratory, and instrumental data, primarily the results of high-resolution computed tomography (HRCT).

At the first stage, this algorithm requires thorough history taking to rule out other known IPD causes, for example, occupational and household exposures, connective tissue diseases, and drug toxicity. In addition, a differential diagnosis should be made with diseases that have similar clinical symptoms, for example, chronic heart failure: shortness of breath, fatigue, cough, crepitations in the lungs, and weight loss [2, 8].

The next step is to conduct HRCT and analyze the data. Improvement of modern diagnostic methods and introduction of HRCT into clinical practice made it possible to significantly expand verification of IPF diagnosis at the onset of the disease [9, 10]. Spiral computed tomography of the lungs is now a gold standard for IPF diagnosis. IPF is characterized by a HRCT pattern, which manifests itself as UIP and includes the following signs: reticular changes, honeycombing, and distribution of pathological changes mainly in the subpleural and posterior basal parts. The criteria for evaluating HRCT data are based on disease probability. The Fleischner Society identified four main radiological patterns of UIP: typical, probable, indeterminate, and not associated with IPF, characteristic of another ILD (not IPF) [11].

A typical UIP pattern in HRCT is characterized by the presence of honeycomb lung features, reticular changes, and peripheral traction bronchiectasis predominantly in the basal (less often diffuse) and subpleural segments. In the absence of honeycombing and in the presence of other signs, the X-ray pattern of UIP is probable. Signs that are atypical of UIP or exclude it are: pronounced frosted glass signs, areas of consolidation, single pulmonary nodules and dissemination, predominance of changes in the upper and middle segments of the lungs, peribronchovascular distribution of changes, pulmonary cysts in the extracortical areas, and bullous emphysema.

It is important to emphasize that the modern diagnostic algorithm for IPF offers a differentiated approach to the use of invasive diagnostic methods. Thus, a lung biopsy is not required to verify the di-

agnosis in the presence of clinical presentation of IPF and a CT pattern of typical or probable UIP. Surgical lung biopsy (SLB) is recommended in the presence of a CT pattern that is atypical of UIP. In such cases, the diagnosis is made on the grounds of the HRCT data combined with histological findings.

Therefore, precise adherence to the diagnostic algorithm and correct interpretation of the HRCT data are prerequisites for IPF diagnosis.

The Tomsk region has developed routing of patients with suspected IPF. If IPF is suspected, the following actions are carried out:

Planned admission to the Pulmonology Department of the Tomsk Regional Clinical Hospital.

At the Department, the patient is examined in accordance with the Federal clinical guidelines for the diagnosis and treatment of idiopathic pulmonary fibrosis (2016), excluding other interstitial lung lesions. The diagnosis is made after discussing all the data obtained by a council of doctors.

After the initial diagnosis of IPF, all documents (with the HRCT scans of the chest organs) are submitted to federal experts to confirm or exclude the diagnosis of IPF. They include patients in the registry of patients with IPF in the Russian Federation.

Once the diagnosis is confirmed, treatment is prescribed. It has become more accessible in the Russian Federation after the registration of new antifibrotic medications.

The presented clinical case is a successful example of adhering to this algorithm.

CLINICAL CASE

Patient K., 67 years old, first went to the Pulmonology Department of the Tomsk Regional Clinical Hospital in October 2015 with complaints of generalized weakness, heart failure, shortness of breath of a mixed nature when walking up to 10 meters and climbing 10 stairs (2 points on the MRC scale), episodic increases in the body temperature to 38.5 °C, and coughing up of gray phlegm up to 30 ml per day. According to the medical history, cough had been bothering the patient for the last 15 years, while shortness of breath of a mixed nature appeared 5 years ago.

Medical history. In 2015, the patient was diagnosed with ischemic heart disease (IHD): paroxysmal atrial fibrillation, supraventricular extrasystoles, paroxysmal supraventricular tachycardia. Chronic heart failure, stage IIa. Stage 3 hypertension, class IV risk of cardiovascular complications. The patient constantly takes sotahexal 80 mg, ¼ tab 2 times a day and

cardiomagnyl 75 mg 1 once a day. Strumectomy was performed in 1995 for diffuse toxic goiter, the patient constantly takes L-thyroxine 100 mg daily. The patient suffers from varicose veins of the lower extremities and has no bad habits.

Objective status. The condition is of moderate severity. Body temperature is 36.3 °C. Pulse – 100 BPM, respiratory rate – 20 breaths per minute, SpO₂ – 96%, blood pressure – 110 / 70 mm Hg. Consciousness is clear, the patient has full awareness. Skin is of moderate humidity with diffuse cyanosis. Drumstick fingers and watch-glass nails are noted. The tongue with a white and yellow coating is moist. The thorax is cylindrical. A box sound is heard in percussion. The lower border of the lungs is one rib higher. Breathing is hard with crepitations in the middle and lower parts on both sides. The heart tones are evenly weakened, the rhythm is normal. The liver edge is palpable along the costal margin. The abdomen is soft and painless. The spleen is not palpable. No peripheral edema is noted. Signs of varicose veins in the lower extremities are observed.

Spirography. Abnormal pulmonary ventilation of the 1st degree mainly according to the restrictive pattern. The HRCT data: the presentation corresponds to UIP. Taking into account the presence of clinical signs typical of IPF and a CT pattern of UIP, a lung biopsy was not performed.

The patient was diagnosed with IPF with a slowly progressive course with the formation of a “honeycomb lung”. Upon discharge from the hospital, the patient was recommended follow-up examinations. The patient was advised to use only symptomatic therapy, since at that time antifibrotic medications with proven effectiveness in IPF (nintedanib (Vargatef) and pirfenidone (Esbriet)) were not available in the Russian Federation. Glucocorticoids and cytostatics used for many years to treat this disease were not recommended as a permanent therapy, since they negatively affect its course [2].

Repeated treatment and admission to the Pulmonology Department of the Tomsk Regional Clinical Hospital in February 2017 was caused by deterioration of the condition (progression of shortness of breath).

Objective status. No significant dynamic changes. The degree of oxygen saturation of the blood (SpO₂) decreased from 96 (2015) to 91%, which characterizes the presence of type I respiratory failure and indicates a downward trend in the disease. Spirography and body plethysmography parameters did not show significant changes.

Spirography of 27.02.2017. Vital capacity (VC) 85%, FVC 84%, forced expiratory volume in the first second (FEV1) 60%, FEV1 / FVC 76.4%.

Body plethysmography of 02.03.2017. The structure of the total lung capacity (TLC) is not altered. Bronchial resistance is normal. VC 93.5%; TLC 82.8%; residual volume (RV) 78.2%; R tot 43.1%.

Spiral CT of the chest of 25.03.2017 (Fig. 1–3). The chest is of usual shape and size. There are no destructive changes in the structure of the ribs, vertebrae, shoulder blades, and clavicles. Moderately pronounced reticular changes are detected in the lungs due to interstitial thickening. Frosted glass signs in the areas of reticular changes are determined. Traction bronchiectasis and honeycombing in the cortical regions are pronounced. The distribution of changes is cortical basal. The diaphragm is high, the volume of the lower lobes is reduced.

The lumina of large bronchi are not changed. The interstitial slits and sinuses are free, there is no fluid in the pleural cavities. The adipose tissue of the mediastinum has a homogeneous structure and increased volume (lipomatosis). The lymph nodes in the mediastinum and in the roots of the lungs are moderately enlarged and do not merge into conglomerates. The atria and ventricles of the heart are of normal size and shape. The aorta and its branches have signs of atherosclerosis, the superior vena cava is within the age norm. The esophagus is normally located, its walls are not thickened, the lumen is expanded, an esophageal hiatus hernia of the diaphragm is noted.

Conclusion: the CT pattern is characteristic of usual interstitial pneumonia.

The results of the spiral CT of the chest were sent for consideration to Igor E. Tyurin, chief freelance expert in radiology of the Ministry of Health of the Russian Federation. The answer was received in the electronic form in the IPF registries on 26.05.2017. Conclusion: the pattern of UIP.

Taking into account the long-term use of drugs for the comorbid pathology, including L-thyroxine (100 mg) since 1995, a differential diagnosis was made between IPF and drug-induced fibrosing alveolitis. Therefore, the council decided to prescribe a trial therapy with prednisone *per os* 30 mg / day for a month, followed by a reduced maintenance dose (10 mg / day), which the patient took for a year (from 10.03.2017 to 01.03.2018) without improvements in the clinical presentation and CT findings.

Afterwards, the patient continued to undergo follow-up examinations. The principal diagnosis: idio-

pathic pulmonary fibrosis with a slowly progressive course with the formation of the “honeycomb lung”. Complication: chronic pulmonary heart disease, compensation stage. Type I respiratory failure.

Concomitant diagnosis: IHD: paroxysmal atrial fibrillation. Supraventricular extrasystole, paroxysmal supraventricular tachycardia. Stage 1 chronic heart failure. Stage 3 hypertension, class IV risk of cardiovascular complications. Diffuse nodular goiter, condition after strumectomy in 1995, postoperative hypothyroidism. Varicose veins in the lower extremities, chronic venous insufficiency.

The condition progressively worsened, shortness of breath increased. In February 2019, due to the deterioration of the patient's condition (increased shortness of breath, an increase in the body temperature to 38 °C, a decrease in blood oxygen saturation to SpO₂ 89%), she was admitted to the Tomsk Regional Clinical Hospital. Differential diagnosis was made between the exacerbation of IPF and community-acquired pneumonia. According to the current data, despite gradual progression of IPF, the course of the disease can be complicated by acute periods of worsening disease symptoms that are not associated with a respiratory tract infection, pulmonary embolism, decompensated heart failure, and other known factors. These episodes of acute deterioration of the IPF course are considered as IPF exacerbations. It is crucial to determine the cause of deterioration in IPF, since it is associated with the choice of therapy. Exacerbation of IPF, as a rule, requires more glucocorticoids (up to pulse therapy), while decompensation of concomitant pathology and pulmonary embolism require appropriate treatment. Respiratory tract infections require anti-infective therapy. For instance, the presence of community-acquired pneumonia requires mandatory prescription of antibiotics.

The analysis of the clinical presentation showed that the increased shortness of breath was not accompanied by an increase in other manifestations of heart failure. Echocardiography revealed signs of moderate pulmonary hypertension. No abnormal local contractility was detected. Global left ventricular function remained within normal limits.

The situation was regarded as a severe course of left-sided community-acquired multilobar pneumonia, caused by *Actinobacter boumonii*. Background diagnosis: idiopathic pulmonary fibrosis. Stage of the honeycomb lung, progressive course. The prescribed antibiotic therapy led to improvement in the clinical and radiological presentation and stabilized the condition.

On March 15, 2018, the patient was consulted by Professor Sergey N. Avdeev, chief freelance pulmonologist of the Ministry of Health of the Russian Federation. The diagnosis of IPF was confirmed. Palliative therapy with nintedanib (Vargatef) was recommended.

In February 2019, the patient started taking nintedanib (Vargatef) at 150 mg 2 times a day within the palliative care program. In December 2019, the patient had a planned admission (follow-up) to the Tomsk Regional Clinical Hospital. No deterioration of the condition during treatment with nintedanib (Vargatef) for 10 months was identified. No exacerbations of the disease were detected. The patient tolerated the therapy well. Parameters of arterial oxygen saturation were stable (SpO₂ 91–92%). Spirography and body plethysmography parameters also did not reveal a downward trend.

Spirography of 13.12.2019: VC 87%; FVC 87%; FEV1 67%, FEV1 / FVC 84%. Bronchodilator test: negative.

Spiral CT of the chest of 18.12.2019 revealed no changes in comparison with the data of the previous CT examination (30.01.2020, Professor Igor E. Tyurin, a copy from the IPF registries, patient number 1759).

Therefore, treatment with nintedanib (Vargatef) leads to stabilization of the disease course, which is the main goal of IPF treatment.

CONCLUSION

IPF is a steadily progressing disease with an unfavorable prognosis. Its clinical manifestations, primarily shortness of breath on exertion and cough, mimic the symptoms of a number of other pathological conditions. Widespread implementation of modern algorithms for IPF diagnosis and quality improvement of imaging methods, primarily HRCT conducted in differential diagnosis, open up prospects for early diagnosis of this pathology. Timely prescribed antifibrotic therapy (nintedanib, pirfenidone) in IPF can slow down the progression of the pathological process and improve the prognosis.

REFERENCES

1. Akopov A.L., Baranova O.P., Bogdanov A.N., Bondarenko I.B., Dvorakovskaya I.V., Ilkovich M.M., Ilkovich Yu. M., Luchkevich V.S., Novikova L.N., Orlova G.P., Orlov S.V. Interstitial and orphan lung diseases. Moscow: GEOTAR-Media, 2019: 560 (in Russ.).
2. Chuchalin A.G., Avdeev S.N., Aisanov Z.R., Belevsky A.S., Demura S.A., Ilkovich M.M., Kogan E.A., Samsonova M.V., Speranskaya A.A., Tyurin I.E., Chernyaev A.L., Chernyak B.A., Chernyak A.V., Shmelev E.I. Diagnosis and treatment of idiopathic pulmonary fibrosis. Federal guidelines. *Pulmonology*. 2016; 26 (4): 399–419 (in Russ.). DOI: 10.18093/0869-0189-2016-26-4-399-419.
3. Kondoh Y., Cottin V., Brown K.K. Recent lessons learned in the management of acute exacerbation of idiopathic pulmonary fibrosis. *Eur. Respir. Rev.* 2017; 26 (145): 170050. DOI: 10.1183/16000617.0050-2017.
4. Lancaster L., Crestani B., Hernandez P., Inoue Y., Wachtlin D., Loaiza L., Quaresma M., Stowasser S., Richeldi L. Safety and survival data in patients with idiopathic pulmonary fibrosis treated with nintedanib: pooled data from six clinical trials. *BMJ Open Respir. Res.* 2019; 6 (1): e000397. DOI: 10.1136/bmjresp-2018-000397.
5. Fabrellas F.E., Sanchez P.R., Abad S.C., Juan J.G. Prognosis and follow-up of idiopathic pulmonary fibrosis. *Med. Sci. (Basel)*. 2018; 6 (2): 51. DOI: 10.3390/medsci6020051.
6. Kreuter M., Swigris J., Pittrow D., Geier S., Klotsche J., Prasse A., Wirtz H., Koschel D., Andreas S., Claussen M., Grohé C., Wilkens H., Hagmeyer L., Skowasch D., Meyer J.F., Kirschner J., Gläser S., Kahn N., Welte T., Neu-rohr C., Schwaiblmair M., Held M., Bahmer T., Oqueka T., Frankenberger M., Behr J. The clinical course of idiopathic pulmonary fibrosis and its association to quality of life over time: longitudinal data from the INSIGHTS-IPF registry. *Respir. Res.* 2019; 20(1): 59. DOI: 10.1186/s12931-019-1020-3.
7. Crestani B., Huggins J.T., Kaye M., Costabel U., Glaspole I., Ogura T., Song J.W., Stansen W., Quaresma M., Stowasser S., Kreuter M. Long-term safety and tolerability of nintedanib in patients with idiopathic pulmonary fibrosis: results from the open-label extension study, INPULSIS-ON. *Lancet Respir. Med.* 2019; 7 (1): 60–68. DOI: 10.1016/S2213-2600(18)30339-
8. Raghu G., Collard H.R., Egan J.J., Martinez F.J., Behr J., Brown K.K., Colby T.V., Cordier J.F., Flaherty K.R., Lasky J.A., Lynch D.A., Ryu J.H., Swigris J.J., Wells A.U., Ancochea J., Bouros D., Carvalho C., Costabel U., Ebina M., Hansell D.M., Johkoh T., Kim D.S., King T.E. Jr., Kondoh Y., Myers J., Müller N.L., Nicholson A.G., Richeldi L., Selman M., Dudden R.F., Griss B.S., Protzko S.L., Schüne-mann H.J.; ATS/ERS/JRS/ALAT Committee on Idiopathic Pulmonary Fibrosis. An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. *Am. J. Respir. Crit. Care Med.* 2011; 183 (6): 788–824. DOI: 10.1164/rccm.2009-040GL.
9. Travis W.D., Costabel U., Hansell D.M., King T.E. Jr., Lynch D.A., Nicholson A.G., Ryerson C.J., Ryu J.H., Selman M., Wells A.U., Behr J., Bouros D., Brown K.K., Colby T., Collard H.R., Cor-deiro C.R., Cottin V., Crestani B., Drent M., Dudden R.F., Egan J., Flaherty K., Hogaboam C., Inoue Y., Johkoh T., Kim D.S., Kitaichi M., Loyd J., Martinez F.J., Myers J., Protzko S., Raghu G., Richeldi L., Sverzellati N., Swigris J., Valeyre D.; ATS/ERS Committee on Idiopathic Interstitial Pneumonias. An official American Thoracic Society/European Respiratory Society statement: update of the international multidisciplinary classification of the idiopathic interstitial pneumonias. *Am. J. Respir. Crit. Care Med.* 2013; 188 (6): 733–748. DOI: 10.1164/rccm.201308-1483ST.

6. Raghu G., Chen S.Y., Yeh W.S., Maroni B., Li Q., Lee Y.C., Collard H.R. Idiopathic pulmonary fibrosis in US Medicare beneficiaries aged 65 years and older: incidence, prevalence, and survival, 2001–11. *Lancet Respir. Med.* 2014; 2 (7): 566–572. DOI: 10.1016/S2213-2600(14)70101-8.
7. Lynch D.A., Sverzellati N., Travis W.D., Brown K.K., Colby T.V., Galvin J.R., Goldin J.G., Hansell D.M., Inoue Y., Johkoh T., Nicholson A.G., Knight S.L., Raoof S., Richeldi L., Ryerson C.J., Ryu J.H., Wells A.U. Diagnostic criteria for idiopathic pulmonary fibrosis: a Fleischner Society White Paper. *Lancet Respir. Med.* 2018; 6 (2): 138–153. DOI: 10.1016/S2213-2600(17)30433-2.

Authors information

Teteneva Anna V., Dr. Sci. (Med.), Deputy Chief Physician, Medical and Sanitary Unit No. 2; Professor, Division of Introduction into Internal Diseases with a Therapy Course of the Pediatrics Department, Siberian State Medical University, Tomsk, Russian Federation. ORCID 0000-0002-4323-2798.

Kalyuzhin Vadim V., Dr. Sci. (Med.), Professor, Head of the Advanced Therapy Division with Rehabilitation Training, Physiotherapy, and Sports Medicine, Siberian State Medical University, Tomsk, Russian Federation. ORCID 0000-0001-9640-2028.

Chernyavskaya Galina M., Dr. Sci. (Med.), Professor, Advanced Therapy Division with Rehabilitation Training, Physiotherapy, and Sports Medicine, SibMed, Tomsk, Russian Federation. ORCID 0000-0003-0105-2307.

Bespalova Inna D., Dr. Sci. (Med.), Professor, Advanced Therapy Division with Rehabilitation Training, Physiotherapy, and Sports Medicine, SibMed; Acting Head of the Division of Introduction into Internal Diseases with a Therapy Course of the Pediatrics Department, Siberian State Medical University, Tomsk, Russian Federation. ORCID 0000-0002-4513-6329.

Chernogoryuk Georgy E., Dr. Sci. (Med.), Professor, Advanced Therapy Division with Rehabilitation Training, Physiotherapy, and Sports Medicine, Siberian State Medical University, Tomsk, Russian Federation. ORCID 0000-0001-5780-6660.

Zavadovskaya Vera D., Dr. Sci. (Med.), Professor, Acting Head of the Radial Diagnostics and Radial Therapy Division, Siberian State Medical University, Tomsk, Russian Federation.

Zhogina Tatyana V., Cand. Sci. (Med.), Associate Professor, Radial Diagnostics and Radial Therapy Division, Siberian State Medical University, Tomsk, Russian Federation.

Ustyuzhanina Elena A., Cand. Sci. (Med.), Head of the Pulmonology Department, Tomsk Regional Clinical Hospital, Tomsk, Russian Federation.

Kuzin Evgeny V., Pulmonologist, Pulmonology Department, Tomsk Regional Clinical Hospital, Tomsk, Russian Federation.

Varfolomeeva Irina A., Pulmonologist, Pulmonology Department, Tomsk Regional Clinical Hospital, Tomsk, Russian Federation.

Sedlyar Olga V., Pulmonologist, Pulmonology Department, Tomsk Regional Clinical Hospital, Tomsk, Russian Federation.

Medikova Elizaveta A., Post-Graduate Student, Radial Diagnostics and Radial Therapy Division, Siberian State Medical University, Doctor, SibMed Clinic, Tomsk, Russian Federation.

Koshchavtseva Yulia I., Resident, Division of Introduction into Internal Diseases with a Therapy Course of the Pediatrics Department, Siberian State Medical University, Tomsk, Russian Federation.

Potapov Kirill V., Resident, Division of Introduction into Internal Diseases with a Therapy Course of the Pediatrics Department, Siberian State Medical University, Tomsk, Russian Federation.

Karzilov Alexander I., Dr. Sci. (Med.), Corresponding Member of the Russian Academy of Natural History, Professor, Division of Introduction into Internal Diseases with a Therapy Course of the Pediatrics Department, Siberian State Medical University, Tomsk, Russian Federation.

Porovsky Yaroslav V., Dr. Sci. (Med.), Professor, Division of Introduction into Internal Diseases with a Therapy Course of the Pediatrics Department, Siberian State Medical University, Tomsk, Russian Federation.

Solovov Mikhail M., Dr. Sci. (Med.), Associate Professor, Advanced Surgery Division with a Cardiovascular Surgery Course, Siberian State Medical University, Tomsk, Russian Federation.

(✉) **Teteneva Anna V.**, e-mail: anna.dubodelova@mail.ru

Received 10.06.2020

Accepted 28.12.2020

THE 75TH ANNIVERSARY OF THE BIRTH OF VYACHESLAV NOVITSKY

Dedicated to the memory of one of the brightest leaders of Russian science – Vyacheslav V. Novitsky, a world-renowned scientist, a social, political, and cultural figure, a founder and editor-in-chief of the journal “Bulletin of Siberian Medicine”, and Academician of the Russian Academy of Sciences.



On January 7, 2021, Siberian State Medical University ((SSMU), Tomsk, the Russian Academy of Sciences, and the medical community suffered a great loss – in the 75th year of his age, a talented scientist and organizer and an outstanding representative of the scientific intelligentsia, Vyacheslav V. Novitsky, passed away.

Vyacheslav V. Novitsky – Doctor of Medical Sciences, Professor, Academician of the Russian Academy of Sciences, Honored Scientist of the Russian Federation, Honored Worker of Culture of the Russian Federation, Head of the (SSMU Pathophysiology Division (2000–2017), Rector of (SSMU (1997–2014). From 1990 to 2000 he concurrently held the position of a principal researcher at the Research Institute of Pharmacology, Tomsk National Research Medical Center (NRMC) of Siberian Branch of the Russian Academy of Sciences (now the Goldberg Research Institute of Pharmacology and Regenerative Medicine of Tomsk NRMC of the Russian Academy of Sciences).

Vyacheslav Novitsky was born on August 23, 1946 in a family of prominent doctors and teachers. His fa-

ther, Viktor Mikhailovich, was from the Polish nobility exiled to Siberia. After graduating from Tomsk Electromechanical Institute of Railway Engineers, the parents combined teaching work at Tomsk Polytechnic Institute (now – Tomsk Polytechnic University) and Tomsk Institute of Radioelectronics and Electronic Engineering (now – Tomsk State University of Control Systems and Radioelectronics; Viktor Novitsky was Vice-Rector for Academic Affairs there from 1965 to 1978). However, his grandmother, Elena Lazarevna, who belonged to the famous Shershevsky dynasty of medical professors, had a great influence on the upbringing of Vyacheslav Novitsky and the choice of his future profession.

After graduating from Tomsk Secondary School No. 6 with a silver medal, Vyacheslav Novitsky entered the Medical Department of Tomsk Medical Institute (TMI, since 1993 – Siberian State Medical University). After graduating with a degree in General Medicine (1969), having worked in a scientific club at the Pathophysiology Division under the guidance of Professor D.I. Goldberg, and having five or six serious scientific publications and



Student
Vyacheslav
Novitsky

numerous diplomas and certificates, Vyacheslav Novitsky decided to connect his life with the medical university and science. As a student, he became the head of the Pirogov Scientific Student Society at TMI (1967–1972).

At TMI, Vyacheslav Novitsky began to work as a junior researcher at the Central Research Laboratory and as an assistant at the Pathophysiology Division. He went through all the stages of formation as a scientist and teacher, showing enthusiasm and dedication. In 1972, under the supervision of professors D.I. Goldberg and E.D. Goldberg, he defended his candidate of science thesis “Reactions of the blood system with the introduction of 5-fluorouracil and ftorafur in experiment”, in 1986 – his doctoral thesis “Reactivity of the blood system in the early and late periods after the effects of anthracycline anticancer antibiotics” (scientific supervisor – corresponding member of the USSR Academy of Medical Sciences, Professor E.D. Goldberg). The academic title of Professor at the Pathophysiology Division was awarded to Vyacheslav Novitsky in 1989. In 1999, he was elected a Corresponding Member of the Russian Academy of Medical Sciences (RAMS) (specializing in pathophysiology), in 2005 – a Full Member (Academician) of RAMS (since 2014 – Academician of the Russian Academy of Sciences). In February 1988, he became the first Vice-Rector for Scientific Work elected on an alternative basis, in December 1997, he became the first elected Rector of SSMU.

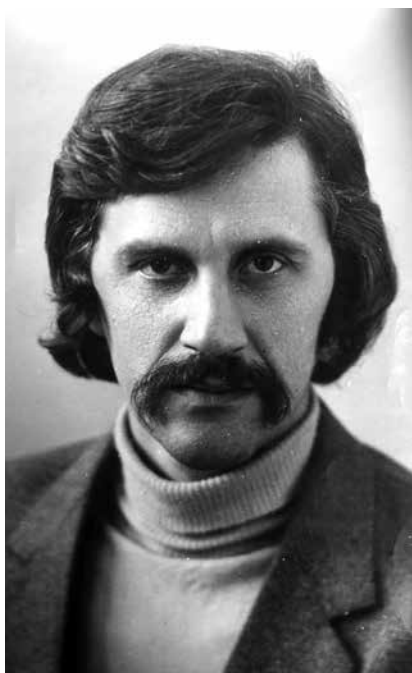
As the Rector of SSMU, Vyacheslav Novitsky considered his main contribution to the development of the university to be raising the university rating from 36th

to 3rd place among medical universities in the Russian Federation. He put into operation educational and laboratory, consultative, and treatment and diagnostic (now the Medical Center “Professor”) buildings of SSMU. The new building of SSMU clinics had been fully completed. Under his rectorship, a modern simulation center was created at SSMU, and the scientific and educational process was significantly modernized. SSMU became the first among medical universities in the Russian Federation to create an innovative infrastructure that implements the cycle of transferring scientific developments to the market – from idea to organization of production. In 2005, for the reforms implemented, Vyacheslav Novitsky was awarded the honorary badge “Rector of the Year”, and the university was awarded the prestigious award “For the European quality of education” by the Independent Public Council of the competition Gold Medal “European Quality”.

However, Vyacheslav Novitsky considered his main achievements as Vice-Rector and Rector of SSMU to be successful implementation of a unique program to preserve the historical heritage of the university, including a gallery of high-relief portraits of prominent scientists on the facade of SSMU clinics, publication and reprint of a two-volume biographical dictionary about SSMU professors, establishment of a unique museum of the history of SSMU divisional scientific schools, and an increase in the share of young teaching staff at the university due to young Doctors of Sciences. Vyacheslav Novitsky always emphasized the importance of adhering to the principle of continuity in science, education, and work of a doctor. He paid great attention to the upbringing and support of talented youth and improving the quality of medical education by introducing the latest achievements in biomedical science into the teaching process.

Vyacheslav Novitsky was a representative of one of the leading Russian scientific and medical schools – Tomsk School of Pathophysiologists, whose members included 6 Academicians and 2 Corresponding Members of the USSR Academy of Medical Sciences, RAMS, RAS, 8 Honored Scientists of the Russian Federation, 2 USSR State Prize laureates, 2 laureates of the Russian Federation Government Prize, 5 laureates of academic awards, more than 100 Doctors of Science, and 400 Candidates of Science.

Being a direct student and follower of the outstanding Russian pathophysiologists D.I. Goldberg and E.D. Goldberg and a successor of traditions of Tomsk classical university pathophysiology school, Vyacheslav Novitsky developed his own scientific direction, within which the issues of dysregulation pathology of hematoipoiesis under the effects of anticancer drugs and other pathogenic factors, as well as fundamental (including mo-



Associate
Professor
Vyacheslav
Novitsky

lecular) mechanisms of damage to red and white blood cells in mental disorders and socially sensitive somatic pathologies of infectious and non-infectious genesis continue to be investigated. The scientific school under the guidance of Vyacheslav Novitsky was 6 times awarded the title of “Leading Scientific School of the Russian Federation” in the competitions for scientific schools held by the Council for Grants awarded by the President of the Russian Federation (2003, 2006, 2008, 2012, 2014, 2016).

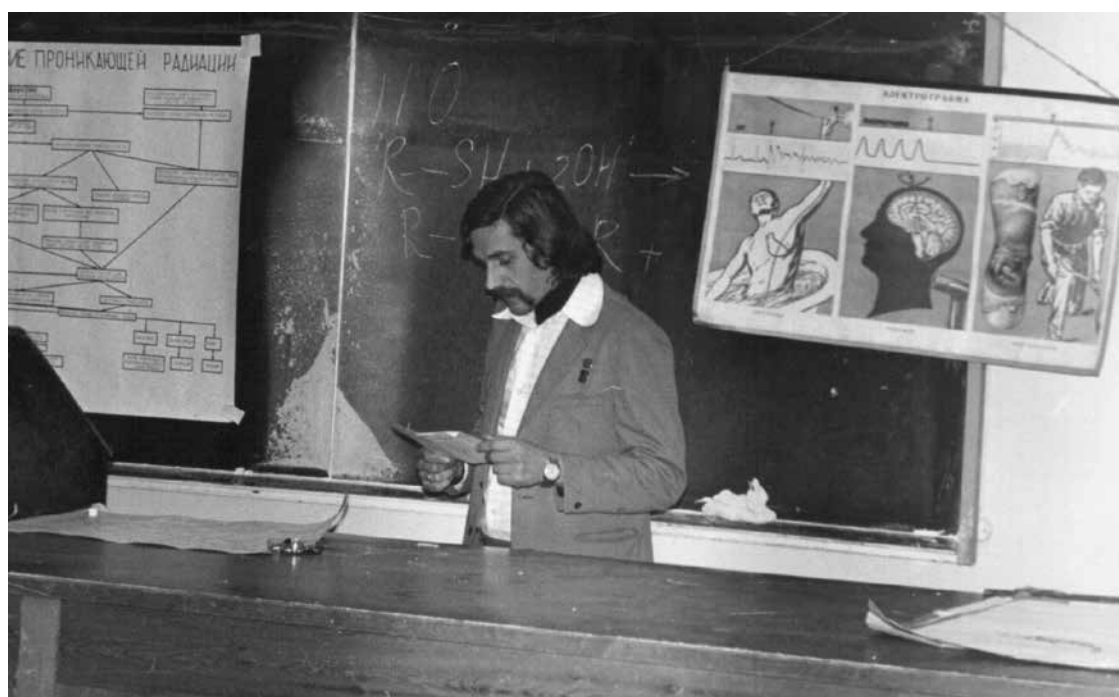
Vyacheslav Novitsky trained 45 Doctors and 108 Candidates of Science, most of whom defended their

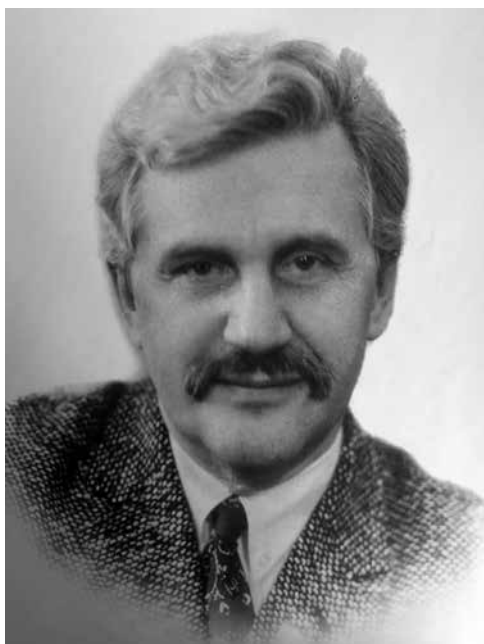
dissertations before the age of 35. In 2015–2016, Vyacheslav Novitsky was included in the TOP-100 of the most cited scientists in the Russian Federation in the field of medicine and healthcare. He was author of over 800 publications in the field of theoretical hematology, pathophysiology, cytology, and cell biology, including 44 monographs, 18 textbooks, 1 manual for doctors, 2 copyright certificates, and 17 Russian Federation patents for inventions. He was a creator of a unique atlas on electron microscopy of erythrocytes, as well as the author and editor of 5 editions of the textbook (1994, 2001, 2006, 2009, 2018) on pathological physiology for medical students (in the Russian and Kazakh languages), co-authors of which are renowned pathophysiologists, representatives of the oldest Moscow, Kazan, Tomsk, Kharkov, and Tbilisi scientific medical schools, and prominent specialists of the RAS institutions.

In 2010, for a series of works “Molecular foundations of blood cell pathology in socially sensitive diseases”, Vyacheslav Novitsky was awarded the Davydovsky prize of the Russian Academy of Medical Sciences in the field of medicine in general pathology (awarded once every 10 years).

Vyacheslav Novitsky is the brightest example of a combination of many outstanding talents – of a scientist, organizer, active social, political, and cultural figure. He was the founder, artistic director, and author of texts and scripts for performances of the popular in 1970–1980 creative association “KOMU” (recreation club for young scientists at Tomsk House of Scientists). In 1996, he was awarded the title “Honored Worker of Culture of the Russian Federation”.

Associate
Professor
Vyacheslav
Novitsky giving
a lecture in
pathophysiology





Vice-Rector Vyacheslav Novitsky

Vyacheslav Novitsky was the owner of numerous state, departmental, and public awards from Russia, Poland, Great Britain, Germany, France, and Belgium for his personal contribution to science, culture, humanism, and progressive politics. He was a Full Member of the Polish Academy of Medicine (since 1998), Full Member

of the Albert Schweitzer World Academy of Medicine (since 1999), Full Member of the International Higher Education Academy of Sciences (since 1997), Soros Professor (1997), and Professor honoris causa of philosophy (in humanities) of the Polish Academy of Medicine (2006).

For his contribution to the development of the Russian democracy, Vyacheslav Novitsky received gratitude and a personalized wristwatch as a memorable gift from President of the Russian Federation Boris Yeltsin (1996). He was deputy of the second (1997–2001), third (200–2006) and fourth (2006–2011) Legislative Duma of the Tomsk Region. In 2000–2006, Vyacheslav Novitsky was twice one of the most influential regional leaders in the Russian Federation. His professional achievements in the field of healthcare and a very outstanding contribution to strengthening of the Russian Federation earned him state awards, such as the Order of Honor (2005) and the Order of Merit for the Motherland, degree IV (2010). Vyacheslav Novitsky was an Honorary Citizen of the city of Tomsk (2006).

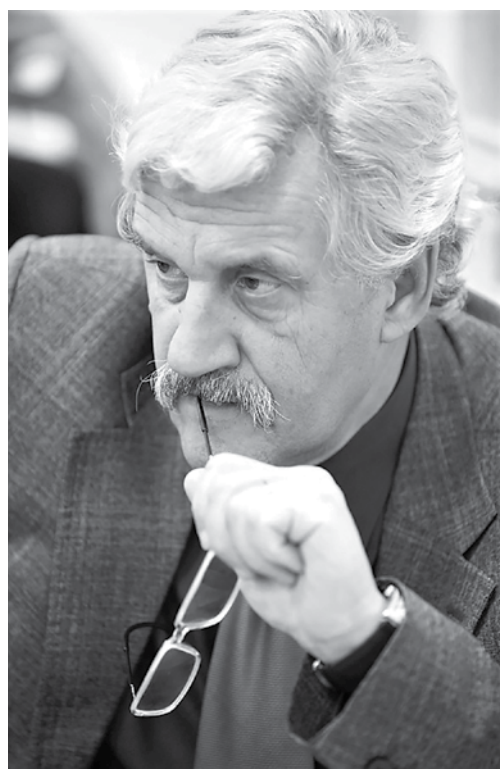
Vyacheslav Novitsky was Deputy Chairman of the Council of Rectors of Medical and Pharmaceutical Universities of the Russian Federation under the Ministry of Health of the Russian Federation (2009–2013), Member of the Expert Council of the Higher Attestation Commission under the Ministry of Education and Science of the Russian Federation (2011–2013), Member of the Scientific Coordination Council of the Federal Target Program under the Ministry of Education and Science of the Russian Federation (2011–2013), Member of the Presidium of SB RAMS (1998–2013), and Member of the Expert Council of the Russian Science Foundation (since 2013). He was also laureate of competitions in the Tomsk Region in the field of education, science, healthcare, and culture (1997, 2004, 2016), winner of the “Person of the Year” competition in the Tomsk Region in the “Scientist of the Year” nomination (2001), laureate of the All-Russian competition “Scientist of the Year” (2010), “Honorary Professor of Siberian State Medical University” (2016), Rector of the Year (All-Russian competitions of 2009, 2012, 2013), winner of the competition of the Governor of the Tomsk Region for professors (2017), chairman of the Dissertation Council D 208.096.01 at SSMU, and creator and editor-in-chief of the journal “Bulletin of Siberian Medicine”.

Everyone who was taught by Vyacheslav Novitsky and worked with him knew him and will remember him as a purposeful person of extraordinary spiritual strength achieving perfect results in any area of focus. He often called himself a “pathological optimist”. However, nothing came as easy as it might seem; his success was the result of great daily work. His work efficiency impressed



many people. They were also surprised by his huge love of life and a desire to create and be in demand, to increase his knowledge and share it with others. He did an incredible amount of good and left a good memory of himself...

The name of Vyacheslav Viktorovich Novitsky can be found on the first in the history of Tomsk avant-garde garden sculpture “The pregnant”, made by the Honored Artist of the Russian Federation N. Gnedykh and installed by SSMU Rector Vyacheslav Novitsky near the University Obstetric Clinic (2005). The memory of Vyacheslav Novitsky will forever live in his books – not only in the books on pathophysiology, but also in one of his last books – his “spontaneous essay” “On miscellaneous issues... Sincerely. Vyacheslav Novitsky” (2016). It became a bible for those interested in the history of Tomsk, Tomsk medicine, and Tomsk intellectuals.



Rector Vyacheslav Novitsky

Textbook
published in 1994



Textbook
published
in 2006



Textbook published in 2014



Textbook
published in 2018



Кафедра патофизиологии. 2008 г.



Модераторы форума
«Развитие Сибири». 2012 г.



Шествие со студентами. 2012 г.



Молодые доктора наук. 2012 г.



Выпускники лечфака

РЕКТОР НАВСЕГДА

ISSN PRINT: 1682-0363
ISSN ONLINE: 1819-3684
Бюллетень сибирской медицины
Bulletin of Siberian Medicine
bulletin
ENG | РУС

Бюллетень сибирской медицины
Расширенный поиск

ГЛАВНАЯ
О ЖУРНАЛЕ
МОЙ КАБИНЕТ
ПОИСК
СВЕЖИЙ НОМЕР
АРХИВ
НОВОСТИ
АРХИВ 2002-2011



Научно-практический рецензируемый журнал
Научно-практический журнал
общемедицинского профиля «Бюллетень
сибирской

медицины/Bulletin of Siberian Medicine» является регулярным рецензируемым печатным изданием, отражающим результаты научных исследований, ориентированных на разработку передовых медицинских технологий.

С целью объединения научной медицинской общественности, распространения актуальной информации и содействия профессиональному росту специалистов журнал публикует оригинальные научные статьи, представляющие результаты экспериментальных и клинических исследований, лекции, научные обзоры, отражающие результаты исследований в различных областях медицины. Приоритет для публикации предоставляется материалам по перспективным направлениям современной медицинской науки:

- молекулярная медицина,
- регенеративная медицина и биоинженерия,
- информационные технологии в биологии и медицине,
- инвазивные медицинские технологии,
- нейронауки и поведенческая медицина,
- фармакология и инновационная фармацевтика,
- ядерная медицина,
- трансляционная медицина.

Журнал выполняет широкий спектр функций, которые в целом дают представление об основных направлениях развития российской медицинской науки и ее достижениях, ее конкурентоспособности и степени интеграции в международное научное сообщество.

Научно-практический рецензируемый журнал «Бюллетень сибирской медицины / Bulletin of Siberian Medicine» издается Сибирским государственным медицинским университетом с 2001 г. при поддержке ТРОО «Академия доказательной доказательной медицины».

Главный редактор – член-корреспондент РАН О.И. Уразова.

Журнал зарегистрирован в Министерстве Российской Федерации по делам печати, телерадиовещания и средств массовых коммуникаций.

Свидетельство ПИ № 77-7366 от 26.03.2001 г.
ISSN 1682-0363

Журнал включен в Перечень периодических научных и научно-технических изданий, выпускаемых в РФ, в которых рекомендуется публикация основных результатов диссертаций на соискание ученой степени доктора и кандидата наук (Перечень ВАК, редакция 01.12.2015).

Индексация:

- РИНЦ (RSCI; Science Index)
- Киберленинка
- DIRECTORY OF OPEN ACCESS JOURNALS
- WoS (ESCI) с 2016 года
- Scopus с 2018 года.

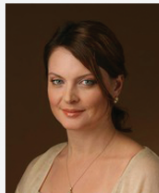
Продолжая традиции первых медицинских журналов, на страницах «Бюллетень сибирской медицины» публикуются

Отправить статью
Правила для авторов
Редакционная коллегия
Рецензирование
Этика публикаций

ПОПУЛЯРНЫЕ СТАТЬИ

Содержание эндотелиальной синтазы оксида азота в плазме после физических нагрузок различного характера

Том 16, № 1 (2017)



ГЛАВНЫЙ РЕДАКТОР
Уразова О.И.

ОБЛАКО ТЕГОВ

адаптация артериальная гипертензия
бронхиальная астма воспаление дети



ISSN 1682-0363 (print)
ISSN 1819-3684 (online)
БЮЛЛЕТЕНЬ СИБИРСКОЙ МЕДИЦИНЫ
2021. Т. 20. № 3. 1–236
Подписной индекс 10650