НАУЧНО-ПРАКТИЧЕСКИЙ ЖУРНАЛ

БСМ

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

БЮЛЛЕТЕНЬ СИБИРСКОЙ МЕДИЦИНЫ

BULLETIN OF SIBERIAN MEDICINE

BSM

Том 20
№ 4. 2021

В издательстве «ГЭОТАР-Медиа» в сентябре 2021 г. вышел в свет новый учебник «Иммунология» академика РАН Р.М. Хаитова

Рецензенты: академики РАН А.Л. Гинцбург, А.Г. Габибов, С.М. Деев, В.В. Зверев, А.Г. Румянцев, Х.П. Тахчиди, В.А. Ткачук, Н.Д. Ющук, профессор Р.И. Атауллаханов.

Учебник «Иммунология», 4-е издание (сентябрь 2021 г.), подготовил известный советский и российский ученый с мировым именем, иммунолог, д-р мед. наук, профессор, академик РАН Рахим Мусаевич Хаитов. Новое издание – это обновленная, переработанная и дополненная версия учебника, который за последние 15 лет стал одним из лучших учебников по иммунологии. Предназначен для студентов вузов медицинского и биологического профилей, врачей, научных сотрудников различных специальностей – иммунологии, аллергологии, микробиологии, вирусологии, инфектологии, вакцинологии и других смежных с ними науках. Учебник «Иммунология» Р.М. Хаитова – это один из лучших в нашей стране, один из самых востребованных и признанных учебников.

Достоинства учебника: хороший, доступный язык изложения; множество иллюстраций, облегчающих понимание и освоение нового материала; простота объяснения очень сложных живых систем и процессов; широкий, практически всеобъемлющий, охват областей иммунологии; современность, представление самых последних достижений в областях науки, которым учит этот учебник; достоверность и научное качество сведений, составляющих содержание учебника; практическая ориентированность научных знаний, всегда направленная на их полезное применение в интересах здоровья человека.

Современное научное знание об иммунитете – это огромный объем сведений. Учебник иммунологии должен помочь студентам медицинских или биологических вузов понять и запомнить самое важное и при этом не утопить обучающихся в изобилии научных фактов, способах их получения, значении этих знаний для нашей иммунной защиты от различных опасностей.

Не вызывает сомнений, что 4-е издание учебника «Иммунология» Р.М. Хаитова будет по достоинству оценено студентами и преподавателями медицинских и биологических вузов, а также исследователями в самых разных областях медицины и биологии.

BULLETIN OF SIBERIAN MEDICINE

Peer-reviewed scientific-practical journal
Issued quarterly

Volume 20, No. 4, 2021

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

FOUNDER AND PUBLISHER:
Siberian State Medical University, Ministry of Healthcare of the Russian Federation

Registered by the Ministry of Mass Media and Communications of the Russian Federation
Certificate of registration No. 77-7366
dated 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 doctoral and Candidate of Sciences theses

Bulletin of Siberian Medicine is indexed in:
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: Siberian State Medical University.
2, Moscow Trakt, Tomsk, 634050,
Russian Federation.
Editors: E.E. Stepanova, Yu.P. Gotfrid
Translator: M.E. Orlova.
Electronic makeup, cover design
L.D. Krivtsova

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

Signed to print on 27.12.2021
Format 60 × 84/8. Offset print.
Coated paper. Times font.
P.s. 28,5. C.p.s. 28,0.
500 copies. Order No 803.

The price – free.
Date of publication 29.12.2021.

БЮЛЛЕТЕНЬ СИБИРСКОЙ МЕДИЦИНЫ

Научно-практический журнал
Выходит 4 раза в год

Том 20, № 4, 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 27.12.2021 г.
Формат 60 × 84/8. Печать офсетная.
Бумага мелованная. Гарнитура «Times».
Печ. л. 28,5. Усл. печ. л. 28,0.
Тираж 500 экз. Заказ 803.

Цена – свободная.
Дата выхода в свет 29.12.2021.

СОДЕРЖАНИЕ

CONTENTS

УДК 612.179:616.12-008:577.152.6
https://doi.org/10.20538/1682-0363-2021-4-6-10

The role of protein kinase C and PI3-kinase in the mechanism of the cardioprotective effect of remote ischemic postconditioning

Mukhomedzyanov A.V., Naryzhnaya N.V., Maslov L.N.

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

ABSTRACT

Background. Acute myocardial infarction (AMI) with ST segment elevation is associated with high incidence of complications. Mortality from AMI is about 5%, which has not decreased in recent years. Revascularization provides recovery of coronary blood flow, but also contributes to the occurrence of reperfusion injury to the heart. Remote ischemic postconditioning (RIPostC) is a promising, non-invasive method that can effectively and safely reduce the infarct size.

The aim of the study was to investigate the role of protein kinase C and PI3-kinase in the development of the infarct-limiting effect of remote ischemic postconditioning.

Materials and methods. The study was performed on Wistar rats. Coronary artery occlusion (45 min) and reperfusion (2 h) were performed. The infarct size (IS) and the size of area at risk (AAR) were assessed. RIPostC was modeled by applying tourniquets to the hind limbs in the hip joint immediately after the restoration of coronary blood flow. All inhibitors were administered intravenously 10 min before reperfusion.

Results. In the control group, the IS / AAR ratio was 44%. RIPostC reduced the IS / AAR ratio by about 50%. Preliminary administration of the protein kinase C inhibitor chelerythrine and the PI3-kinase inhibitor wortmannin eliminated the cardioprotective effect of RIPostC.

Conclusion. The mechanism of the infarct-limiting effect of RIPostC is implemented through activation of protein kinase C and PI3-kinase.

Key words: heart, ischemia, reperfusion, remote ischemic postconditioning.

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 article was supported by the Russian Foundation for Basic Research Grant No. 21-515-53003. The section dedicated to PI3 was framed within the state assignment AAAA-A15-115120910024-0. The study was performed using the equipment of the Center for Collective Use “Medical Genomics”.

Conformity with the principles of ethics. The study was approved by the Ethics Committee at the Cardiology Research Institute, Tomsk National Research Medical Center (Protocol No. of 23.02.2020).

For citation: Mukhomedzyanov A.V., Naryzhnaya N.V., Maslov L.N. The role of protein kinase C and PI3-kinase in the mechanism of the cardioprotective effect of remote ischemic postconditioning. *Bulletin of Siberian Medicine.* 2021; 20 (4): 6–10. https://doi.org/10.20538/1682-0363-2021-4-6-10.

✉ *Mukhomedzyanov Aleksander V.,* e-mail: sasha_m91@mail.ru

Участие протеинкиназы С и PI3-киназы в механизме кардиопротекторного эффекта дистантного посткондиционирования

Мухомедзянов А.В., Нарыжная Н.В., Маслов Л.Н.

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

РЕЗЮМЕ

Введение. Острый инфаркт миокарда (ОИМ) с подъемом сегмента ST отличается высокой частотой осложнений. Смертность от ОИМ составляет около 5% и в последние годы не снижается. Реваскуляризация обеспечивает восстановление коронарного кровотока, но также способствует возникновению реперфузионных повреждений сердца. Дистантное посткондиционирование (ДПост) является многообещающим неинвазивным методом, способным эффективно и безопасно уменьшить размер инфаркта миокарда.

Цель – изучение участия протеинкиназы С и PI3-киназы в реализации инфаркт-лимитирующего эффекта ДПост.

Материалы и методы. Исследование выполнено на 48 самцах крыс линии Вистар. Осуществляли коронарооккклюзию (45 мин) и реперфузию (2 ч). Оценивали размер зоны некроза и зоны риска. Дистантное посткондиционирование моделировали путем наложения жгутов на задние конечности в области тазобедренного сустава сразу послу восстановления коронарного кровотока. Все ингибиторы вводили внутривенно за 10 мин до реперфузии.

Результаты. В контрольной группе отношение зона инфаркта/зона риска (ЗИ/ЗР) составило 44%. Дистантное посткондиционирование уменьшало соотношение ЗИ/ЗР в 1,5 раза. Предварительное введение ингибитора протеинкиназы С хелеритрина или ингибитора PI3-киназы вортманнина устраняло кардиопротекторный эффект ДПост.

Заключение. Механизм инфаркт-лимитирующего эффекта ДПост реализуется через активацию протеинкиназы С, PI3-киназы.

Ключевые слова: сердце, ишемия, реперфузия, дистантное посткондиционирование.

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

Источник финансирования. Статья подготовлена при поддержке гранта РФФИ № 21-515-53003. Раздел, посвященный PI3, оформлен в рамках государственных назначений АААА-А15-115120910024-0. Работа выполнена на оборудовании Центра коллективного пользования «Медицинская геномика».

Соответствие принципам этики. Исследование одобрено локальным этическим комитетом НИИ кардиологии Томского НИМЦ (протокол № 207 от 23.02.2020).

Для цитирования: Мухомедзянов А.В., Нарыжная Н.В., Маслов Л.Н. Участие протеинкиназы С и PI3-киназы в механизме кардиопротекторного эффекта дистантного посткондиционирования. *Бюллетень сибирской медицины.* 2021; 20 (4): 6–10. https://doi.org/10.20538/1682-0363-2021-4-6-10.

INTRODUCTION

The mortality rate for acute myocardial infarction (AMI) is about 5% and has not decreased in recent years[1, 2].Revascularization of an infarct-related coronary artery is an important therapeutic intervention for myocardial infarction [3]. However, recovery of coronary blood flow also has adverse consequences manifested through reperfusion injury of the heart affecting the final infarct size and further prognosis [4]. Currently, in clinical practice there are no highly effective drugs for preventing reperfusion injury of the heart [5]. Literature data suggest that remote ischemic postconditioning (RIPostC) is a promising,

non-invasive method to effectively and safely reduce the infarct size and reduce the risk of developing complications.

RIPostC was discovered in 2005 by a research group led by Prof. J. Vinten-Johansen [6]. RIPostC consists in an increase in myocardial tolerance to prolonged reperfusion after exposure to short-term ischemia-reperfusion of another organ at the time of cardiac reperfusion. It was found that RIPostC helps reduce the infarct size by 50% [7]. However, the molecular mechanisms underlying this effect remain poorly understood.

The aim of this study was to investigate the role of protein kinase C and PI3-kinase in the infarction-limiting effect of remote ischemic postconditioning.

MATERIALS AND METHODS

Male Wistar rats (n = 48), weighing 250–300 g, were used in in the study. All procedures related to keeping and using the animals were carried out in accordance with Directive 2010/63/EU of the European Parliament and of the Council adopted of 22 September, 2010 on the protection of animals used for scientific purposes. The study was approved by the Ethics Committee at the Cardiology Research Institute of Tomsk NRMC. All painful procedures were performed on anesthetized animals.

The rats were anesthetized with α-chloralose (60 mg / kg, intraperitoneally) and ventilated with a SAR-830/P ventilator via a tracheostomy tube. Heart rate was recorded using the Data Acquisition Unit MP35. The infarction-limiting effect of RIPostC and its intracellular mechanisms were studied using a model of 45-minute coronary occlusion and 120-minute reperfusion *in vivo* [8]. The quantitative assessment of the myocardium damage was determined by the ratio of the infarct size to the area at risk (IS / AAR) [8]. The area at risk is considered to be a part of the myocardium that was exposed to ischemia during coronary occlusion. RIPostC was modeled by applying tourniquets to the hind limbs in the hip joint, immediately after restoration of coronary blood flow. The time of ischemia and reperfusion for each phase was 3 cycles of 5 minutes.

The experiment used the following pharmacological agents: a protein kinase C inhibitor chelerythrine was injected at a dose of 0.3 mg / kg [9], a PI3-kinase inhibitor wortmannin was administered at a dose of 25 μg / kg [10]. The inhibitors were administered into the femoral vein 10 min before reperfusion (35 minutes after the onset of coronary occlusion). Chelerythrine and wortmannin were dissolved in 0.1 ml of DMSO and then diluted in 0.9 ml of 20% 2-hydroxypropyl-β-cyclodextrin. The animals of the control group were intravenously injected with a mixture of DMSO / 2-hydroxypropyl-β-cyclodextrin. The rats were removed from the experiment 2 hours after the onset of reperfusion by excision of the heart from the thoracic cavity for subsequent staining and determination of the IS / AAR ratio.

The data were statistically processed using the Statistica 13 software. The Mann – Whitney test was used to assess reliability of the results obtained. The data were presented as mean and standard deviation $M \pm SD$. The threshold significance level p was assumed to be 0.05.

RESULTS

After 45-minute coronary occlusion and 120-minute reperfusion, the IS / AAR index in the control group was 44%. The use of RIPostC helped reduce the IS / AAR ratio by 1.5 times (Figure). Therefore, RIPostC increases resistance of the heart to reperfusion injury. Further research was aimed at studying the signaling mechanism underlying the cardioprotective effect of RIPostC. Thus, preliminary administration (10 minutes before reperfusion and RIPostC) of the protein kinase C inhibitor chelerythrine eliminated the infarct-limiting effect of RIPostC. The use of the PI3-kinase inhibitor wortmannin showed a similar effect (Figure).

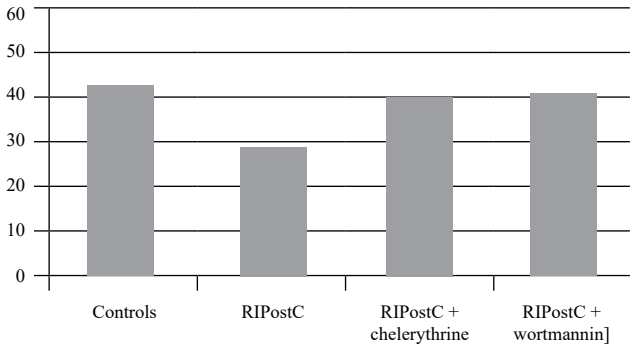


Figure. IS / AAR ratio: *p <0.05 compared to the control

No significant changes in the heart rate were found in the control group during the experiment. No significant changes in the heart rate were observed in the group in which RIPostC was simulated 45 minutes after coronary occlusion (Table). Therefore, the use of RIPostC does not affect the heart rate.

Table

Heart rate values in the model of coronary occlusion and reperfusion, *M* ± *SD*

Group	Observation period				
	Before coronary occlusion	10 minutes before reperfusion	Before reperfusion	30 minutes after reperfusion	2 hours after reperfusion
Control, *n* = 12	364.4 ± 3.5	362.3 ± 3.8	358.7 ± 2.9	353.4 ± 4.3	349.7 ± 5.2
RIPostC, *n* = 12	366.8 ± 3.1	364.8 ± 3.6	363.5 ± 3.2	357.6 ± 4.1	351.5 ± 4.9

DISCUSSION

Literature data suggest that RIPostC has a profound infarct-limiting effect [7]. The results obtained in our research confirmed these data. Literature data indicate that the cardioprotective effect of RIPostC is associated with the activation of protein kinase C, MEK-kinase, and PI3-kinase [7, 11, 12]. Based on these data, we suggested that these kinases can be involved in the mechanism of the infarct-limiting effect of RIPostC. Indeed, according to the results obtained, protein kinase C and PI3-kinase are involved in the mechanism of the cardioprotective effect of RIPostC, which indicated the similarity of the molecular mechanisms of preconditioning and RIPostC.

According to some data, the cardioprotective effect of RIPostC is a consequence of the appearance of a hydrophobic peptide with a molecular weight of 30 kDa in the blood of experimental animals; this substance is released into the blood from ischemic limbs [13]. The researchers suggest that this humoral factor differs from the known endogenous peptides with infarct-limiting effect (opioid peptides, bradykinin) and significantly exceeds them in molecular weight. Several studies showed that RIPostC has a neuroprotective effect [14]. Therefore, it can be asserted that a distinctive feature of the humoral factor is its ability to penetrate the blood – brain barrier. It can be assumed that the cardioprotective effect of RIPostC may be associated with central mechanisms as well. In turn, to date, there is no clear understanding of what molecular mechanisms underlie the effect of RIPostC.

CONCLUSION

The presented data indicate that remote ischemic postconditioning can increase the resistance of the heart to reperfusion injury. The infarct-limiting effect of RIPostC is implemented through the activation of protein kinase C and PI3-kinase.

REFERENCES

1. Vaidya S.R., Devarapally S.R., Arora S. Infarct related artery only versus complete revascularization in ST-segment elevation myocardial infarction and multi vessel disease: a meta-analysis. *Cardiovasc. Diagn. Ther*. 2017; 7 (1): 16–26. DOI: 10.21037/cdt.2016.08.06.
2. Zhou Y., Chen S., Zhu X., Gui J., Abusaada K. Prior beta blockers use is independently associated with increased in patient mortality in patients presenting with acute myocardial infarction. *Int. J. Cardiol.* 2017; 243: 81–85. DOI: 10.1016/j.ijcard.2017.03.004.
3. McCartney P.J., Berry C. Redefining successful primary PCI. *Eur. Heart J. Cardiovasc. Imaging*. 2019; 20 (2): 133–135. DOI: 10.1093/ehjci/jey159.
4. Ndrepepa G. Improving myocardial injury, infarct size, and myocardial salvage in the era of primary PCI for STEMI. *Coron. Artery Dis*. 2015; 26 (4): 341–355. DOI: 10.1097/mca.0000000000000220.
5. Maslov L.N., Barbarash O.L. Pharmacological Approaches to Limiting the Infarct Zone Size in Patients with Acute Myocardial Infarction: Analysis of Clinical Data. *Experimental and Clinical Pharmacology.* 2018; 81 (3): 75–82 (in Russ.). DOI: 10.30906/0869-2092-2018-81-3-34-41.
6. Kerendi F., Kin H., Halkos M.E., Jiang R., Zatta A.J., Zhao Z.Q., Guyton R.A., Vinten-Johansen J. Remote postconditioning. Brief renal ischemia and reperfusion applied before coronary artery reperfusion reduces myocardial infarct size via endogenous activation of adenosine receptors. *Basic Res. Cardiol*. 2005; 100 (5): 404–412. DOI: 10.1007/s00395-005-0539-2.
7. Heusch G. Molecular basis of cardioprotection: signal transduction in ischemic pre-, post-, and remote conditioning. *Circ. Res*. 2015; 116 (4): 674–699. DOI: 10.1161/circresaha.116.305348.
8. Maslov L.N., Naryzhnaya N.V., Tsibulnikov S.Yu., Kolar F., Zhang Y., Wang H., Gusakova A.M., Lishmanov Yu.B. Role of endogenous opioid peptides in the infarct size-limiting effect of adaptation to chronic continuous hypoxia. *Life Sci*. 2013; 93 (9–11): 373–379. DOI: 10.1016/j.lfs.2013.07.018.
9. Maslov L.N., Lishmanov Yu.B., Oeltgen P.R., Barzakh E.I., Krylatov A.V., Govindaswami M., Brown S.A. Activation of peripheral δ_2 opioid receptors increases cardiac tolerance to ischemia/reperfusion injury: Involvement of protein kinase C, NO-synthase, K_{ATP} channels and the autonomic nervous system. *Life Sci*. 2009; 84 (19–20): 657–663. DOI: 10.1016/j.lfs.2009.02.016.
10. Fettiplace M.R., Kowal K., Ripper R., Young A., Lis K., Rubinstein I., Bonini M., Minshall R., Weinberg G. Insulin signaling in bupivacaine-induced cardiac toxicity: sensitization during recovery and potentiation by lipid emulsion. *Anesthesiology*. 2016; 124 (2): 428–442. DOI: 10.1097/aln.0000000000000974.
11. Cohen M.V., Downey J.M. Signalling pathways and mechanisms of protection in pre- and postconditioning: historical

perspective and lessons for the future. *Br. J. Pharmacol*. 2015; 172 (8): 1913–3192. DOI: 10.1111/bph.12903.
12. Hausenloy D.J., Yellon D.M. Ischaemic conditioning and reperfusion injury. *Nat. Rev. Cardiol*. 2016; 13 (4): 193–209. DOI: 10.1038/nrcardio.2016.5.
13. Breivik L., Helgeland E., Aarnes E.K., Mrdalj J., Jonassen A.K. Remote postconditioning by humoral factors in effluent from ischemic preconditioned rat hearts is mediated via PI3K/Akt-dependent cell-survival signaling at reperfusion. *Basic Res. Cardiol*. 2011; 106 (1): 135–145. DOI: 10.1007/s00395-010-0133-0.
14. Liang D., He X.B., Wang Z., Li C., Gao B.Y., Wu J.F., Bai Y.L. Remote limb ischemic postconditioning promotes motor function recovery in a rat model of ischemic stroke via the up-regulation of endogenous tissue kallikrein. *CNS Neurosci. Ther*. 2018; 24 (6): 519–527. DOI: 10.1111/cns.12813.

Authors information

Mukhomedzyanov Aleksander V., Junior Researcher, Laboratory of Experimental Cardiology, Cardiology Research Institute, Tomsk NRMC, Tomsk, Russian Federation. ORCID 0000-0003-1808-556X.

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.

Maslov Leonid N., Dr. Sci. (Med.), Professor, Head of the Laboratory of Experimental Cardiology, Cardiology Research Institute, Tomsk NRMC, Tomsk, Russian Federation. ORCID 0000-0002-6020-1598.

(✉) **Mukhomedzyanov Aleksander V.,** e-mail: sasha_m91@mail.ru

Received 20.08.2020
Accepted 28.12.2020

УДК 618.15-085.37.017:618.15-006.52
https://doi.org/10.20538/1682-0363-2021-4-11-17

The effect of vaginal probiotic therapy on the outcome of papillomavirus infection

Oleynik V.V.[1], Kremleva E.A.[1, 2], Sgibnev A.V.[1, 2]

[1] *Orenburg State Medical University*
6, Sovetskaya Str., Orenburg, 460000, Russian Federation

[2] *Institute of Cellular and Intracellular Symbiosis, Ural Branch of the Russian Academy of Sciences*
11, Pionerskaya Str.,Orenburg, 460000, Russian Federation

ABSTRACT

Aim. To study the effect of vaginal probiotic therapy on the outcome of human papillomavirus (HPV) infection.

Materials and methods. The study included HPV-infected patients: 29 patients with normal vaginal flora and 146 patients with a deficiency of vaginal lactobacilli, of which 117 patients received vaginal probiotic therapy. In samples obtained before and after the therapy, the effect of the probiotic on the change in the ratio of living, apoptotic, and necrotic vaginal epithelial cells after preliminary exposure to oxidative stress was studied.

Results. It was found that probiotics reduce the number of infected epithelial cells that survived the oxidative damage and shift the balance of cell death forms towards apoptosis. Vaginal probiotic therapy in patients with a deficiency of lactobacilli increased the frequency of HPV elimination by 2.5 times and reduced the likelihood of treatment failure from 1.5 to 4 times, depending on the viral load. The probiotic therapy made the structure of HPV outcomes in Lactobacillus-deficient patients similar to that in patients with normal vaginal flora.

Conclusion. Vaginal probiotic therapy improves outcomes of HPV infection in patients with a deficiency of lactobacilli by reducing the number of survived infected cells and shifting the cell death pattern towards apoptosis.

Key words: apoptosis, epithelial cells, human papillomavirus, lactobacilli, Lactobacillus casei subsp. rhamnosus LCR35, probiotics, vagina.

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.

Conformity with the principles of ethics. All patients included in the research signed an informed consent to participate in the study. The study was approved by the Human Research Ethics Committee at Orenburg State Medical University (Protocol No. 149 of 05.10.2016).

For citation: Oleynik V.V., Kremleva E.A., Sgibnev A.V. The effect of vaginal probiotic therapy on the outcome of papillomavirus infection. *Bulletin of Siberian Medicine.* 2021; 20 (4): 11–17. https://doi.org/10.20538/1682-0363-2021-4-11-17.

✉ *Sgibnev Andrey V.,* e-mail: sgibnew72@yandex.ru

Влияние интравагинальной пробиотической терапии на течение папилломавирусной инфекции

Олейник В.В.[1], Кремлева Е.А.[1, 2], Сгибнев А.В.[1, 2]

[1] *Оренбургский государственный медицинский университет (ОрГМУ)*
Россия, 460000, г. Оренбург, ул. Советская, 6

[2] *Институт клеточного и внутриклеточного симбиоза (ИКВС), Уральское отделение Российской академии наук (УрО РАН)*
Россия, 460000, г. Оренбург, ул. Пионерская, 11

РЕЗЮМЕ

Цель – оценить влияние интравагинальной пробиотической терапии на течение папилломавирусной инфекции.

Материалы и методы. Исходы папилломавирусной инфекции оценивали у 29 пациенток с нормоценозом и 146 – с дефицитом нормофлоры, из них 117 женщин дополнительно интравагинально получали пробиотик. В пробах, полученных до и после терапии, изучали соотношение живых, апоптотических и некротических вагинальных эпителиоцитов после предварительной стимуляции апоптоза пероксидом водорода.

Результаты. Пробиотики снижают количество выживших инфицированных клеток и смещают баланс форм клеточной гибели в сторону апоптоза. Применение пробиотика у пациенток с дефицитом лактофлоры увеличило частоту элиминации вируса папилломы в 2,5 раза и повысило эффективность терапии в 1,5–4 раза в зависимости от вирусной нагрузки. Пробиотическая терапия у пациенток с дефицитом лактобацилл приблизила структуру исходов папилломавирусной инфекции к таковой группы пациенток с нормоценозом.

Заключение. Интравагинальная пробиотическая терапия улучшает исходы папилломавирусной инфекции у пациенток с дефицитом лактофлоры за счет снижения количества выживших инфицированных клеток и смещения структуры клеточной смерти в сторону апоптоза.

Ключевые слова: *Lactobacillus casei subsp. rhamnosus LCR35*, апоптоз, вирус папилломы человека, влагалище, лактобациллы, пробиотик, эпителиальные клетки.

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. Все пациенты подписали информированное согласие на участие в исследовании. Исследование одобрено локальным этическим комитетом ОрГМУ (протокол № 149 от 05.10.2016).

Для цитирования: Олейник В.В., Кремлева Е.А., Сгибнев А.В. Влияние интравагинальной пробиотической терапии на течение папилломавирусной инфекции. *Бюллетень сибирской медицины.* 2021; 20 (4): 11–17. https://doi.org/10.20538/1682-0363-2021-4-11-17.

INTRODUCTION

Human papillomavirus (HPV) is the most common sexually transmitted infection which contributes to the development of female genital tract cancer [1]. In most cases, papillomavirus is successfully eliminated, but in about 10% of infected women, HPV persists and can cause HPV-associated diseases of the genital tract, including cervical cancer [2]. It is not completely clear why HPV is eliminated in some cases but persists in other. Some of the likely reasons for this include individual differences in the state of the mucosal immune system [2, 3] and features of the microbial flora in the genital organs.

Sexually transmitted infections and bacterial vaginosis, in which the species composition of microorganisms in the vagina changes significantly, create a suitable environment for expansion of the virus [4–6]

and development of HPV-associated precancerous conditions and cancer [7]. The main manifestation of the imbalance in the vaginal microbial ecosystem is a pronounced deficiency of lactobacilli which protect the female reproductive tract from many pathogens, including viruses due to production of antimicrobial compounds and modulation of the mucosal immune system [2, 5].

Therefore, the concept of modifying vaginal bacterial communities using pre- and probiotics as a potential prospect for HPV infection management is widely discussed [8]. There are already successful examples of using probiotics for elimination of pathogens. In particular, the use of probiotics can prevent recurrence of vaginal yeast infection or bacterial vaginosis and increase the efficiency of metronidazole therapy for *Trichomonas vaginalis*; however, data on the possibility of using probiotics to control HPV infection are controversial.

The aim of the study was to investigate the effect of vaginal probiotic therapy on the progression of HPV infection. To achieve this aim, we studied how vaginal probiotic therapy affects the survival rate and the cell death pattern in primary cultures of vaginal epithelial cells, as well as the outcome of HPV infection.

MATERIALS AND METHODS

The study was conducted in accordance with the standards of good clinical practice and the principles of the Declaration of Helsinki, with the approval of the Human Research Ethics Committee at Orenburg State Medical University of the Ministry of Health of Russia (Protocol No. 149 of 05.10.2016). The study included 175 female patients with HPV infection who met the inclusion and exclusion criteria. The inclusion criteria were the following: high-oncogenic-risk HPV infection; age 18–40 years; a written informed consent to participate in the research and take pharmaceuticals. The exclusion criteria: smoking; immunodeficiency disorders; somatic symptom disorders at sub- and decompensated stages; menstrual dysfunction; pregnancy and lactation; taking hormonal contraceptives; individual allergic reaction to the drugs used in the study; therapy with antimicrobials or immunomodulators during the month preceding the study; a need for destructive methods of treating cervical pathology.

All the patients were treated with Allokin-Alpha® (State Research Institute of Highly Pure Biopreparations of the Federal Medical and Biological Agency) according to the following scheme: 1 mg subcutaneously, every other day, 6 injections per course, according to the manufacturer's instructions. In addition, patients with a deficiency of vaginal lactobacilli received a vaginal probiotic Lactoginal® (Laboratoires LYOCENTRE, SAS, France) containing *Lactobacillus casei subsp. rhamnosus LCR35* for 21 days according to the following scheme: 1 capsule 2 times a day for 7 days, then 1 capsule once a day for 14 days. The control group included 29 patients with a deficiency of vaginal lactobacilli who refused from vaginal probiotic therapy and received only immunomodulatory therapy. In accordance with the initial viral load, the patients were divided into three groups: 1) HPV $\leq 10^3$ cells, 2) HPV $10^{4–5}$ cells, 3) HPV $> 10^5$ cells, which were additionally divided into subgroups in accordance with the state of the vaginal flora: 1) normal flora; 2) flora deficient in lactobacilli.

Before treatment, all patients underwent a gynecological examination, cytology, and colposcopy, as well as an examination of the vaginal flora; the HPV copy number was measured, and the ratio of living, apoptotic, and necrotic vaginal epithelial cells was assessed. One month after the start of the treatment, the state of normal flora and the ratio of living, apoptotic, and necrotic vaginal epithelial cells were studied. Six months after the treatment, a gynecological examination, cytology, and colposcopy were carried out, and the HPV viral load was measured. All studies were performed on day 7–8 of the menstrual cycle. The progression of HPV infection was assessed by changes in the viral load and the cytology and colposcopy findings.

The presence and level of HPV were determined using a reagent kit for real-time polymerase chain reaction (PCR) manufactured by Lytech LLC (Russian Federation) on the MiniOpticon detection system (Bio-Rad, USA). The state of normal flora was assessed using the Nugent and Hay / Ison criteria and the number of bacteria of the genus Lactobacillus, determined by plating bacteria on MRS agar and then culturing in an atmosphere of 5% CO_2 for 48 hours. The belonging of microorganisms to the genus Lactobacillus was confirmed by real-time PCR with genus-specific primers [9].

The primary culture of stratified squamous epithelial cells was obtained by scraping the vaginal portion of the cervix after removing exfoliated cells with sterile normal saline. The cultures were transported in thermal containers in 1 ml of Hanks' Balanced Salt Solution (HBSS). To eliminate the accompanying microbial flora, the epithelial cells were washed once with a tenfold volume of phosphate-buffered saline.

The state of the epithelial cells was assessed after preliminary stimulation of apoptosis by triple exposure to H_2O_2 (50 μM) with an interval of 1 hour [10]. The ratio of apoptotic, living, and necrotic cells was determined by fluorescence microscopy using a kit containing Annexin V–Fluorescein with Propidium Iodide (BioVision, USA) according to the manufacturer's instructions.

Data are presented as absolute and relative (abs. (%)) incidence (outcomes of infection) and mean and standard deviation (state of epithelial cells). GraphPad Prism 6.0 software was used for statistical analysis and normal distribution testing. Normality of data distribution was tested using the Kolmogorov – Smirnov test. To assess the significance of differences between the incidence of different outcomes, the Fisher's exact test was used. The Mann – Whitney test was used for the ratio of apoptotic, living, and necrotic cells. The threshold for the statistical significance was set as a two-tailed *p*-value of 0.05.

RESULTS

Depending on the results of determining the viral load and the state of the vaginal flora, the patients were divided into the following groups: 29 patients with a normal number of lactobacilli and 29 patients with a deficiency of lactobacilli, who refused from probiotic therapy and were treated with immunomodulators, as well as 117 patients with a deficiency of vaginal lactobacilli receiving both immunomodulatory and probiotic therapy.

Table

Characteristics of patients according to the state of normal flora, HPV viral load, and the type of therapy

Viral load, DNA copies / 10^5 cells	Number of patients			Total
	with a deficiency of lactobacilli		with a normal number of lactobacilli	
	treated without probiotics	treated with probiotics		
$\leq 10^3$	47		13 (21.67%)	60
	9 (15.00%)	38 (63.33%)		
10^{4-5}	52		9 (14.75%)	61
	11 (18.03%)	41 (67.21%)		
$>10^5$	47		38 (70.37%)	54
	9 (16.67%)			

No significant differences in the basic demographic and anthropometric parameters between the groups were identified. By the end of the study, 6 women had dropped out: 4 due to failure to appear for the final follow-up examination and 2 (HPV> 10^5) due to indications for destructive therapy. Therefore, data on the structure of HPV outcomes will be presented for 141 patients in the treatment group and 28 patients in the control group.

Effect of probiotic therapy on the ratio of living, apoptotic, and necrotic vaginal epithelial cells

In samples from the patients with lactobacilli deficiency receiving probiotic therapy, the proportion of living infected cells remained practically unchanged, while the proportion of apoptotic cells increased, and the proportion of necrotic cells decreased (Fig.1). These processes were more pronounced in patients with high viral load.

In samples obtained from the patients with lactobacilli deficiency who refused from probiotic therapy (except for those who had high viral load), an increase in the survival rate of infected cells was observed (Fig.1). Reduction of the number of dead cells took place due to a decrease in necrosis, while the relative proportion of apoptotic cell death increased (Fig. 1). In the group of patients with high viral load, the changes were minimal and did not have statistical significance.

Therefore, inclusion of probiotics in the combination therapy for HPV infection prevented an increase in the proportion of survived infected cells and shifted the balance of cell death forms towards apoptosis.

Effect of vaginal probiotic therapy on outcomes of HPV infection

The results of a final follow-up examination (6 months after the beginning of the observation) showed that the state of the normal flora in the vagina affects the outcomes of HPV infection. Therefore, the frequency of complete HPV elimination in patients with normal flora was 2–2.5 times higher than in patients with deficiency of lactobacilli who did not receive probiotic therapy (Fig. 2). Vaginal probiotic therapy in patients with deficiency of lactobacilli caused an increase in the frequency of HPV elimination by 2.5 times and reduced the likelihood of therapy failure from 1.5 to 4 times, depending on the viral load (Fig. 2).

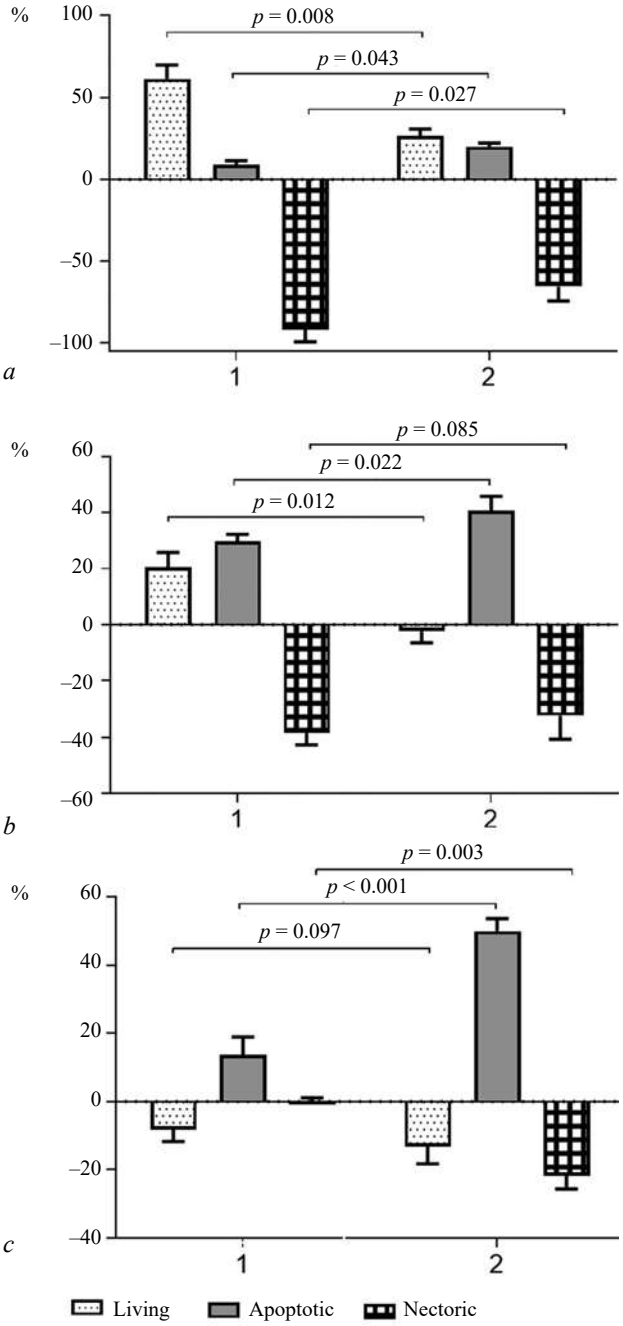


Fig. 1. Change in the ratio of living, apoptotic, and necrotic vaginal epithelial cells under the effect of therapy: Y-axis – compared with the baseline level (before therapy), %; X-axis – type of therapy: without a probiotic (1), with a probiotic (2). Baseline viral load, DNA copies / 10^5 epithelial cells: $\leq 10^3$ (a*),* $10^{4–5}$ (*b),* $>10^5$ *(c)*

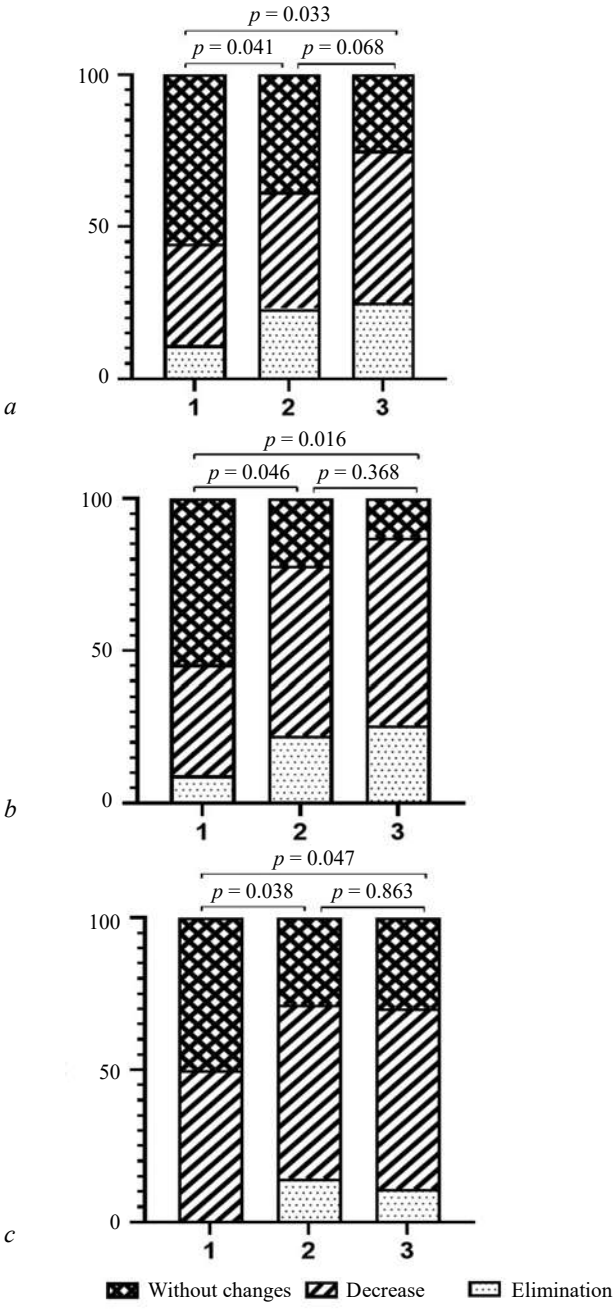


Fig. 2. Structure of HPV infection outcomes. Y-axis – proportion of outcomes, %; X-axis – the state of normal flora and the type of therapy: lactobacilli deficiency, without a probiotic (1), normal flora, without a probiotic (2), deficiency of lactobacilli, with a probiotic (3). Baseline viral load, DNA copies / 10^5 epithelial cells: $\leq 10^3$ (*a),* $10^{4–5}$ (*b),*; $>10^5$ (*c*)

The structure of HPV infection outcomes in Lactobacillus-deficient patients receiving probiotic therapy was similar to that in the group of patients with normal flora, and sometimes even exceeded it. Thus, inclusion of vaginal probiotic therapy in treatment of HPV-associated diseases improves their outcomes.

DISCUSSION

Therefore, the use of probiotics in Lactobacillus-deficient HPV-infected patients increases the likelihood of virus elimination. This effect can be determined by several mechanisms. Firstly, the stimulating effect of probiotics on synthesis of host defense fac-

tors [11] and an increase in their biological activity is known [12]. Secondly, metabolites of probiotic strains have a direct antiviral effect [13]. Thirdly, lactobacilli are able to selectively suppress cancer epithelial cells by increasing apoptosis without affecting healthy epithelial cells [14]. Our study showed that probiotic therapy also reduced the survival rate of infected cells by increasing apoptosis, while the proportion of other cell death forms decreased. At the same time, the shift in the balance of cell death forms towards apoptosis was most pronounced in samples obtained from patients with high viral load. Apparently, the shift in the cell death pattern from necrosis to apoptosis is also important for the elimination of the virus. It is known that necrosis, unlike apoptosis, is accompanied by the release of inflammatory mediators [15], and this adversely alters the environment of lactobacilli [16] and induces carcinogenesis [17].

We found that the effect of a probiotic containing the *LCR35* strain on HPV elimination is similar to the effect of normal flora and even slightly exceeds it. Perhaps this is determined by a higher concentration of lactobacilli during vaginal use of the probiotic or individual features of the probiotic strain *LCR35*. The first hypothesis is supported by the data that an oral probiotic does not affect HPV outcomes [18], since it cannot provide the required level of lactobacilli in the vagina. The second hypothesis is supported by data on the ability of the probiotic strain *LCR35* to potentiate the effect of antimicrobial drugs and significantly increase the likelihood of pathogen elimination [19]. In addition, a direct inhibitory effect of surfactants and lactate produced by the probiotic strain on reproduction of the papilloma virus is possible [11, 20].

CONCLUSION

Therefore, vaginal probiotic therapy improves the outcomes of HPV infection by reducing the number of survived infected cells and shifting the balance of cell death forms towards apoptosis.

REFERENCES

1. Drolet M., Bénard É., Boily M.C., Ali H., Baandrup L., Bauer H., Beddows S., Brisson J., Brotherton J.M.L., Cummings T., Donovan B., Fairley C.K., Flagg E.W., Johnson A.M., Kahn J.A., Kavanagh K., Kjaer S.K., Kliewer E.V., Lemieux-Mellouki P., Markowitz L., Mboup A., Mesher D., Niccolai L., Oliphant J., Pollock K.J., Soldan K., Sonnenberg P., Tanton C., Brisson M. Population-level impact and herd effects following human papillomavirus vaccination programmes: a systematic review and meta-analysis. *The Lancet Infectious Diseases*. 2015; 15 (5): 565–580. DOI: 10.1016/S1473-3099(14)71073-4.
2. Murall C.L., Rahmoun M., Selinger C., Baldellou M., Berna C., Bonneau M., Bernat C., Bonneau M., Boué V., Buisson M., Christophe G., D'Auria G., Taroni F.D., Foulongne V., Froissart R., Graf C., Grasset S., Groc S., Hirtz C., Jaussent A., Lajoie J., Lorcy F., Picot E., Picot M.-C., Ravel J., Reynes J., Rousset T., Seddiki A., Teirlinck M., Tribout V., Tuaillon E., Waterboer T., Jacobs N., Bravo I.G., Segondy M., Boulle N., Alizon S. Natural history, dynamics, and ecology of human papillomaviruses in genital infections of young women: protocol of the PAPCLEAR cohort study. *BMJ Open*. 2019; 9 (6): e025129. DOI: 10.1136/bmjopen-2018-025129.
3. Huh W.K. Human papillomavirus infection: a concise review of natural history. *Obstetrics & Gynecology*. 2009; 114 (1): 139–143. DOI: 10.1097/AOG.0b013e3181ab6878.
4. Da Silva C.S., Adad S.J., de Souza M.A.H., Barcelos A.C.M., Terra A.P.S., Murta E.F.C. Increased frequency of bacterial vaginosis and *Chlamydia trachomatis* in pregnant women with human papillomavirus infection. *Gynecologic and Obstetric Investigation*. 2004; 58 (4): 189–193. DOI: 10.1159/000079822.
5. Kero K., Rautava J., Syrjänen K., Grenman S., Syrjänen S. Association of asymptomatic bacterial vaginosis with persistence of female genital human papillomavirus infection. *European Journal of Clinical Microbiology & Infectious Diseases*. 2017; 36 (11): 2215–2219. DOI: 10.1007/s10096-017-3048-y.
6. Gillet E., Meys J.F., Verstraelen H., Bosire C., De Sutter P., Temmerman M., Broeck D.V. Bacterial vaginosis is associated with uterine cervical human papillomavirus infection: a meta-analysis. *BMC Infectious Diseases*. 2011; 11 (1): 10. DOI: 10.1186/1471-2334-11-10.
7. Norenhag J., Du J., Olovsson M., Verstraelen H., Engstrand L., Brusselaers N. The vaginal microbiota, human papillomavirus and cervical dysplasia: a systematic review and network meta-analysis. *BJOG: an International Journal of Obstetrics and Gynaecology*. 2019. DOI: 10.1111/1471-0528.
8. Mitra A., MacIntyre D.A., Marchesi J.R., Lee Y.S., Bennett P.R., Kyrgiou M. The vaginal microbiota, human papillomavirus infection and cervical intraepithelial neoplasia: what do we know and where are we going next? *Microbiome.* 2016; 4 (1): 58. DOI: 10.1186/s40168-016-0203-0.
9. Balashov S.V., Mordechai E., Adelson M.E., Sobel J.D., Gygax S.E. Multiplex quantitative polymerase chain reaction assay for the identification and quantitation of major vaginal lactobacilli. *Diagnostic Microbiology and Infectious Disease*. 2014; 78 (4): 321–327. DOI: 10.1016/j.diagmicrobio.2013.08.004.
10. Pletyushkina O.Yu., Fetisova E.K., Lyamzaev K.G., Ivanova O.Yu., Domnina L.V., Vysokikh M.Yu., Pustovidko A.V., Alekseevsky A.V., Alekseevsky D.A., Vasilyev Yu.M., Merphy M.P., Chernyak B.V., Skulachev V.P. Hydrogen peroxide produced inside mitochondria takes part in cell-to-cell transmission of the apoptotic signal. *Biochemistry.* 2006; 71 (1): 75– 84 (in Russ.).
11. Arena M.P., Capozzi V., Russo P., Drider D., Spano G., Fiocco, D. Immunobiosis and probiosis: antimicrobial activity of lactic acid bacteria with a focus on their antiviral and antifungal properties. *Applied Microbiology and Biotechnology*. 2018; 102 (23): 9949–9958. DOI: 10.1007/s00253-018-9403-9.

12. Sgibnev A.V., Kremleva E.A. Modification of enzymatic and antimicrobial activity of lysosomes by metabolites of vaginal lactobacilli. *Journal of Microbiology, Epidemiology, and Immunobiology*. 2018; 4: 21–27 (in Russ.). DOI: 10.36233/0372-9311- 2018-4-21-27.
13. Cadieux P., Burton J., Gardiner G., Braunstein I., Bruce A.W., Kang C.Y., Reid G. Lactobacillus strains and vaginal ecology. *JAMA*. 2002; 287 (15): 1940–1941. DOI: 10.1001/jama.287.15.1940.
14. Motevaseli E., Shirzad M., Akrami S.M., Mousavi A.S., Mirsalehian A., Modarressi M.H. Normal and tumour cervical cells respond differently to vaginal lactobacilli, independent of pH and lactate. *J. Med. Microbiol*. 2013; 62 (7): 1065–1072. DOI: 10.1099/jmm.0.057521-0.
15. Bordignon V., Di Domenico E., Trento E., D'Agosto G., Cavallo I., Pontone M., Pimpinelli F., Mariani L., Ensoli F. How human papillomavirus replication and immune evasion strategies take advantage of the host DNA damage repair machinery. *Viruses*. 2017; 9 (12): 390. DOI: 10.3390/v9120390.
16. Kremleva E.A., Sgibnev A.V. Proinflammatory cytokines as regulators of vaginal microbiota. *Bulletin of Experimental Biology and Medicine*. 2016; 162 (1): 75–78. DOI: 10.1007/s10517-016-3549-1.
17. Mangino G., Chiantore M.V., Iuliano M., Fiorucci G., Romeo G. Inflammatory microenvironment and human papillomavirus-induced carcinogenesis. *Cytokine & Growth Factor Reviews.* 2016; 30: 103–111. DOI: 10.1016/j.cytogfr.2016.03.007.
18. Ou Y.C., Fu H.C., Tseng C.W., Wu C.H., Tsai C.C., Lin H. The influence of probiotics on genital high-risk human papilloma virus clearance and quality of cervical smear: a randomized placebo-controlled trial. *BMC Womens Health*. 2019; 19 (1): 103. DOI: 10.1186/s12905-019-0798-y.
19. Sgibnev A.V., Kremleva E.A., Shchetinina Yu.S., Cherkasova Yu. I. Combined sue of antimicrobials and probiotics as a way to enhance the efficacy of therapy for genital infections. *Obstetrics and Gynecology*. 2018; 4: 113–118 (in Russ.). DOI: 10.18565/aig.2018.4.113-118.
20. Gao Y., Liu W., Wang W., Zhang X., Zhao X. The inhibitory effects and mechanisms of 3, 6-O-sulfated chitosan against human papillomavirus infection. *Carbohydrate Polymers*. 2018; 198: 329–338. DOI: 10.1016/j.carbpol.2018.06.096.

Authors information

Oleynik Victoria V., Assistant, Department of Obstetrics and Gynecology, Orenburg State Medical University, Orenburg, Russian Federation. ORCID 0000-0003-4774-558X.

Kremleva Elena A., Dr. Sci. (Med.), Principal Researcher, Laboratory for Studying the Mechanisms of Human Microbiocenosis Formation; Institute of Cellular and Intracellular Symbiosis, Ural Branch of RAS; Professor, Department of Obstetrics and Gynecology, Orenburg State Medical University, Orenburg, Russian Federation. ORCID 0000-0003-1916-784X.

Sgibnev Andrey V., Dr. Sci. (Biology), Associate Professor, Head of the Laboratory for Studying the Mechanisms of Human Microbiocenosis Formation; Institute of Cellular and Intracellular Symbiosis, Ural Branch of RAS; Professor, Department of Chemistry, Orenburg State Medical University, Orenburg, Russian Federation. ORCID 0000-0003-1866-1678.

(✉) **Sgibnev Andrey V.,** e-mail: sgibnew72@yandex.ru

Received 13.08.2020
Accepted 28.12.2020

УДК 616.2-002.182-07
https://doi.org/10.20538/1682-0363-2021-4-18-24

Clinical and morphological phenotypes in intrathoracic sarcoidosis

Palchikova I.A.[1], Denisova O.A.[2], Chernyavskaya G.M.[2], Purlik I.L.[2], Kalacheva T.P.[2], Naumov A.O.[2], Soloviev M.M.[2]

[1] *Tomsk Regional Clinical Hospital (TRCH)*
96, I.Chernykh Str., Tomsk, 634063, Russian Federation

[2] *Siberian State Medical University (SSMU)*
2, Moscow Trakt, Tomsk, 634050, Russian Federation

ABSTRACT

Aim. To study clinical and morphological phenotypes in different variants of the course of intrathoracic sarcoidosis and isolate new phenotypes.

Materials and methods. The study included 121 patients with intrathoracic sarcoidosis aged 21–66 years (50.4% were men, 49.6% were women, the average age at the time of the disease onset was 38 years) over the period 2007–2019. During the examination, patients' complaints were studied thoroughly, and the diagnosis was histologically verified in all cases. During an extended histological examination, the quantitative and qualitative composition of biopsy specimens was investigated. The number of granulomas in the field of vision and the content of giant cells, macrophages, lymphocytes, neutrophils, and eosinophils in them were studied. Qualitative parameters were assessed for the presence of hyalinosis, Schaumann bodies, necrosis, stamping, calcification, fibrosis, and vasculitis. All patients were retrospectively divided into two clinical groups depending on the outcomes of the disease: group 1 included patients with a favorable course of sarcoidosis, proceeding without relapses and signs of progression; group 2 encompassed patients with an unfavorable course of the disease with relapses and progression, requiring long-term administration of systemic glucocorticoids.

Results. The analysis showed that among all general clinical manifestations, only the presence of dyspnea, skin manifestations, and weight loss occurred significantly more often in the patients with an unfavorable course of intrathoracic sarcoidosis ($p = 0.04$; 0.02; and 0.01, respectively). Among morphological parameters, a large number of macrophages was significantly more frequent in the biopsy specimens in this group of patients ($p < 0.01$).

Key words: sarcoidosis, morphology, phenotypes of intrathoracic sarcoidosis.

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. All patients signed an informed consent to participate in the study. The study was approved by the local Ethics Committee at SSMU (Protocol No. 5045 of 28.11.2005).

For citation: Palchikova I.A., Denisova O.A., Chernyavskaya G.M., Purlik I.L., Kalacheva T.P., Naumov A.O., Soloviev M.M. Clinical and morphological phenotypes in intrathoracic sarcoidosis. *Bulletin of Siberian Medicine.* 2021; 20 (4): 18–24. https://doi.org/10.20538/1682-0363-2021-4-18-24.

✉ *Kalacheva Tatiana P.,* e-mail: tatyana-kalachyova@yandex.ru

Клинико-морфологические фенотипы при внутригрудном саркоидозе

Пальчикова И.А.[1], Денисова О.А.[2], Чернявская Г.М.[2], Пурлик И.Л.[2], Калачева Т.П.[2], Наумов А.О.[2], Соловьев М.М.[2]

[1] *Томская областная клиническая больница (ТОКБ)*
Россия, 634063, г. Томск, ул. И. Черных, 96

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

РЕЗЮМЕ

Цель – изучить клинические и морфологические параллели при разных вариантах течения саркоидоза органов дыхания с выделением новых фенотипов.

Материалы и методы. В исследование включен 121 пациент с саркоидозом органов дыхания в возрасте 21–66 лет (50,4% мужчин и 49,6% женщин, средний возраст обследованных на момент дебюта заболевания составил 38 лет) в период наблюдения 2007–2019 гг. В ходе обследования детально изучались жалобы пациентов, диагноз подтвержден гистологически во всех случаях. При расширенном гистологическом исследовании изучался количественный и качественный состав биоптата. Исследовалось количество гранулем в полях зрения, а также содержание в них таких показателей, как гигантские клетки, макрофаги, лимфоциты, нейтрофилы и эозинофилы. Оценивались качественные параметры на наличие гиалиноза, телец Шауманна, некроза, штампованности, кальциноза, фиброза и васкулита. Все пациенты ретроспективно были разделены на две клинические группы в зависимости от исходов заболевания. В первую группу вошли пациенты с благоприятным течением саркоидоза, протекающим без рецидивов и признаков прогрессирования; во вторую – с неблагоприятным течением заболевания, с рецидивами и прогрессированием, потребовавшие курсового и длительного назначения системных глюкокортикостероидов.

Результаты. Проведенный анализ показал, что среди всех общих клинических проявлений только наличие одышки, кожные проявления и потеря веса встречались достоверно чаще у пациентов с неблагоприятным течением внутригрудного саркоидоза ($p = 0,04$; 0,02 и 0,01 соответственно). Из морфологических параметров у этой группы пациентов в биоптатах значимо чаще встречалась бóльшая численность макрофагов $p < 0,01$).

Заключение. Предикторами неблагоприятного течения саркоидоза органов дыхания являются повышенная численность макрофагов в биоптате и более значимая потеря веса пациента.

Ключевые слова: саркоидоз, морфология, фенотипы внутригрудного саркоидоза.

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. Все пациенты подписали информированное согласие на участие в исследовании. Исследование одобрено локальным этическим комитетом СибГМУ (протокол № 5045 от 28.11.2005).

Для цитирования: Пальчикова И.А., Денисова О.А., Чернявская Г.М., Пурлик И.Л., Калачева Т.П., Наумов А.О., Соловьев М.М. Клинико-морфологические фенотипы при внутригрудном саркоидозе. *Бюллетень сибирской медицины.* 2021; 20 (4): 18–24. https://doi.org/10.20538/1682-0363-2021-4-18-24.

INTRODUCTION

Sarcoidosis is a granulomatous disease with characteristic granulomas in the lungs, lymph nodes, and other organs. It is known that the gold standard for making the diagnosis of sarcoidosis is biopsy taken from the affected lesion [1–3]. Granulomas are mainly composed of epithelioid cells, lymphocytes, and giant cells. Alveolar macrophages, neutrophils, and eosinophils are also involved in the formation of granulomas. In the cytoplasm of giant cells, cholesterol-containing crystalloid inclusions, asteroid bodies, and calcified Schaumann bodies are often found [3, 4].

Immunopathological processes occurring in the patient's body determine not only the clinical course, but also the prognosis of the disease. When assessing the course of sarcoidosis, an integrated approach is required, taking into account the clinical and morphological manifestations of the disease. Sarcoidosis is an extremely heterogeneous disease with an unpredictable clinical course [5].

Intensive studies on sarcoidosis in recent years have led to doubts about the correctness of criteria for assessing the course of the disease. Opinions have been increasingly expressed that, when assessing the course of sarcoidosis, it is necessary to abandon the generally accepted radiographic staging system and take into account a set of parameters [6]. In this regard, in recent years, much attention has been paid to isolating phenotypes in sarcoidosis. In the literature, there are few works on assessment of morphological parameters for predicting the course of sarcoidosis [6, 7]. Basically, the search for prognostic markers of sarcoidosis is based on laboratory and instrumental data. A number of scientists have investigated bronchoalveolar lavage and its cellular composition to determine the activity of intrathoracic sarcoidosis [8–10]. Other researchers have studied various parameters of blood serum [11–13].

Current research is focused on the study of histocompatibility complex genes. Since the accurate diagnosis of sarcoidosis is based on the results of a histological examination, it is necessary to look for reliable morphological markers that would reflect a relationship with the clinical manifestations of the disease, and, possibly, isolation of any new phenotypes. It is quite likely that for each clinical variant of the disease, there is a combination of morphological parameters which will make it possible to distinguish groups of patients with an unfavorable and recurrent course of intrathoracic sarcoidosis. In the future, it will allow to predict the course and outcome of the disease, as well as to prescribe therapy at an earlier stage with ineffective follow-up, since clinically silent pulmonary sarcoidosis can have fatal consequences. Therefore, a more detailed study of the morphometric parameters of granulomas is necessary, which will make it possible to predict the course of sarcoidosis and distinguish various clinical and morphological phenotypes of the disease.

The aim of the study was to investigate clinical and morphological characteristics in different variants of the course of intrathoracic sarcoidosis and isolate new phenotypes of the disease.

MATERIALS AND METHODS

The study included 121 patients with intrathoracic sarcoidosis aged 21–66 years (50.4% were men, 49.6% were women, the average age of the patients at the time of the disease onset was 38 years). The patients were examined at the Department of Pulmonology and Consultative and Diagnostic Clinic of Tomsk Regional Clinical Hospital in 2007–2019. A written informed consent was obtained from all patients who took part in the study. Visits to the sarcoidologist took place according to the Federal Clinical Guidelines [14]. All patients underwent a full range of examinations, including analysis of medical history and clinical and epidemiological data, as well as instrumental and laboratory methods.

During the examination, patients' complaints were studied thoroughly. In 100% of cases, we performed videothoracoscopy with targeted biopsy from the affected lesion in the lungs and from the intrathoracic lymph nodes with subsequent pathomorphological study of the specimens. The diagnosis was histologically verified in all patients. During the histological examination, the quantitative and qualitative composition of the biopsy specimens was studied. Using visual microscopy (Leica DM 3000 microscope, Leica Microsystems, Germany), the total number of cells was counted in 10 fields of view at 100-fold magnification according to the generally accepted technique. The number of granulomas in the fields of vision and the content of giant cells, macrophages, lymphocytes, neutrophils, and eosinophils in them (% from 100 in the field of vision) were studied. Such morphological signs as hyalinosis, the presence of Schaumann bodies, necrosis, stamping, calcification, fibrosis, and vasculitis were assessed qualitatively.

Calcification and vasculitis in biopsy specimens were extremely rare; they were observed only in 2 cases. No fibrosis was found in the studied material. In this regard, these parameters were not taken into account in further calculations. All patients were retrospectively divided into two clinical groups depending on the outcomes of the disease during the observation period 2007–2019 (6.4 to 11.6 years). The first group included patients with a favorable course of sarcoidosis, proceeding without relapses and signs of progression (clinical, radiologic, and functional), without the use of systemic glucocorticoids (SGC) or their accidental prescription in small doses and a short course (85 people in total, 35 men and 50 women). The second group consisted of patients with an unfavorable course of the disease, with relapses and progression,

who required a course of long-term CGS administration (a total of 36 people, 26 men and 10 women). The patient groups were matched by sex and age. The criteria for exclusion from the study were severe comorbidities in patients, such as chronic heart failure, complicated or decompensated diabetes mellitus, cancer, tuberculosis, kidney disease with kidney failure, and other lung diseases with respiratory failure determined by pulseoximetry.

Statistical processing of the data was carried out using Statistica 10.0 software (StatSoft, USA). To compare two independent samples, the nonparametric Mann – Whitney test was used, the mean values were calculated; a nonparametric cross tabulation analysis was performed with the calculation of the Pearson's $\chi 2$ contingency coefficient. If the expected phenomenon had a value from 5 to 9, the Pearson's $\chi 2$ test was performed with Yates' correction. If the expected phenomenon was less than 5, the Fisher's exact test was used for the analysis. Statistical calculations included Spearman's rank-order correlation, association analysis, and intergroup difference analysis. The strength of correlations was characterized by a weak positive relationship at values of r from 0.18 to 0.26; by a moderate positive relationship at r from 0.28 to 0.44; by a weak negative relationship at r from –0.18 to –0.26; and a moderate negative relationship at r from –0.28 to –0.44. The analysis of the confidence intervals (CI) of the compared parameters was carried out with the determination of the values of the upper and lower quantiles, and the risk ratio (RR) was calculated. The differences were considered statistically significant at $p < 0.05$.

RESULTS

The analysis of clinical symptoms in the examined patients revealed a variety of complaints: asthenic syndrome of varying severity (41.3% of cases), sweating (29.8% of cases), dyspnea (24% of cases), cough (48% of cases), chest pain (18.2% of cases), heart arrhythmia (20% of cases), pain in the joints (18.2% of cases), any skin manifestations, except for lupus pernio (21.5% of cases), altered peripheral lymph nodes (18.2% of cases), weight loss (13%), fever (29.8%). The acute course of sarcoidosis in the form of Löfgren's syndrome occurred in only 8.3% of cases (of which 6.6% of patients belonged to the group with a favorable course of the disease and 1.7% of patients were from the group with an unfavorable course of sarcoidosis without a significant difference), in most cases the disease had a chronic course.

The inter-group analysis showed that among all the general clinical manifestations, only the presence of dyspnea, skin manifestations, and weight loss occurred statistically significantly more often in patients with an unfavorable course of sarcoidosis ($p = 0.04$, 0.02, and 0.01, respectively). It should also be noted that in patients with an unfavorable course of sarcoidosis, bronchitis was also more common at the onset of the disease ($p = 0.05$, chi2 = 3.57). For the rest of the complaints in the vast majority of patients, no significant differences between the groups were detected.

The findings of our study allowed us to note that one of the most typical symptoms for sarcoidosis of any course was weight loss. It was found that in the group of patients with a favorable course of the disease, weight loss was small – on average, up to 1 kg. On the contrary, with an unfavorable course of sarcoidosis, patients had a more pronounced weight loss of 4 kg or more (up to a maximum of 40 kg). It was established that the presence of weight loss increased the risk of developing an unfavorable course of sarcoidosis by more than 3 times: RR 3.6, 95% CI 2.3–5.6. The data are presented in Table 1.

Table 1

The presence of weight loss in intrathoracic sarcoidosis in the groups, $M \pm m$

Parameter	Favorable course ($n = 85$)	Unfavorable course ($n = 36$)	p
Weight loss in kilograms	0.34 ± 0.19	3.94 ± 1.7	0,01

In accordance with the stated aim of the study, we compared the data of morphological studies in patients with different variants of the course of intrathoracic sarcoidosis. Quantitative differences between the groups in terms of the number of macrophages, lymphocytes, and neutrophils in the biopsy specimens were identified. The presence of qualitative features did not differ significantly between the groups. The data are presented in Tables 2 and 3.

It was noted that in patients with an unfavorable course of the disease, a greater number of macrophages in the biopsy specimens was significantly more frequent. In patients with a favorable course of the disease, a larger number of lymphocytes and neutrophils was more common. It was found that the predominance of macrophages in the biopsy material increased the risk of developing an unfavorable course of sarcoidosis by 1.4 times: RR 1.4, 95% CI 0.8–2.5.

Table 2

Quantitative morphological parameters of biopsy specimensin the groups throughout the course of sarcoidosis, $M \pm m$

Parameter	Favorable course (n = 85)	Unfavorable course (n = 36)	p
Granulomas, number in the field of vision	1.47 ± 0.1	1.6 ± 0.15	0.492642
Giant cells, %/100 in the field of vision	1.17 ± 0.11	1.33 ± 0.18	0.499801
Macrophages, %/100 in the field of vision	65.5 ± 1.8	78.5 ± 2.1	0.000041**
Lymphocytes, %/100 in the field of vision	30 ± 1.5	19.2 ± 1.8	0.000076**
Neutrophils, %/100 in the field of vision	3.36 ± 0.4	1.3 ± 0.4	0.001734**
Eosinophils, %/100 in the field of vision	1.0 ± 0.2	0.6 ± 0.27	0.225057

* $p < 0.05$; ** $p < 0.01$.

Table 3

Manifestations of activity according to morphological studies in the groups throughout the course of sarcoidosis

Sign	Favorable course				Unfavorable course				Statistical analysis
	present		absent		present		absent		
	абс.	%	абс.	%	абс.	%	абс.	%	
Hyalinosis	36	42.4	49	57.6	18	50	18	50	$p = 0.44$ $\chi^2 = 0.60$
Necrosis	18	21.2	67	78.8	8	22.2	28	77.7	$p = 0.89$ $\chi^2 = 0.02$* Yates' = 0.01
Schaumann bodies	14	16.5	71	83.5	10	27.8	26	72.2	$p = 0.15$ $\chi^2 = 2.03$
Stamping	44	51.8	41	48.2	20	55.6	16	44.4	$p = 0.70$ $\chi^2 = 0.15$
Total	85				36				

* $\chi 2$ (Chi-square test)

Therefore, it is obvious that the unfavorable course of intrathoracic sarcoidosis is accompanied by an increased number of macrophages in the histological examination of the biopsy specimens and greater weight loss in patients. A favorable course of the disease is associated with a relative weight loss (less than 1 kg) and a larger number of neutrophils and lymphocytes in the biopsy material.

DISCUSSION

A comprehensive analysis of clinical symptoms and morphological data between the groups different in the course of the disease showed that the cellular composition of granulomas is of great importance in assessing the prognosis of sarcoidosis. The analysis in the groups revealed that a significantly larger number of macrophages in the biopsy specimens was observed with an unfavorable and recurrent course of intrathoracic sarcoidosis. Macrophages are known to play an essential role in implementation of the immune response in sarcoidosis. These cells produce proinflammatory cytokines, such as interleukins (IL)-12, IL-6, IL-8, tumor necrosis factor alpha (TNFα), and chemokines. Chemokines, in turn, attract natural killer cells, neutrophils, and naïve T cells (Th0) to the focus of inflammation [11–13].

The above-described inflammatory mediators lead to alveolitis, granuloma formation, and tissue damage, and cytokine levels in bronchoalveolar lavage fluid and blood serum of patients can serve as markers of inflammation in sarcoidosis [14]. It was also found that in patients with an unfavorable course of sarcoidosis, weight loss (more than 3 kg) was more common. Moreover, a relationship was established according to the Spearman's rank-order correlation between the number of macrophages in the biopsy specimens and a greater weight loss in patients with an unfavorable course of the disease ($r = 0.39$, $p < 0.05$). Additionally, a relationship was identified between the number of lymphocytes and a relatively small decrease in weight in patients with a favorable course of sarcoidosis ($r = 0.32$, $p < 0.01$).

It should be noted that in modern studies, the authors not only study in detail the pathogenesis and morphogenesis of sarcoidosis, but also draw parallels between the clinical course, radiologic findings, and other data, thereby highlighting new phenotypes of the disease. M.A. Judson [15] distinguishes three groups

of patients: group 1 – patients with thoracic lymphadenopathy and silent pathological process in the lung tissue; group 2 – patients with minor lymphadenopathy and predominantly perivascular, peribronchial, and subpleural localization of granuloma complexes, as well as along the interlobar pleura, group 3 – patients with large granulomatous foci in the interstitial and peribronchial tissues, with various fibrotic changes (from moderate changes to severe cysts and bullae).

When comparing microscopic features, the author noted that in groups 1 and 2 the granulomatous inflammation was localized along the lymph-collecting vessels in the perivascular and peribronchial tissues. At the same time, in the patients of group 1, development of sclerotic changes, necrosis, and alveolitis was not detected, but in about 50% of cases, Schaumann bodies were found in giant cells, and granulomas had "stamped" appearance. In group 2, alveolitis and bronchiolitis of varying degrees were revealed, as well as zones of moderate interstitial lung disease and development of mild to moderate sclerotic changes. The patients of group 3 differed significantly: large granulomatous foci in the interstitium were observed (80% of cases), alveolitis and bronchiolitis were pronounced to varying degrees, and moderate to severe interstitial lung disease was detected. Granulomas were localized in the interstitial and peribronchial tissues, some of the granulomas affected the bronchiolo-alveolar region and led to pronounced fibrosis [15].

In another study, the author distinguished phenotypes of sarcoidosis according to the gender – age principle, determining the course of the disease in each group according to clinical symptoms [16]: 1) men from 18 to 35 years old; 2) men from 36 to 60 years old; 3) women from 18 to 35 years old; 4) women from 36 to 60 years old; 5) persons over 61 years of age. Preliminary data allow to consider histological studies with determination of the cellular composition of granulomas promising, since they can help predict a possible scenario for development of the course of intrathoracic sarcoidosis.

CONCLUSION

The data obtained indicate that the risk of developing an unfavorable course of intrathoracic sarcoidosis is associated with an increased number of macrophages in the histological examination and clinically significant weight loss in patients. The predominance of a large number of lymphocytes and neutrophils in sarcoid granulomas is typical of a favorable course of sarcoidosis. Based on the above-stated data, it is possible to distinguish two different clinical and morphological variants of the course of intrathoracic sarcoidosis: 1) macrophage-dominant sarcoidosis with severe weight loss (typical of a recurrent and unfavorable course of sarcoidosis); 2) lymphocyte-dominant sarcoidosis with minimal weight loss (typical of a favorable course of sarcoidosis).

Therefore, there is a close relationship between the clinical and morphological data of the study of the lungs and lymph nodes in pulmonary sarcoidosis. Morphological examination of biopsy specimens from the affected lesion provides significant information and complements the clinical presentation, allowing to predict the course of intrathoracic sarcoidosis. The predominance of macrophages in the biopsy material increases the risk of an unfavorable and recurrent course of sarcoidosis by 1.4 times, and the presence of significant weight loss increases the risk of an unfavorable course of the disease by more than 3 times.

With ubiquitous use of histological research methods in the diagnosis of sarcoidosis, it is necessary to search for new phenotypes of the disease course and identify new classification subtypes, taking into account morphological criteria. The presence of certain morphological markers may affect treatment and outcome of the disease. The data obtained in this study, undoubtedly, can be used to carry out morphological diagnosis in intrathoracic sarcoidosis and applied in real clinical practice.

REFERENCES

1. Baranova O.P., Ilkovich M.M., Speranskaya A.A. Difficulties in diagnosis of intrathoracic sarcoidosis. *Practical Medicine.* 2011; 3–1 (50): 58–62 (in Russ.).
2. Kalacheva T.P., Fedosenko S.V., Denisova O.A., Chernyavskaya G.M., Poponina T.M., Naumov A.O., Palchikova I.A., Dudko G.V., Karnaushkina M.A. Clinical and functional features of the course of intrathoracic sarcoidosis in real clinical practice. *Pilmonology.* 2020; 30 (2): 204–212 (in Russ.). DOI: 10.18093/0869-0189-2020-30-2-204-212.
3. Gudim L.N., Postnikova L.B., Kostrov V.A., Mironov A.A., Kubysheva N.I. The quality of life and physical performance in patients with intrathoracic sarcoidosis. *Archives of Internal Medicine.* 2020; 10 (1): 47–56 (in Russ.). DOI: 10.20514/2226-6704-2020-10-1-47-56.
4. Shklyaev A.E., Semyonovykh E.A., Ivanova L.V., Vedyokhina A.N. Lofgren's syndrome: clinical case. *Archives of Internal Medicine.* 2019; 9 (4): 308–312 (in Russ.). DOI: 10.25514/2226-6704-2019-9-4-308-312.
5. Sarcoidosis. Clinical guidelines. M.: PPO; 2019 (in Russ.). URL: http://spulmo.ru/obrazovatelnyeresursy/federalnye-klinicheskie-rekomendatsii/. (date of entry: 22.09.2019).
6. Vizel I.Yu., Vizel A.A. Analysis of the effect of prednisolone in patients with intrathoracic sarcoidosis: results of a retrospec-

tive study. *Pulmonology.* 2016; 26 (3): 316−322 (in Russ.). DOI: 10.18093/0869-0189-2016-26-3-316-322.
7. Shmalts A.A., Gorbachevsky S.V. Opportunities and limitations for initial combination non-specific therapy for pulmonary hypertension in the Russian Federation. *Therapeutic Archives.* 2020; 92 (12): 80−85 (in Russ.). DOI: 10.26442/00403660.2020.12.000840.
8. Pilzak K., Żebrowska A., Sikora M. et al. Physical functioning and symptoms of chronic fatigue in sarcoidosis patients. *Adv. Exp. Med. Biol.* 2018; 1040: 13−21. DOI: 10.1007/5584_2017_85.
9. Vizel A.A., Vizel A.Yu. Review of reports and publications on sarcoidosis at the annual conference of the American Thoracic Society (ATS 2016). *RMJ.* 2017; 3: 206−210 (in Russ.).
10. Bahmer T., Watz H., Develaska M. et al. Physical activity and fatigue in patients with sarcoidosis. *Respiration.* 2018; 95 (1):18−26. DOI: 10.1159/000481827.
11. Lazareva N.M., Baranova O.P., Kudryavtsev I.V., Arsentyeva N.A., Lyubimova N.E., Ses T.P., Ilkovich M.M., Totolyan A.A. Features of the cytokine profile in sarcoidosis. *Medical Immunology.* 2020; 22 (5): 993−002 (in Russ.). DOI: 10.15789/1563-0625-FOC-2064.
12. Vizel I.Yu., Shmelev E.I., Vizel A.A., Ganibaeva G.S. Comparison of newly diagnosed sarcoidosis cases in young and old age. *RMJ.* 2018; 10 (I): 16−20 (in Russ.).
13. Karadallı M.N., Boşnak-Güçlü M., Camcıoğlu B., Kokturk N., Türktaş H. Effects of Inspiratory Muscle Training in Subjects with Sarcoidosis: A Randomized Controlled Clinical Trial. *Respir. Care.* 2016; 61 (4): 483−494. DOI: 10.4187/respcare.04312.
14. Kostorz S., Jastrzębski D., Sikora M., Zebrovska A., Margas A., Stepanik D., Swinder H., Ziora D. Predominance of comorbidities in the detriment of daily activity in sarcoidosis patients. *Advs. Exp. Medicine, Biology – Neuroscience and Respiration.* 2018; 1040: 7−12. DOI: 10.1007/5584_2017_87.
15. Judson M.A. Quality of life in sarcoidosis. Seminars in respiratory and critical care medicine. 2017; 38 (4): 546−558. DOI: 10.1055/s-0037-1602589.
16. Chernikov A.Yu., Zemlyanskikh L.G. Phenotypes of sarcoidosis. *Pulmonology.* 2012; (5): 53−55 (in Russ.). DOI: 10.18093/0869-0189-2012-0-5-53-55.

Authors contribution

Palchikova I.A. – conception and design; carrying out of research, formation of the database, analysis and interpretation of data; drafting of the article. Denisova O.A. – conception and design; formation of the database, analysis and interpretation of data. Chernyavskaya G.M. – conception and design; analysis and interpretation of data; final approval of the manuscript for publication. Purlik I.L. – carrying out of research; analysis and interpretation of data. Kalacheva T.P. – conception and design; drafting of the manuscript. Naumov A.O., Soloviev M.M. – analysis and interpretation of data.

Authors information

Palchikova Inna A., Pulmonologist, Tomsk Regional Clinical Hospital, Tomsk, Russian Federation. ORCID 0000-0003-4968-1110.

Denisova Olga A., Dr. Sci. (Med.), Assistant of the Department of Advanced-Level Therapy with a Course of Physical Rehabilitation, and Sports Medicine, Rheumatologist, Therapeutic Clinic, Siberian State Medical University, Tomsk, Russian Federation. ORCID 0000-0003-1652-9622.

Chernyavskaya Galina M., Dr. Sci. (Med.), Professor, Department of Advanced-Level Therapy with a Course of Rehabilitation, Physiotherapy, and Sports Medicine, Siberian State Medical University, Tomsk, Russian Federation. ORCID 0000-0003-0105-2307.

Purlik Igor L., Dr. Sci. (Med.), Professor, Department of Pathological Anatomy, Siberian State Medical University, Tomsk, Russian Federation. ORCID 0000-0003-3757-0173.

Kalacheva Tatyana P., Cand. Sci. (Med.), Associate Professor, Department of General Medical Practice and Polyclinic Therapy, Siberian State Medical University, Tomsk, Russian Federation. ORCID 0000-0002-4292-7723.

Naumov Andrey O., Cand. Sci. (Med.), Associate Professor, Department of Healthcare Organization and Public Health, Siberian State Medical University, Tomsk, Russian Federation. ORCID 0000-0001-6532-2420.

Soloviev Mikhail M., Dr. Sci. (Med.), Professor, Department of Advanced-Level Surgery with a Course of Cardiovascular Surgery, Siberian State Medical University, Tomsk, Russian Federation. ORCID 0000-0002-9497-1013.

(✉) **Kalacheva Tatiana P.,** e-mail: tatyana-kalachyova@yandex.ru

Received 03.11.2021
Accepted 25.05.2021

УДК 612.179:616.12-008:577.152.6
https://doi.org/10.20538/1682-0363-2021-4-25-31

The effects of NETosis on fibrinolysis in colon cancer patients

Parshina A.A.[1]., Tsybikov N.N.[1], Tereshkov P.P.[1], Karavaeva T.M.[1], Maksimenya M.V.[1]

Chita State Medical Academy (CSMA)
39a, Gorkogo Str., Chita, 672090, Russian Federation

ABSTRACT

Aim. To investigate formation of neutrophil extracellular traps (NETs) and their impact on fibrinolysis in patients with colon cancer.

Materials and methods. The study was performed in two groups. The experimental group consisted of patients with stage 2–3 non-metastatic colon cancer (n = 17, average age – 67 years). The control group included healthy volunteers matched by sex and age (n = 30, average age – 68 years). An experimental model was created from the whole blood. It included platelet-poor plasma and an isolated culture of neutrophils, previously induced to NETosis by adding 100 nmol PMA. The samples were incubated for 4 hours, then the test tubes were centrifuged to pellet cells and their remnants, and the plasma was transferred for subsequent examination. The plasma incubated with intact neutrophils was used as a control. The levels of interleukin-8 (IL-8) and P-selectin glycoprotein ligand-1 (PSGL-1) were used to determine the degree of cell activation. NETosis was confirmed by enzyme-linked immunosorbent assay (ELISA) and fluorescent microscopy. Fibrinolysis was assessed using the thrombodynamics test. The results were compared with the levels of fibrinolytic system components measured by flow cytometry.

Results. In the control group, NETosis induction contributed to pronounced neutrophil activation that was accompanied by an increase in the IL-8, PSGL-1, and plasminogen levels, a decrease in PAI-1, and enhancement of fibrinolysis, compared with the intact samples. Higher levels of IL-8, PSGL-1, plasminogen, and PAI-1 and intensified fibrinolysis were detected in the intact samples. However, PMA-induced NETosis did not result in an increase in the degree of activation and significant changes in the given parameters.

Conclusion. NETosis promotes both formation and lysis of fibrin clots. However, in cancer patients, suicidal NETosis does not contribute to fibrinolysis due to intracellular protease depletion, which may be one of the mechanisms causing hypercoagulation and insufficient fibrinolysis in cancer.

Key words: cancer, neutrophil extracellular traps, fibrinolysis, immunothrombosis, NETosis, hypercoagulation.

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 Russian Foundation for Basic Research (Project No. 19-34-90050).

Conformity with the principles of ethics. All patients signed an informed consent to participate in the study. The study was approved by Chita State Medical Academy (Protocol No. 86 of 01.11.2017).

For citation: Parshina A.A., Tsybikov N.N., Tereshkov P.P., Karavaeva T.M., Maksimenya M.V. The effects of NETosis on fibrinolysis in colon cancer patients. *Bulletin of Siberian Medicine.* 2021; 20 (4): 25–31. https://doi.org/10.20538/1682-0363-2021-4-25-31.

✉ *Parshina Anastasia A.,* e-mail: a.parshina.csma@gmail.com

Влияние нетоза на лизис фибринового сгустка при раке толстого кишечника

Паршина А.А., Цыбиков Н.Н., Терешков П.П., Караваева Т.М., Максименя М.В.

Читинская государственная медицинская академия (ЧГМА)
Россия, 672090, г. Чита, ул. Горького 39а

РЕЗЮМЕ

Цель – изучение особенностей формирования нейтрофильных внеклеточных ловушек, а также их влияния на фибринолиз у лиц, имеющих злокачественные новообразования толстого кишечника.

Материалы и методы. Группу пациентов составили лица с впервые выявленным раком толстого кишечника 2–3-й стадии без метастаза (17 человек, средний возраст 67 лет); контрольную группу – доноры, сопоставимые по полу и возрасту, не имеющие злокачественных опухолей (30 человек, средний возраст 68 лет). Из цельной крови создали экспериментальную модель, включавшую бедную тромбоцитами плазму крови и изолированную культуру нейтрофилов, предварительно индуцированных к нетозу внесением 100 нмоль PMA, инкубировали 4 ч, клетки осаждали центрифугированием, плазму отбирали для дальнейшего исследования. В качестве контроля использовали плазму, инкубированную с интактными нейтрофилами. О степени активации клеток судили по уровню интерлейкина (IL) 8 и PSGL-1. Нетоз подтверждали измерением уровня нуклеосом и флуоресцентной микроскопией. Оценку фибринолиза проводили в тесте тромбодинамики. Результаты сопоставляли с концентрацией компонентов фибринолитической системы, измеренных методом проточной цитометрии.

Результаты. В контроле индукция нетоза вызывает выраженную активацию нейтрофилов, сопровождающуюся повышением уровня IL-8, PSGL-1, плазминогена, снижением PAI-1 и усилением фибринолиза, в сравнении с интактными образцами. У пациентов зафиксирован больший, чем в группе контроля, уровень IL-8, PSGL-1, плазминогена, PAI-1 и показателей фибринолиза в интактных образцах. При этом индукция нетоза не привела к увеличению степени активации и значимому изменению данных показателей.

Заключение. Гибель нейтрофилов путем нетоза в местах тромбообразования может способствовать как формированию, так и растворению фибринового сгустка. Однако у лиц со злокачественными новообразованиями «смертельный» нетоз не приводит к локальному увеличению фибринолитического потенциала ввиду истощения внутриклеточных резервов протеаз нейтрофилов, что может являться одним из механизмов развития гиперкоагуляции и недостаточности фибринолиза при онкопатологии.

Ключевые слова: рак, нейтрофильные внеклеточные ловушки, фибринолиз, иммунотромбоз, нетоз, гиперкоагуляция.

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

Источник финансирования. Исследование выполнено при финансовой поддержке РФФИ (проект № 19-34-90050).

Соответствие принципам этики. Все пациенты подписали информированное согласие на участие в исследовании. Исследование одобрено ЧГМА (протокол № 86 от 01.11.2017).

Для цитирования: Паршина А.А., Цыбиков Н.Н., Терешков П.П., Караваева Т.М., Максименя М.В. Влияние нетоза на лизис фибринового сгустка при раке толстого кишечника. *Бюллетень сибирской медицины.* 2021; 20 (4): 25–31. https://doi.org/10.20538/1682-0363-2021-4-25-31.

INTRODUCTION

The hypercoagulable state and thrombotic events are known to accompany malignancy. Thrombophilia in cancer is based on synthesis and release of procoagulants by tumor cells, as well as a complex of reactions, including activation of and (or) damage to the endothelium, activation of platelets and immune cells, and synthesis of cytokines. Neutrophils, being effectors of innate immunity, are essentially involved in inflammatory processes and hemostatic responses. Indeed, neutrophils and NETosis products – neutrophil

extracellular traps (NETs) – are detected in thrombi of various localizations [1]. However, in numerous studies on the prothrombotic properties of NETs [2–7], the possible role of NETs in fibrinolysis, especially in cancer, remains understudied [8, 9].

MATERIALS AND METHODS

The study was carried out in two groups. The experimental group consisted of patients with newly diagnosed, stage 2–3 non-metastatic colon cancer (n = 17, average age – 67 years). The control group included healthy volunteers matched by sex and age (n = 30, average age – 68 years). Preliminary selection of donors was carried out on the basis of a questionnaire. Then, the absence of cancer in the control group was estimated by the results of a clinical examination. Measurement of body temperature and complete blood count were performed in each donor. Donor exclusion criteria were the following: 1) body temperature > 37 °C; hemoglobin (HGB) < 100 g/l, red blood cells (RBC) < 3.5×10^{12}/l, white blood cells (WBC) > 10×10^{9}/l, erythrocyte sedimentation rate (ESR) > 12 mm per hour; 2) intake of anticoagulants / platelet antiaggregants, chemotherapy; 3) acute inflammation or exacerbation of chronic inflammation, inherited blood disorders, diabetes mellitus, traumas or surgeries in the last 6 months.

All patients and healthy donors signed an informed consent to participate in the study. 25 ml of peripheral venous blood was collected in tubes with 3.2% sodium citrate solution as an anticoagulant once. To obtain platelet-poor plasma (PPP), whole venous blood was centrifuged for 15 min at 1,500 rpm, and then the plasma was taken for repeated centrifugation at 3,000 rpm for 10 min. 90% of prepared PPP was transferred into separate tubes.

To isolate neutrophilic granulocytes (NG), Ficoll – Urographin density gradient centrifugation was performed (1.077 / 1.093). The granulocyte layer was transferred into separate tubes, washed with phosphate-buffered saline (PBS), and then centrifuged to pellet cells. The remained red blood cells were lysed (VersaLyse, Beckman Coulter, USA), isolated neutrophils were washed again with PBS, and the supernatant was discarded. Then, 4 ml of RPMI1640 was added to the cell pellet and mixed, and a suspension of isolated neutrophils was obtained. Cell viability was assessed by staining with a 0.4% methylene blue solution; the culture with the viability of at least 98% was used. The number of cells was adjusted to $4.5–6 \times 10^{6}$/ml.

Then 1 ml of the suspension was added into two separate test tubes and centrifuged to pellet cells. The culture medium was removed. After that, 5µl of PBS and then 1 ml of PPP were added to the cell pellet in the first test tube, mixed, and, thus, a suspension of intact neutrophils was prepared. 5µl (100 nm) of phorbol-12-myristate-13-acetate (PMA) (Sigma Aldrich, USA) was added to the cell pellet in the second test tube to induce NETosis, then 1 ml of PPP was added, mixed, and, thus, a suspension of neutrophils stimulated for NETosis was obtained. The obtained samples were labeled in the following way: PPP – platelet-poor plasma; INT – plasma containing intact neutrophils; PMA – plasma containing PMA-stimulated neutrophils.

All samples were incubated for 4 hours at 37 °C. Then the INT and PMA samples were centrifuged to pellet cells and their remnants, and the plasma was transferred into separate test tubes for further research. Each plasma sample was assessed using the thrombodynamics test (HemaCor, Russia): lysis onset time (LOT; min), lysis progression (LP; %/min), and lysis time estimation (LTE; min) were recorded. Fibrinogen (Fib), plasminogen / plasmin system (PLS), tissue plasminogen activator (tPA), plasminogen activator ingibitor-1 (PAI-1), P-selectin glycoprotein ligand-1 (PSGL-1), and IL-8 in each plasma sample were determined using multiplex assay kits (Human Thrombosis Panel, BioLegend, USA; Human Fibrinolysis Panel, BioLegend, USA) by flow cytometry. NETosis induction was estimated by the level of extracellular DNA (ecDNA), measured by ELISA (Cell Death Detection Kit ELISA^{plus}, Roche, Germany). Additionally, NETs were visualized using fluorescence microscopy.

For this purpose, 1 ml of prepared INT and PMA samples were placed into the Poly-L-Lysine-coated (Sigma Aldrich, USA) cell culture plates and incubated at 37 °C for 4 hours. After that, the plates were washed with PBS three times, the adherent cells were stained with SYTOX Green (Beckman Coulter, USA), and NETs were visualized using the ZOE Fluorescent Cell Imager (BioRad, USA), green channel, objective ×20.

Statistical processing of the results was performed using Microsoft Excel and Statistica 10 (StatSoft Inc., USA) software. The data obtained are presented as the median and the interquartile range Me [Q_1; Q_3]. The Wilcoxon signed-rank test was used to compare the results within the groups, and the Mann – Whitney U-test was used for comparison between the experimental and control groups. The differences were considered significant at $p^* < 0.05$.

RESULTS

NETosis in the control and experimental groups was confirmed by an increase in the level of ecDNA in the PMA samples compared with the INT samples ($p_1 = 0.008$ and $p_2 = 0.05$, Table). Additionally, microscopy of NG cultures made it possible to visualize NETs. The figure demonstrates intact neutrophils and NETs in the control group (*a* and *b*, respectively) and in cancer patients (*c* and *d*, respectively). Neutrophil-specific IL-8 and free PSGL-1 were considered as activation markers. The levels of IL-8 and PSGL-1 in the control group were the highest in the PMA samples; however, no significant increase in the indicated molecules in the corresponding plasma samples was detected in the experimental group (Table). The effect of NETs on fibrinolysis was evaluated in the control group: increased LP and reduced LTE were registered (Table). In the experimental group, no significant changes in fibrinolysis parameters were recorded.

Changes in the level of NETosis-associated fibrinolytic system components were detected in the control group: a significant increase in plasminogen and a decrease in PAI-1 were found in the PMA samples compared with the intact ones. In the experimental group, changes in the levels of fibrinolytic system components were not significant (Table).

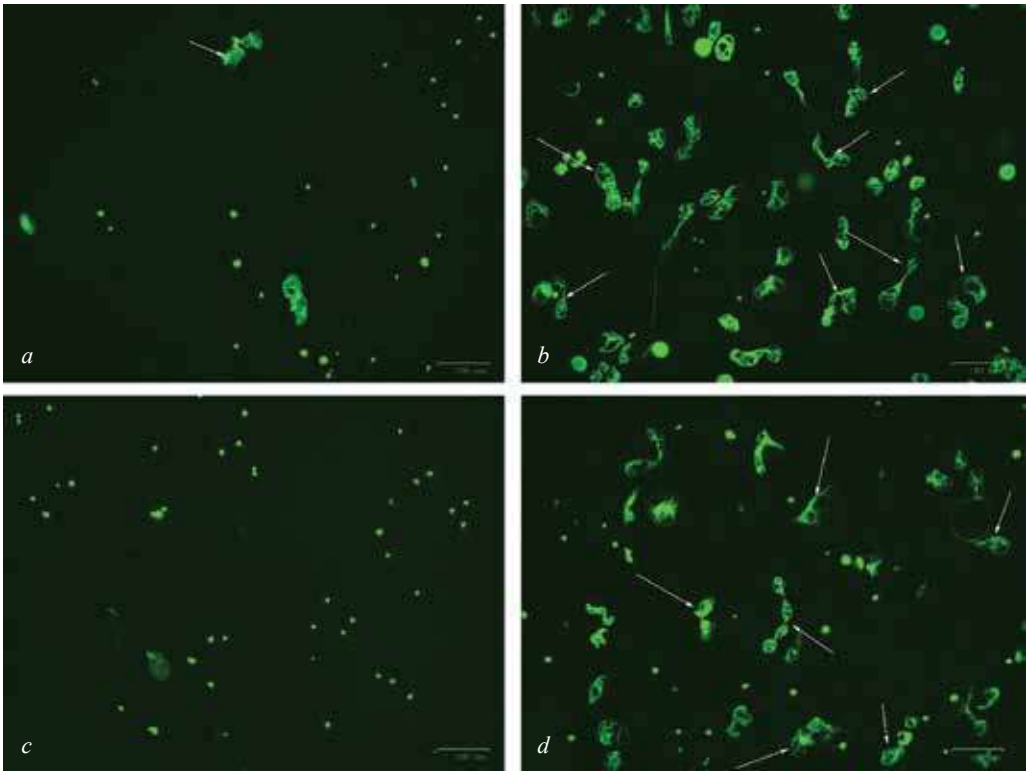


Figure. Intact neutrophils: in the control group (*a*), in the experimental group (*c*); neutrophil extracellular traps: in the control group (*b*); in the experimental group (*d*). SYTOX Green staining, objective ×20. White arrows mark neutrophil extracellular traps

Table

Parameters of cell activation and the effect of intact and PMA-stimulated neutrophils on fibrinolysis and the level of fibrinolytic system components, *Me* [Q_1; Q_3]

Parameter	Control group			*p*	Experimental group	
	Value		p_1		Value	p_2
ecDNA, OD	INT	0.38 [0.22; 0.48]	0.008*	0.1	0.26 [0.18; 0.33]	0.01*
	PMA	0.5 [0.4; 0.6]		0.03*	0.34 [0.26; 0.41]	
PSGL-1, pg / ml	INT	865 [620.5; 1018.7]	0.01*	0.3	781.25 [431.8; 926.5]	0.2
	PMA	1236.7 [1071.2; 1340.5]		0.05*	791.5 [538.8; 1254.3]	
IL-8, pg / ml	INT	2422 [880.8; 4852.5]	0.007*	0.7	3150.7 [2007; 5470.8]	0.6
	PMA	7836.2 [5422.5; 10786.]		0.07	1677 [1006.8; 7777]	
LOT, min	INT	30.2 [28.3; 40.7]	0.7	0.02*	24.8 [16.7; 28.6]	0.5
	PMA	29 [28.1; 36.7]		0.01*	24.1 [19; 25.5]	

Table (continued)

Parameter	Control group			p	Experimental group	
	Value		p_1		Value	p_2
LP, %/min	INT	2.7 [2.2; 2.9]	0.01*	0.0003*	8.8 [5.1; 12.4]	0.4
	PMA	3 [2.9; 3.8]		0.001*	9.2 [4.7; 17.1]	
LTE, min	INT	40.6 [36.8; 50.6]	0.02*	0.002*	16.9 [12.6; 22.4]	0.8
	PMA	35.2 [27.8; 37.1]		0.001*	16 [12.4; 24.8]	
PLS, mcg/ml	INT	547.1 [475; 732.5]	0.008*	0.007*	805.1 [783.7; 825.6]	0.9
	PMA	767.7 [662; 864.1]		0.7	820 [787.9; 845]	
tPA, pg/ml	INT	63.5 [61.6; 64.7]	0.4	0.0001*	440.5 [227.5; 607.75]	0.5
	PMA	61.3 [59.7; 65.7]		0.0001*	389.7 [247; 672]	
AI-1, pg/ml	INT	2101.5 [508; 2681.3]	0.008*	0.001*	6376.2 [5258.5; 9428.8]	0.6
	PMA	1328.2 [1044.4; 2157.8]		0.0002*	7956.7 [5332.5; 10645.6]	
Fib, mcg/ml	INT	2882.7 [1877.3; 3142.6]	0.9	0.04*	3203.4 [2749.6; 6229.9]	0.6
	PMA	2355.8 [1745; 3206.6]		0.08	3384.7 [2723.7; 8997]	

Note: INT – plasma sample after incubation of intact neutrophils; PMA – plasma sample after incubation of PMA-stimulated neutrophils; ecDNA – extracellular DNA, OD – optical density; p – level of statistical significance between the control and experimental groups (Mann – Whitney U-test); p_1 – level of statistical significance between the INT and PMA samples in the control group (Wilcoxon signed-rank test); p_2 – level of statistical significance between the INT and PMA samples in the experimental group (Wilcoxon signed-rank test); * – statistically significant difference (at $p < 0.05$).

DISCUSSION

The results obtained demonstrate different effect of NETosis induction in the investigated groups. Lower ecDNA level in the plasma samples in the experimental group indicates that less neutrophils underwent NETosis in the presented experimental model (Table). Presumably, it could be caused by alteration of intracellular signaling due to predominance of other signaling pathways. It is known that cancer cells are capable of regulating the activity of leukocytes, contributing to tumor progression [10–14]. Different cytokine microenvironment might be a factor determining NETosis propensity and (or) the prevailing signaling pathway (suicidal or vital NETosis) [15].

PMA induced lytic (suicidal) NETosis accompanied by the release in the plasma of not only chromatin, but also all intracellular proteases, which activate both the plasminogen system and non-specific proteolysis of fibrin. Our previous study [16] demonstrates failure of NETs-associated fibrin plate lysis in the presence of the protease inhibitor aprotinin. Besides a higher number of NETosis-affected neutrophils, a more pronounced effect of NETs formation on fibrinolysis in the control group could be explained by the ability of the crucial NETosis enzyme PAD4 to modify the fibrinogen structure. It results in impaired fibrin formation and changes in its structure [17, 18]. In this study, altered fibrin structure could be one of the factors underlying fibrinolysis boost in the PMA samples in the control group (Table).

No increase in fibrinolysis in cancer patients could be associated with cell activation failure (low ecDNA and PSGL-1), higher concentration and activity of PAI-1, and prevalence of procoagulant factors in malignancy. Additionally, in cancer, initially high activity of intact neutrophils suggests lower intracellular protease level due to their fast and persistent release into the extracellular space, which, therefore, causes their insufficient increase in suicidal NETosis (that also concerns IL-8). Considering literature and our own data, it could be assumed that NETs formed following suicidal or vital NETosis have different effects on clotting and fibrinolysis due to the differences in the amount of released chromatin and the final composition of extracellular traps, which depends on NETosis inductor signal [15]. Hence, it is more relevant to *in vivo* studies. However, that should become the objective of further investigations.

CONCLUSION

NETs formation can provide not only a thrombogenic effect, implemented due to interactions of nucleosomes with platelets, endothelium, and coagulation factors, but also a fibrinolytic effect, based on the plasminogen activation system and non-specific lysis of insoluble fibrin by neutrophil granule proteases, which, along with thrombi phagocytosis by other leukocyte types, provides final recanalization of blood vessels. However, cancer disrupts the coagulation / fibrinolytic balance. It contributes to thrombophilia by the development of a local and systemic inflammatory response, alteration of the NETs formation pathway

and, therefore, prevalence of prothrombotic effects of ecDNA over the fibrinolytic activity of NG. When combined with an increased concentration of fibrinolysis inhibitors, it eventually contributes to the development of a chronic hypercoagulable state typical of cancer.

REFERENCES

1. Brinkmann V., Reichard U., Goosmann C., Fauler B., Uhlemann Y., Weiss D.S., Weinrauch Y., Zychlinsky A. Neutrophil extracellular traps kill bacteria. *Science.* 2004; 303 (5663): 1532–1535. DOI: 10.1126/science.1092385.
2. Fuchs T.A., Brill A., Duerschmied D., Schatzberg D., Monestier M., Myers D.D. Jr., Wrobleski S.K., Wakefield T.W., Hartwig J.H., Wagner D.D. Extracellular DNA traps promote thrombosis. *PNAS*. 2010; 107 (36): 15880–15888. DOI: 10.1073/pnas.1005743107.
3. Martinod K., Demers M., Fuchs T.A., Wong S.L., Brill A., Gallant M., Hu J., Wang Y., Wagner D.D. Neutrophil histone modification by peptidylarginine deiminase 4 is critical for deep vein thrombosis in mice. *PNAS*. 2013; 110 (21): 8674–8679. DOI: 10.1073/pnas.1301059110.
4. Czaikoski P.G., Mota J. M.S.C., Nascimento D.C., Sônego F., Castanheira F.V.e S., Melo P.H., Scortegagna G.T., Silva R.L., Barroso-Sousa R., Souto F.O., Pazin-Filho A., Figueiredo F., Alves-Filho J.C., Cunha F.Q. Neutrophil extracellular traps induce organ damage during experimental and clinical sepsis. *PLoS One.* 2016; 11 (2): e148142. DOI: 10.1371/journal.pone.0148142.
5. Budnik I., Brill A. Immune factors in deep vein thrombosis initiation. *Trends in Immunology*. 2018; 39 (8): 610–623. DOI: 10.1016/j.it.2018.04.010.
6. Grover S.P., Mackman N. Neutrophils, NETs, and immunothrombosis. *Blood*. 2018; 132 (13): 1360–1361. DOI: 10.1182/blood-2018-08-868067.
7. Iba T., Hashiguchi N., Nagaoka I., Tabe Y., Murai M. Neutrophil cell death in response to infection and its relation to coagulation. *Journal of Intensive Care*. 2013; 1 (1): 13. DOI: 10.1186/2052-0492-1-13.
8. Fedotkina Yu.A., Panchenko E.P. Thrombosis in oncology. Part 1. *Atherothrombosis*. 2017; 1: 11–15 (in Russ.). DOI: 10.21518/2307-1109-2017-1-11-15.
9. Falanga A., Schieppati F., Russo L. Pathophysiology 1. Mechanisms of thrombosis in cancer patients. In: Soff G. (eds) Thrombosis and hemostasis in cancer. *Cancer Treatment and Research*. 2019; 179: 11–36. DOI: 10.1007/978-3-030-20315-3_2.
10. Chennakrishnaiah S., Meehan B., D'Ast E., Montermini L., Lee T.H., Karatzas N., Buchanan M., Tawil N., Choi D., Divangahi M., Basik M., Rak J. Leukocytes as a reservoir of circulating oncogenic DNA and regulatory targets of tumor-derived extracellular vesicles. *J. Thromb. Haemost*. 2018; 16 (9): 1800–1813. DOI: 10.1111/jth.14222.
11. Tashireva L.A., Perelmuter V.M., Manskikh V.N., Denisov E.V. Savelyeva O.E., Kaigorodova E.V., Zavyalova M.V. Types of immune-inflammatory reactions as algorithms for cell interaction under conditions of reparative regeneration and tumor growth. *Biochemistry*. 2017; 82 (5): 732–748 (in Russ.).
12. Demers M., Wong S.L., Martinod K., Gallant M., Cabral E., Wang Y., Wagner D.D. Priming of neutrophils toward netosis promotes tumor growth. *Oncoimmunology.* 2016; 5 (5): e1134073. DOI: 10.1080/2162402X.2015.1134073.
13. Shaul M.E., Fridlender Z.G. Cancer-related circulating and tumor-associated neutrophils –subtypes, sources and function. *FEBS J.* 2018; 285 (23): 4316–4342. DOI: 10.1111/febs.14524.
14. Leal A.C., Mizurini D.M., Gomes T., Rochael N.C., Saraiva E.M., Dias M.S., Werneck C.C., Sielski M.S., Vicente C.P., Monteiro R.Q. Tumor-derived exosomes induce the formation of neutrophil traps: Implications for the establishment of cancer-associated thrombosis. *Sci. Rep.* 2017; 7 (1): 6438. DOI: 10.1038/s41598-017-06893-7.
15. Thiam H.R., Wong S.L., Wagner D.D., Waterman C.M. Cellular Mechanisms of NETosis *Annu. Rev. Cell Dev. Biol.* 2020; 36: 191–218. DOI: 10.1146/annurev-cellbio-020520-111016.
16. Parshina A.A., Tsybikov N.N. The impact of neutrophil extracellular traps on plasma clotting and fibrinolysis in colon cancer patients *in vitro*. *Transbaikal Medical Bulletin*. 2019; 4: 90–96 (in Russ.).
17. Damiana T., Damgaard D., Sidelmann J.J., Nielsen C.H., de Maat M.P., Münster A.M.B., Palarasah Y. Citrullination of fibrinogen by peptidylarginine deiminase 2 impairs fibrin clot structure. *Clinica Chimica Acta*. 2020; 501: 6–11. DOI: 10.1016/j.cca.2019.10.033.
18. Zhou Y., Chen B., Mittereder N., Chaerkady R., Strain M., An. L.-L., Rahman S., Ma W., Low C.P., Chan D., Neal F., Bingham C.O., Sampson K., Darrah E., Siegel M.R., Hasni S., Andrade F., Vousden K.A., Mustelin T., Sims G.P. Spontaneous secretion of the citrullination enzyme PAD2 and cell surface exposure of PAD4 by neutrophils. *Frontiers in Immunology*. 2017; 8: 1200. DOI: 10.3389/fimmu.2017.01200.

Authors contribution

Parshina A.A. – conception and design, carrying out of the experiment, analysis and interpretation of data, drafting of the manuscript. Tsybikov N.N. – conception, substantiation of the manuscript, critical revision of the manuscript for important intellectual content, final approval of the manuscript for publication. Tereshkov P.P. – carrying out of the experiment, critical revision of the manuscript for important intellectual content. Karavaeva T.M. – analysis and interpretation of data, critical revision of the manuscript for important intellectual content. Maksimenya M.V. – critical revision of the manuscript for important intellectual content.

Authors information

Parshina Anastasia A., Post-Graduate Student, Department of Pathological Physiology, Chita State Medical Academy, Chita, Russian Federation. ORCID 0000-0002-1458-2385.

Tsybikov Namzhil N., Dr. Sci. (Med.), Professor, Head of the Department of Pathological Physiology, Chita State Medical Academy, Chita, Russian Federation. ORCID 0000-0002-0975-2351.

Tereshkov Pavel P., Cand. Sci. (Med.), Principal Researcher, Laboratory of Clinical and Experimental Biochemistry and Immunology, Research Institute of Molecular Medicine, Chita State Medical Academy, Chita, Russian Federation. ORCID 0000-0002-8601-3499.

Karavaeva Tatiana M., Cand. Sci. (Med.), Senior Researcher, Laboratory of Clinical and Experimental Biochemistry and Immunology, Research Institute of Molecular Medicine, Chita State Medical Academy, Chita, Russian Federation. ORCID 0000-0002-0487-6275.

Maksimenya Maria V., Cand. Sci. (Biology), Senior Researcher, Laboratory of Clinical and Experimental Biochemistry and Immunology, Research Institute of Molecular Medicine, Chita State Medical Academy, Chita, Russian Federation. ORCID 0000-0001-6308-3411.

(✉) **Parshina Anastasia A.,** e-mail: a.parshina.csma@gmail.com

Received 06.08.2020
Accepted 28.12.2020

УДК 616.314-002:577.161.2:612.017.1
https://doi.org/10.20538/1682-0363-2021-4-32-38

The association between parameters of oral mucosal immunity and 25-hydroxyvitamin D in patients with rampant caries

Putneva A.S., Karavaeva T.M., Maksimenya M.V., Tereshkov P.P., Mishchenko M.N., Fefelova E.V., Tsybikov N.N., Parshina A.A.

Chita State Medical Academy (CSMA)
39a, Gorkogo Str., Chita, 672090, Russian Federation

Aim. To determine the saliva level of immunoregulatory proteins in patients with rampant caries and 25-hydroxyvitamin D (25(OH)D) deficiency and evaluate the association of their concentration with 25(OH)D plasma level.

Materials and methods. The study was performed in two groups. The experimental group included 15 patients aged 20–22 years with rampant caries and the 25(OH)D plasma level of < 20 ng / ml. The control group encompassed 15 healthy age-matched volunteers with the 25(OH)D plasma level of 20–100 ng / ml. The concentrations of B7.2 (CD86), free active TGF-β1, CTLA-4, PD-1, Tim-3, LAG-3, IGFBP-4, and ICAM-1 were assessed using flow cytometry. The levels of LL-37 and secretory immunoglobulin A (sIgA) were measured using ELISA. The Spearman's rank correlation coefficient was used to reveal a correlation between the indicated proteins and the 25(OH)D plasma level.

Results. A decrease in B7.2 (CD86), PD-1, Tim-3, sIgA, and LL-37 and elevation of IGFBP-4 and ICAM-1 saliva levels were detected in patients with rampant caries and 25-hydroxyvitamin D deficiency. A positive Spearman's rank correlation coefficient was revealed between plasma 25(OH)D and saliva levels of free active TGF-β1, CTLA-4, B7.2 (CD86), LL-37, and sIgA. A negative correlation was revealed between 25(OH)D and ICAM-1.

Conclusion. 25(OH)D deficiency in patients with rampant caries is associated with decreased levels of B7.2 (CD86), PD-1, Tim-3, sIgA, and LL-37 and elevated levels of IGFBP-4 and ICAM-1 in the saliva.

Key words: rampant caries, 25-hydroxyvitamin D deficiency, mucosal immunity.

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. All individuals signed an informed consent to participate in the study. The study was approved by the local Ethics Committee at Chita State Medical Academy (Protocol No. 9 of 24.06.2019).

For citation: Putneva A.S., Karavaeva T.M., Maksimenya M.V., Tereshkov P.P., Mishchenko M.N., Fefelova E.V., Tsybikov N.N., Parsina A.A. The association between parameters of oral cavity mucosal immunity and 25-hydroxy-vitamin D3 plasma level in patients with multiple tooth decay. *Bulletin of Siberian Medicine.* 2021; 20 (4): 32–38. https://doi.org/10.20538/1682-0363-2021-4-32-38.

✉ *Putneva Aleksandra S.,* e-mail: putnevaas@yandex.ru

Взаимосвязь некоторых параметров мукозального иммунитета полости рта с уровнем витамина D у пациентов с множественным кариесом

Путнева А.С., Караваева Т.М., Максименя М.В., Терешков П.П., Мищенко М.Н., Фефелова Е.В., Цыбиков Н.Н., Паршина А.А.

Читинская государственная медицинская академия (ЧГМА)
Россия, 672090, г. Чита, ул. Горького 39а

РЕЗЮМЕ

Цель – оценить содержание иммунорегуляторных молекул в слюне у лиц с множественным кариесом и дефицитом 25(OH)D_3 и определить взаимосвязи их величин с концентрацией 25(OH)D_3 в крови.

Материалы и методы. Обследованы две группы лиц в возрасте 20–22 лет. В одну включены 15 человек с кариесом и уровнем 25(OH)D_3 менее 20 нг/мл, в другую (контрольную) – 15 здоровых человек с содержанием 25(OH)D_3 30–100 нг/мл. В ротовой жидкости определены концентрации растворимых форм молекул B7.2 (CD86), Free Active TGF-b1, CTLA-4, PD-1, Tim-3, LAG-3, IGFBP-4, ICAM-1 методом проточной цитофлуометрии, количество кателицидина LL-37, секреторного иммуноглобулина А (IgA) методом иммуноферментного анализа. Между определяемыми показателями рассчитан критерий корреляции Спирмена.

Результаты. У лиц с кариесом и дефицитом витамина D выявлено снижение значений Free Active TGF-b1, B7.2 (CD86), PD-1, Tim-3, sIgA, кателицидина LL-37 и повышение уровня IGFBP-4 и ICAM-1 в слюне. Обнаружено наличие прямых корреляционных связей между количеством 25(OH)D_3 в крови, с одной стороны, и значениями Free Active TGF-b1, CTLA-4, B7.2 (CD86), секреторного IgA, пептида LL-37 – с другой. Зафиксирована отрицательная взаимосвязь между величинами 25(OH)D_3 и ICAM-1.

Заключение. На фоне дефицита витамина D при множественном кариесе в ротовой жидкости регистрируются низкие концентрации Free Active TGF-b1, B7.2 (CD86), PD-1, Tim-3, секреторного IgA, кателицидина LL-37 по сравнению с контролем, но увеличены значения IGFBP-4 и ICAM-1.

Ключевые слова: множественный кариес, гиповитаминоз D, мукозальный иммунитет.

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. Все пациенты подписали информированное согласие на участие в исследовании. Исследование одобрено локальным этическим комитетом ЧГМА (протокол № 9 от 24.06.2019).

Для цитирования: Путнева А.С., Караваева Т.М., Максименя М.В., Терешков П.П., Мищенко М.Н., Фефелова Е.В., Цыбиков Н.Н., Паршина А.А. Взаимосвязь некоторых параметров мукозального иммунитета полости рта с уровнем витамина D у пациентов с множественным кариесом. *Бюллетень сибирской медицины.* 2021; 20 (4): 32–38. https://doi.org/10.20538/1682-0363-2021-4-32-38.

INTRODUCTION

Dental caries is a multifaceted, dynamic pathological process accompanied by demineralization and proteolysis of dental hard tissues [1]. This chronic disease is widespread among both adults and children [2]. Around the world, there are approximately 2.4 billion people with untreated dental caries [3], which in turn determines the importance of developing prognostic methods and finding ways to eliminate its causes.

Dental caries is formed due to complex internal and external causes, such as dietary habits (frequency of sugar consumption), acidogenic bacteria, and impaired local immunity [2]. Numerous salivary proteins are involved in innate and adaptive immune responses in the oral mucosa. Resistance or susceptibility to dental caries significantly correlates with changes in the salivary protein level that modulate the oral microflora. Therefore, the saliva protein profile is a sensitive indicator of dental health and a non-invasive prognostic biomarker [4].

A number of studies showed that rampant caries develops in patients with a lack of vitamin D [5]. The active form of vitamin D, calcitriol, is one of the main hormones that regulate calcium and phosphorus metabolism and provide mineralization of dental hard tissues [6]. Additionally, this biologically active substance can modulate activity of immune cells and functioning of the immune system and contribute to synthesis and release of antimicrobial peptides, in particular, cathelicidin LL-37 [7–9].

Our earlier study revealed the association between low level of 25(OH)D in the blood serum of patients with moderate to severe caries. In our opinion, this confirms the literature data that a lack of bioactive vitamin D plays an important role in the development of dental caries.

The aim of this study was to assess the level of some immunoregulatory salivary proteins in patients with rampant caries and vitamin D deficiency and to determine the relationship between these values and the concentration of 25(OH)D in the blood.

MATERIALS AND METHODS

The study involved 30 male ChSMA students aged 20–22 years who were divided into two groups. The first group (control) included 15 healthy people (the Decayed, Missing, and Filled Teeth (DMFT) index was 0.00 (0.00; 0.00)) with a normal vitamin D level (30–100 ng / ml). The second group included 15 people with rampant caries (DMFT index was 10.3 (9.5; 11.5)) and vitamin D deficiency (25(OH)D was less than 20 ng / ml). The groups were formed taking into account the "Clinical guidelines of the Russian Association of Endocrinologists on diagnosis, and prevention of vitamin D deficiency in adults (2016)". The serum level of 25(OH)D was determined using the chemiluminescent immunoassay (Immunoassay Analyzer Access 2, Beckman Coulter, USA).

All participants signed an informed consent to take part in the study. The study adhered to the ethical principles of The Declaration of Helsinki of the World Medical Association (as amended in 2013). The levels of soluble membrane proteins (membrane protein of the immunoglobulin superfamily, the product of the *CD86* gene, B7.2 (CD86), free active TGF-β1, co-inhibitory receptors (cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4), programmed cell death-1 (PD-1), T-cell immunoglobulin and mucin domain-3 (Tim-3), lymphocyte-activation gene-3 (LAG-3), insulin-like growth factor binding protein-4 (IGFBP-4), and intercellular adhesion molecule-1 (ICAM-1)) were determined in the oral fluid collected from all the participants using the Human Immuno-Oncology Checkpoint Protein Panel 1 bead-based multiplex panel (Biolegend, USA).

The analysis of the oral fluid was performed without dilution, all stages of the study were conducted according to the kit instructions (https://www.biolegend.com/Files/Images/media_assets/pro_detail/datasheets/750000504_HU_Immune_Checkpoint_Panel_1_Manual_R01.pdf). The results were evaluated using a flow cytometer Cytoflex LX (Beckman Coulter, USA).

Additionally, the levels of antimicrobial peptide cathelicidin LL-37 and secretory immunoglobulin A (sIgA) were assessed by enzyme-linked immunosorbent assay (ELISA) using Hycult Biotechnology (Denmark) and IFA-BEST (Russian Federation) reagent kits, respectively. The data are presented as the median and the interquartile range $Me(Q_{25}; Q_{75})$; the Mann – Whitney test was used to compare two independent samples. The non-parametric Spearman's rank-order correlation coefficient was calculated for the correlation analysis. A p value of < 0.05 was considered statistically significant. The Statistica 10 software packages were used for statistical processing of the data.

RESULTS AND DISCUSSION

The analysis of the research results showed that with rampant caries, free active TGF-β1 was significantly reduced in the saliva (by 67.82%) compared with the control group (Figure).

Tooth pulp is known to contain a number of TGF isoforms that are either expressed by odontoblasts, macrophages, and T- and B-lymphocytes in the tooth pulp at the plasma membrane and/or are bound to the extracellular matrix [10]. It was revealed that TGF-β1 induces synthesis of type III collagen in ontoblasts, regulates transcription of non-collagenous proteins (dentin sialophosphoprotein (DSPP), dentin matrix protein 1 (DMP1)) [10], and is crucial for the reparative process and development of teeth by regulating proliferation and differentiation of cells [10–12]. It is assumed that the physiological function of TGF-β in mature odontoblasts contributes to formation of secondary dentin, mineralization of intact and healthy teeth, as well as to degradation of the matrix in case of injury [11]. Probably, TGF-β1 is also involved in antimicrobial protection of the tooth, although this mechanism is not fully understood.

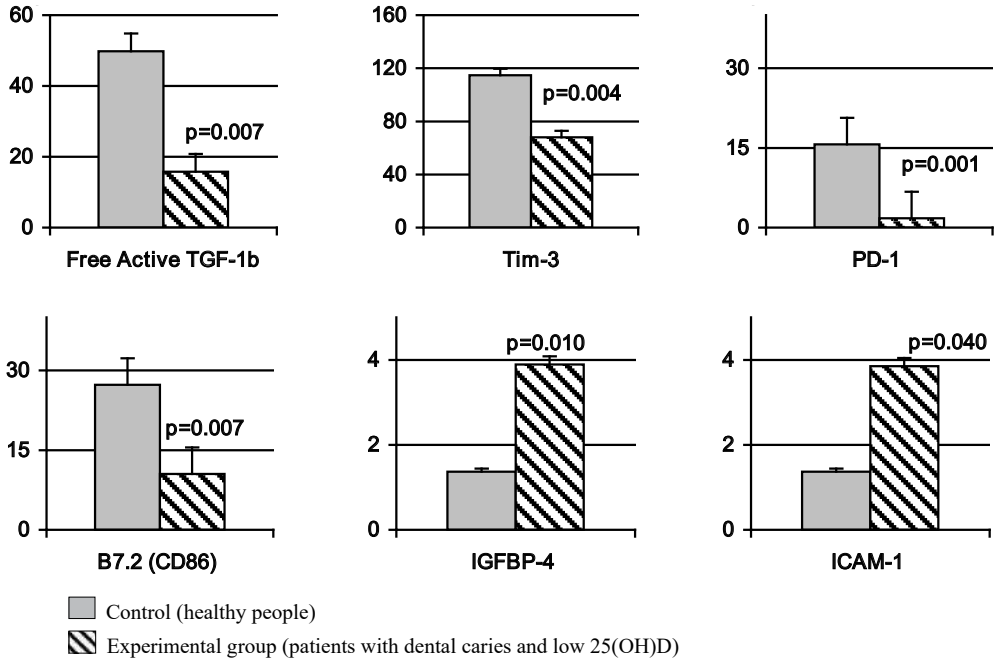


Figure. The saliva level of soluble membrane proteins regulating the immune response, pg / ml

We found a direct moderate relationship ($r = 0.67$; $p < 0.001$) between the levels of free active TGF-β1 in the saliva and 25(OH)D in the blood in individuals with rampant caries and vitamin D deficiency. No such correlation was found in the control group. There are also data in the literature on the association between TGF-β1 and vitamin D in patients with some diseases [13] and on the role of vitamin D in TGF-β1metabolism [14].

Co-stimulatory and co-inhibitory molecules, also referred to as immune checkpoints, play and important role in the development of an adequate cellular immune response. There are several other pathways of co-stimulation and co-inhibition of T-cells. Co-stimulation is provided by binding B7.2 (CD86) protein to CD28 localized at the T-cell membrane. The co-inhibitory signal, which limits the cellular immune response, is induced by receptors that include molecules of the CTLA-4 family (Tim-3, LAG-3, and TIGIT). The expression of these molecules begins at the membrane of T-cells after activation of the latter, by binding to B7.2 (CD86) protein, they inhibit the formation of effector T-cells.

The second component of the immune checkpoint is a co-inhibitory receptor PD-1, whose function is somewhat different from that of CTLA-4. Both receptors suppress proliferation of T-cells, their survival, and cytokine synthesis. However, CTLA-4 suppresses the cellular immune response in the early phase primarily in lymphoid tissues, while PD-1 does the same in the late phase in peripheral tissues [15]. Co-inhibitory receptors are crucial for maintaining immune homeostasis and preventing the development of autoimmunity, while providing effective immune responses to destroy pathogenic microorganisms [15].

In our study, the values of the following parameters were significantly reduced in the group of people with rampant caries and vitamin D deficiency (compared with the control group): PD-1 – by 83.82% ($p = 0.001$), Tim-3 – by 40.75% ($p = 0.004$), and B7.2(CD86) – by 61.47% ($p = 0.007$) (Figure). The LAG-3 levels only showed a downward trend and were 50.11% lower than in the control group.

A correlation analysis revealed a direct correlation between the values of CTLA-4 in the saliva and 25(OH)D ($r = 0.63$; $p = 0.010$) in the blood serum, as well as between the values of B7.2(CD86) and 25(OH)D ($r = 0.70$; $p < 0.001$) in patients with dental caries and vitamin D deficiency. Moreover, in this group, a direct relationship was found between the values of CTLA-4 and B7.2(CD86) in the saliva ($r = 0.77$; $p < 0.001$). In the control group, correlations were found only between the values of CTLA-4 and B7.2(CD86) ($r = 0.56$; $p = 0.019$).

Our study also identified an increased level of IGFBP-4 by 187.39% ($p = 0.01$) in the saliva in patients with rampant caries compared with the control (Figure). IGFBP-4 is a protein that modulates the

effect of insulin-like growth factor-1 (IGF-1). Insulin-like growth factor, its receptors, and binding proteins (IGFBPs) are critical for adequate development, tissue growth, metabolism, and homeostasis [16]. Additionally, IGF-1 is the most abundant growth factor in the bone matrix [16], with its molecular mechanisms being involved in osteogenic differentiation. However, there is a lack of information about the functions of six types of its high-affinity IGF-binding proteins (IGFBP 1–6). Available studies are mainly focused on the role of IGFBP-4 and IGFBP-5 in bone tissue formation [17].

We also found that the saliva ICAM-1 values in patients with caries increased by 181.02% ($p = 0.04$) compared with the control group (Figure). Interestingly, this group showed a negative relationship *(*$r = –0.56$; $p = 0.024$) between the values of the vitamin D metabolite and the values of ICAM-1; in the control group, the relationship was weaker ($r = –0.44$; $p = 0.047$). ICAM-1 is a protein regulating interactions between immune cells and vascular endothelium [18], thus providing strong adhesion of white blood cells to the vessel wall and facilitating penetration of these cells into the intima. Inflammation increases the expression of ICAM-1 [18]. There are studies showing that the level of sICAM-1 in the blood correlated with the severity of periodontitis [https://www.ncbi.nlm.nih.gov/nlmcatalog?term=%22J+Periodontol%22%5bTitle+Abbreviation%5dhttps://pubmed.ncbi.nlm.nih.gov/23688098/19]. C.L. Greillera et al. demonstrated that vitamin D metabolites attenuate RV-induced expression of ICAM-1 [20].

In addition to other protective mechanisms, human saliva contains several immunoglobulins, which account for about 5–15% of all salivary proteins [21]. The main subclass of immunoglobulins found in the saliva is IgA (50–60%), which acts as the first line of defense [4]. Several studies examined the relationship between salivary immunoglobulins and caries formation. A. Bagherian et al. [22] and T.K. Fidalgo et al. [23] found higher IgA concentrations in this disease, but another study reported an negative relationship between the level of this immunoglobulin in the saliva and the intensity of caries in children aged 3–6 years [24]. At the same time, no correlations were found between the concentration of this immunoglobulin and the intensity of the pathological process [22].

In our study, we registered that in patients with rampant caries the IgA level in the saliva decreased by 77.62% ($p < 0.001$) compared with the control group, and the concentration of LL-37 decreased by 62.5% ($p = 0.045$) (Table). The correlation analysis revealed a positive relationship between the values of the vitamin D metabolite and the sIgA ($r = 0.88$; $p = 0.001$) and cathelicidin LL-37 ($r = 0.52$; $p = 0.037$) levels in both experimental and control groups.

Table

The saliva concentration of antimicrobial peptides in the oral cavity of patients with rampant caries and 25(OH)D deficiency, *Me* (Q_{25}; Q_{75})

Parameter	Control group, $n = 15$	Experimental group, $n = 15$
Secretory IgA, g/l	55.00 (50.00; 61.46)	12.31 (9.11; 15.53) $p < 0.001$
Cathelicidin LL-37, ng/ml	0.56 (0.37; 0.72)	0.21 (0.13; 0.38) $p = 0.045$

Note: p – the significance level compared with the control group.

Cathelicidins are antimicrobial peptides essential for innate immunity in the oral cavity [25, 26]. S. Davidopoulou et al. found that the concentration of LL-37 in the saliva was lower in children with highly active dental caries compared with children without this pathology [27]. A bioactive form of vitamin D affects a Th-2 immune response, diminishing the expression of Th-1 cytokines and stimulating release of Th-2 cytokines, in particular, IL-4 [9], which might influence the production of immunoglobulins, including IgA.

Therefore, literature data and the results of this study indicate an imbalance of mucosal immunity as an important factor in the development of dental caries. Additionally, in our opinion, vitamin D deficiency in the body can contribute to the development of this pathology along with decreased levels of free active TGF-β1, B7.2(CD86), PD-1, Tim-3, sIgA, and cathelicidin LL-37 in the saliva and increased levels of IGFBP-4 and ICAM-1. Prescription of vitamin D may alleviate the detected disorders in the oral mucosal immunity.

CONCLUSION

In vitamin D deficiency and rampant caries, low levels of immunoregulatory free active TGF-β1, B7.2 (CD86), PD-1, Tim-3, sIgA, and cathelicidin LL-37 and high concentrations of IGFBP-4 and ICAM-1 are found in the oral fluid compared with the control group.

REFERENCES

1. Petersen P.E. Challenges to improvement of oral health in the 21st century – The approach of the WHO Global Oral Health Programme. *Int. Dent. J.* 2004; 54 (6; Suppl. 1): 329–343. DOI: 10.1111/j.1875-595X.2004.tb00009.x.

2. Takahashi N., Nyvad B. The role of bacteria in the caries process: ecological perspectives. *J. Dent. Res.* 2011; 90 (3): 294–303. DOI: 10.1177/0022034510379602.
3. Simon-Soro A., Sherriff A., Sadique S., Ramage G., Macpherson L., Mira A., Culshaw S., Malcolm J. Combined analysis of the salivary microbiome and host defence peptides predicts dental disease. *Sci. Rep.* 2018; 8 (1): 1484. DOI: 10.1038/s41598-018-20085-x.
4. Hemadi A.S., Huang R., Zhou Y., Zou J. Salivary proteins and microbiota as biomarkers for early childhood caries risk assessment. *Int. J. Oral Sci.* 2017; 9 (11): e1. DOI: 10.1038/ijos.2017.35.
5. Deane S., Schroth R.J., Sharma A., Rodd C. Combined deficiencies of 25-hydroxyvitamin D and anemia in preschool children with severe early childhood caries: A case-control study. *Paediatr. Child. Health.* 2018; 23 (3): e40–e45. DOI: 10.1093/pch/pxx150.
6. Akhpolova V.O., Brin V.B. Calcium exchange and its normal regulation. *Journal of Fundamental Medicine and Biology*. 2017; 2: 38–47 (in Russ.).
7. Firsova I.V., Mokrova E.A., Zavodovsky B.V., Makedonova Yu.A. Vitamin D and its role in the development of dental diseases (Review article). *Modern Problems of Science and Education. Surgery*. 2014; 6: 1047 (in Russ.).
8. Bivona G., Agnello L., Ciaccio M. The immunological implication of the new vitamin D metabolism. *Cent. Eur. J. Immunol.* 2018; 43 (3): 331–334. DOI: 10.5114/ceji.2018.80053.
9. Mak A. The impact of vitamin D on the immunopathophysiology, disease activity, and extra-musculoskeletal manifestations of systemic lupus erythematosus. *Int. J. Mol. Sci.* 2018; 19 (8): 2355. DOI: 10.3390/ijms19082355.
10. Niwa T., Yamakoshi Y., Yamazaki H., Karakida T., Chiba R., Hu J.C.-C., Nagano T., Yamamoto R., Simmer J.P., Margolis H.C., Gomi K. The dynamics of TGF-β in dental pulp, odontoblasts and dentin. *Sci. Rep.* 2018; 8 (1): 4450. DOI: 10.1038/s41598-018-22823-7.
11. Jain A., Bahuguna R. Role of matrix metalloproteinases in dental caries, pulp and periapical inflammation: An overview. *Journal of Oral Biology and Craniofacial Research.* 2015; 5 (3): 212–228. DOI: 10.1016/j.jobcr.2015.06.015.
12. Chan C.P., Lan W.H., Chang M.C., Chen Y.J., Lan W.C., Chang H.H., Jeng J.H. Effects of TGF-βs on the growth, collagen synthesis and collagen lattice contraction of human dental pulp fibroblasts in vitro. *Arch. Oral Biol*. 2005; 50 (5): 469–479. DOI: 10.1016/j.archoralbio.2004.10.005.
13. Isik S., Ozuguz U., Tutuncu Y.A., Erden G., Berker D., Acar K., Aydin Y., Akbaba G., Helvaci N., Guler S. Serum transforming growth factor-beta levels in patients with vitamin D deficiency. *European Journal of Internal Medicine*. 2011; 23 (1): 93–97. DOI: 10.1016/j.ejim.2011.09.017.
14. Fischer K.D., Agrawal D.K. Vitamin D regulating TGF-β induced epithelial-mesenchymal transition. *Respir. Res.* 2014; 15 (1): 146. DOI: 10.1186/s12931-014-0146-6.
15. Joller N., Kuchroo V.K. Tim-3, Lag-3, and TIGIT. *Curr. Top. Microbiol. Immunol*. 2017; 410: 127–156. DOI: 10.1007/82_2017_62.
16. Smith T.J. Insulin-like growth factor-I regulation of immune function: a potential therapeutic target in autoimmune diseases? *Pharmacol. Rev*. 2010; 62 (2): 199–236. DOI: 10.1124/pr.109.002469.
17. Alkharobi H., Alhodhodi A., Hawsawi Y., Alkafaji H., Devine D., El-Gendy R., Beattie J. IGFBP-2 and -3 co-ordinately regulate IGF1 induced matrix mineralisation of differentiating human dental pulp cells. *Stem Cell Res*. 2016; 17 (3): 517–522. DOI: 10.1016/j.scr.2016.09.026.
18. Naeini A.E., Moeinzadeh F., Vahdat S., Ahmadi A., Hedayati Z.P., Shahzeidi S. The effect of vitamin D administration on intracellular adhesion molecule-1 and vascular cell adhesion molecule-1 levels in hemodialysis patients: a placebo-controlled, double-blinded clinical trial. *J. Res. Pharm. Pract*. 2017; 6 (1): 16–20. DOI: 10.4103/2279-042X.200994.
19. Liu J., Duan J., Wang Y., Ouyang X. Intracellular adhesion molecule-1 is regulated by porphyromonas gingivalis through nucleotide binding oligomerization domain-containing proteins 1 and 2 molecules in periodontal fibroblasts. *J. Periodontol*. 2014; 85 (2): 358–368. DOI: 10.1902/jop.2013.130152.
20. Greillera C.L., Suria R., Jolliffea D.A., Kebadzeb T., Hirsmanbd A.G., Griffithsc Ch.J., Johnstonbc S.L., Martineauac A.R. Vitamin D attenuates rhinovirus-induced expression of intercellular adhesion molecule-1 (ICAM-1) and platelet-activating factor receptor (PAFR) in respiratory epithelial cells. *The Journal of Steroid Biochemistry and Molecular Biology.* 2019; 187: 152–159. DOI: 10.1016/j.jsbmb.2018.11.013.
21. Van Nieuw Amerongen A., Bolscher J.G.M., Veerman E.C. Salivary proteins: protective and diagnostic value in cariology? *Caries Res.* 2004; 38 (3): 247–253. DOI: 10.1159/000077762.
22. Bagherian A., Asadikaram G. Comparison of some salivary characteristics between children with and without early childhood caries. *Indian J. Dent. Res.* 2012; 23(5): 628–632. DOI: 10.4103/0970-9290.107380.
23. Fidalgo T.K., Freitas-Fernandes L.B., Ammari M., Mattos C.T., Ribeiro de Souza I.P., Maia L.C. The relationship between unspecific s-IgA and dental caries: a systematic review and meta-analysis. *J. Dent.* 2014; 42 (11): 1372–1381. DOI: 10.1016/j.jdent.2014.07.011.
24. Shifa S., Muthu M.S., Amarlal D., Prabhu Rathna V. Quantitative assessment of IgA levels in the unstimulated whole saliva of caries-free and caries-active children. *J. Indian Soc. Pedodont. Prev. Dent.* 2008; 26 (4): 158–161. DOI: 10.4103/0970-4388.44031.
25. Khurshid Z., Naseem M., Yahya I., Asiri F., Mali M., Khan R.S., Sahibzada H.A., Zafar M.S., Moin S.F., Khan E. Significance and diagnostic role of antimicrobial cathelicidins (LL-37) peptides in oral health. *Biomolecules*. 2017; 7 (4): 80. DOI: 10.3390/biom7040080.
26. Murakami M., Ohtake T., Dorschner R.A., Gallo R.L. Cathelicidin antimicrobial peptides are expressed in salivary glands and saliva. *J. Dent. Res.* 2002; 81 (12): 845–850. DOI: 10.1177/154405910208101210.
27. Davidopoulou S., Diza E., Menexes G., Kalfas S. Salivary concentration of the antimicrobial peptide LL-37 in children. *Arch. Oral. Biol.* 2012; 57 (7): 865–869. DOI: 10.1016/j.archoralbio.2012.01.008.

Authors contribution

Putneva A.S., Karavaeva T.M., Maksimenya M.V., Tereshkov P.P., Tsybikov N.N. – conception and design of the study. Putneva A.S., Tereshkov P.P., Mishchenko M.N., Fefelova E.V. – collection and processing of clinical and biological material. Tereshkov P.P., Putneva A.S., Parshina A.A. – statistical processing, graphic design. Putneva A.S., Karavaeva T.M., Maksimenya M.V. – drafting of the article. Tsybikov N.N., Fefelova E.V., Tereshkov P.P., Mishchenko M.N., Parshina A.A. – editing of the manuscript. Putneva A.S., Karavaeva T.M., Maksimenya M.V., Tereshkov P.P., Mishchenko M.N., Fefelova E.V., Tsybikov N.N., Parshina A.A. – final approval of the manuscript for publication.

Authors information

Putneva Aleksandra S., Post-Graduate Student, Division of Pathological Physiology, ChSMA, Chita, Russian Federation. ORCID 0000-0003-3225-1333.

Karavaeva Tatyana M., Cand. Sci. (Med.), Senior Researcher, Laboratory of Clinical and Experimental Biochemistry and Immunology, Research Institute of Molecular Medicine; Associate Professor, Division of Chemistry and Biochemistry, ChSMA, Chita, Russian Federation. ORCID 0000-0002-0487-6275.

Maksimenya Mariya V., Cand. Sci. (Med.), Senior Researcher, Laboratory of Clinical and Experimental Biochemistry and Immunology, Research Institute of Molecular Medicine; Associate Professor, Division of Chemistry and Biochemistry, ChSMA, Chita, Russian Federation. ORCID 0000-0001-6308-3411.

Tereshkov Pavel P., Cand. Sci. (Med.), Leading Researcher, Laboratory of Clinical and Experimental Biochemistry and Immunology, Research Institute of Molecular Medicine, Associate Professor, Division of Chemistry and Biochemistry, ChSMA, Chita, Russian Federation. ORCID 0000-0002-8601-3499.

Mishchenko Mariya N., Cand. Sci. (Med.), Assistant, Division of Dentistry, Department of Advanced Staff Training, ChSMA, Chita, Russian Federation. ORCID 0000-0003-4678-0527.

Fefelova Elena V., Cand. Sci. (Med.), Associate Professor, Division of Pathological Physiology, ChSMA, Chita, Russian Federation. ORCID 0000-0002-0724-0352.

Tsybikov Namzhil N., Dr. Sci. (Med.), Professor, Head of the Division of Pathological Physiology, ChSMA, Chita, Russian Federation. ORCID 0000-0002-0975-2351.

Parshina Anastasiya A., Assistant, Division of Pathological Physiology, ChSMA, Chita, Russian Federation. ORCID 0000-0002-1458-2385.

(✉) **Putneva Aleksandra S.,** e-mail: putnevaas@yandex.ru

Received 24.09.2020
Accepted 28.12.2020

УДК 617.55-056.257-06:616-036.2(571.1./5)-053.81
https://doi.org/10.20538/1682-0363-2021-4-39-48

Prevalence of diseases and pathological conditions in young people under 45 years of age with abdominal obesity in Siberia

Ragino Yu.I., Khudyakova A.D., Striukova E.V., Denisova D.V., Shcherbakova L.V.

Research Institute of Internal and Preventive Medicine, Branch of the Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Sciences (IIPM – Branch of IC&G SB RAS)
175/1, B. Bogatkova Str., Novosibirsk, 630089, Russian Federation

ABSTRACT

Aim. To study the prevalence of abdominal obesity in young people aged 25–44 years in Novosibirsk, as well as the prevalence of diseases and pathological conditions in individuals with abdominal obesity.

Materials and methods. We conducted a cross-sectional, population-based study of the population of Novosibirsk aged 25–44 years. The screening examined 1,415 people, including 670 men and 745 women. For all individuals, we evaluated the presence of such conditions as abdominal obesity (AO), arterial hypertension (AH), increased body mass index (BMI), coronary heart disease (according to validated epidemiologic and functional criteria with ECG findings classified according to the Minnesota Code), diabetes mellitus (DM), reduced glomerular filtration rate (GFR), chronic bronchitis (CB), increased blood levels of total cholesterol (hypercholesterolemia) and low-density lipoprotein (LDL) cholesterol (hyper-LDL-cholesterolemia).

Results. The prevalence of AO in the population of Novosibirsk aged 25–44 years was 42.4%: in men – 42.7%, in women – 42.1%. We found that AO had a significant direct effect on the development of AH (odds ratio (OR) = 2.550, 95% confidence interval (CI) 1.899–3.422, p = 0.0001), CB (OR = 1.830, CI 1.326–2.527, p = 0.0001), hypercholesterolemia (OR = 1.486, CI 1.193–1.851, p = 0.0001), hyper-LDL-cholesterolemia (OR = 1.527, CI 1.222–1.907, p = 0.0001) and a reverse effect on reduced GFR (OR = 0.603, CI 0.427–0.852, p = 0.004). In the male population under 45 years of age, AO had a significant direct effect on the development of AH, CB, hypercholesterolemia, and hyper-LDL-cholesterolemia. In the female population under the age of 45, AO had a significant direct effect on the development of DM, AH, CB, and hyper-LDL-cholesterolemia and a reverse effect on the reduced GFR development.

Conclusion. Therefore, in the young Siberian population under 45 years of age, abdominal obesity is associated with the development of common diseases and pathological conditions.

Key words: abdominal obesity, population under 45 years of age, arterial hypertension, chronic bronchitis, hypercholesterolemia, diabetes mellitus.

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 No. AAAA-A17-117112850280-2; statistical processing and analysis were supported by the Russian Science Foundation grant No. 21-15-00022.

Conformity with the principles of ethics. An informed consent to examination and processing of personal data was obtained from all individuals. The study was approved by the local Ethics Committee at IIPM – Branch of IC&G SB RAS (Protocol No. 6/2013 of 25.06.2013).

For citation: Ragino Yu.I., Khudyakova A.D., Striukova E.V., Denisova D.V., Shcherbakova L.V. Prevalence of diseases and pathological conditions in young people under 45 years of age with abdominal obesity in Siberia. *Bulletin of Siberian Medicine.* 2021; 20 (4): 39–48. https://doi.org/10.20538/1682-0363-2021-4-39-48.

✉ *Striukova Evgeniia V.,* e-mail: stryukova.j@mail.ru

Распространенность заболеваний и патологических состояний у молодых людей до 45 лет с абдоминальным ожирением в Сибири

Рагино Ю.И., Худякова А.Д., Стрюкова Е.В., Денисова Д.В., Щербакова Л.В.

Научно-исследовательский институт терапии и профилактической медицины – филиал Федерального исследовательского центра «Институт цитологии и генетики Сибирского отделения Российской академии наук» (НИИТПМ – филиал ИЦиГ СО РАН)
Россия, 630089, г. Новосибирск, ул. Б. Богаткова, 175/1

РЕЗЮМЕ

Цель – изучение распространенности абдоминального ожирения (АО) в популяции молодых людей 25–44 лет г. Новосибирска, а также распространенности терапевтических заболеваний и патологических состояний у лиц с АО.

Материалы и методы. Проведено одномоментное популяционное обследование населения 25–44 лет г. Новосибирска. На скрининге обследованы 1 415 человек, из них 670 мужчин (47,3%) и 745 женщин (52,7%). Беременные и женщины в декретном отпуске не включались в исследование. У обследуемых оценивалось наличие таких заболеваний и патологических состояний, как АО, артериальная гипертензия (АГ), повышенный индекс массы тела, ишемическая болезнь сердца (по валидизированным эпидемиологическим и функциональным критериям с расшифровкой электрокардиограммы по Миннесотскому коду), сахарный диабет (СД), сниженная скорость клубочковой фильтрации (СКФ), хронический бронхит (ХБ), повышенный уровень в крови общего ХС (гиперХСемия), повышенный уровень в крови ХС-ЛНП (гиперХС-ЛНПемия).

Результаты. Распространенность АО в популяции 25–44 лет г. Новосибирска составила 42,4%, у мужчин – 42,7%, у женщин – 42,1%. Обнаружено, что в молодой популяции до 45 лет АО оказывает прямое влияние на развитие АГ (отношение шансов (ОШ) 2,550, 95%-й доверительный интервал (95%-й ДИ) 1,899–3,422, p = 0,0001), ХБ (ОШ = 1,830, 95%-й ДИ 1,326–2,527, p = 0,0001), гиперХСемии (ОШ = 1,486, 95%-й ДИ 1,193–1,851, p = 0,0001), гипер-ХС-ЛНПемии (ОШ = 1,527, 95%-й ДИ 1,222–1,907, p = 0,0001), обратное влияние на развитие сниженной СКФ (ОШ = 0,603, 95%-й ДИ 0,427–0,852, p = 0,004). В мужской популяции до 45 лет АО оказывает прямое влияние на развитие АГ, ХБ, гиперХСемии, гипер-ХС-ЛНПемии. В женской популяции до 45 лет АО оказывает прямое влияние на развитие СД, АГ, ХБ, гипер-ХС-ЛНПемии, и обратное – на развитие сниженной СКФ.

Заключение. Таким образом, в популяции до 45 лет абдоминальное ожирение ассоциировано с развитием распространенных терапевтических заболеваний и патологических состояний.

Ключевые слова: абдоминальное ожирение, популяция в возрасте до 45 лет, артериальная гипертензия, хронический бронхит, гиперхолестеринемия, сахарный диабет.

Конфликт интересов. Авторы заявляют об отсутствии конфликта интересов, связанных с публикацией настоящей статьи.

Источник финансирования. Исследование выполнено в рамках бюджетной темы по государственному заданию № АААА 17-117112850280-2, статистическая обработка и анализ материала проведены при финансовой поддержке гранта Российского научного фонда № 21-15-00022.

Соответствие принципам этики. От всех лиц получено информированное согласие на обследование и обработку персональных данных. Исследование одобрено локальным этическим комитетом НИИТПМ – филиала ИЦиГ СО РАН (протокол № 6/2013 от 25.06.2013).

Для цитирования: Рагино Ю.И., Худякова А.Д., Стрюкова Е.В., Денисова Д.В., Щербакова Л.В. Распространенность заболеваний и патологических состояний у молодых людей до 45 лет с абдоминальным ожирением в Сибири. *Бюллетень сибирской медицины.* 2021; 20 (4): 39–48. https://doi.org/10.20538/1682-0363-2021-4-39-48.

INTRODUCTION

Currently, obesity is a topical issue worldwide. The disease is associated with progressive spread and severe complications, which often cause death of patients at a young age [1, 2].

Recent research on abdominal obesity (AO) around the world has been devoted to the study of its impact on endocrine and cardiovascular pathologies [3–5].

Recent studies show that visceral adipose tissue serves not only for accumulation of energy substrates. It is also a kind of endocrine gland producing many different substances, which act at both local and systemic level. The products of adipocyte (visceral adipose tissue cells) secretion are hormones (leptin, adiponectin, and resistin), proinflammatory cytokines (tumor necrosis factor-alpha, interleukin (IL)-6, IL-8, etc.), and proteins of the renin-angiotensin system. Some of them are involved in the complement system and vascular hemostasis (plasminogen activator inhibitor-1 and others) [6–9]. Based on the known pathological effects of various biological substances secreted by visceral adipocytes, an increased blood level of adipokines/cytokines in AO is assumed to be a significant etiopathogenetic link in the development of many common diseases and pathological conditions.

Novosibirsk (Russian Federation) shows high prevalence of AO and metabolic syndrome (MS) in the population over 45 years of age [10]. The problem of AO is poorly studied in the young working-age population of reproductive age. Therefore, the study is dedicated to the prevalence of AO in the population of Novosibirsk aged 25–44-years, as well as to the prevalence of diseases and pathological conditions in individuals with AO.

MATERIALS AND METHODS

A cross-sectional, population-based study of the population of Novosibirsk was carried out. The study was approved by the local Ethics Committee at IIPM – Branch of IC&G SB RAS (Protocol No. 6/2013 of 25.06.2013). To build a population sample, we used a database of the Territorial Federal Compulsory Medical Insurance Fund for persons aged 25–44 years in one of the districts of Novosibirsk. The district was typical in terms of industrial, social, population, demographic, and transport structures and the level of population migration. Using a random number generator, a random representative sample of 2,500 people was built. Young age groups are known to be among the most rigid ones regarding response, so methods of gradual epidemiological stimulation were used: mail invitations, phone calls, information messages in the mass media. 1,415 people were examined at the screening – 670 men (47.3%) and 745 women (52.7%). The study did not include pregnant women and women on maternity leave. The response was 56.6%. An informed consent to the examination and personal data processing was obtained from all individuals participating in the study.

A team of doctors trained in standardized epidemiological screening methods conducted the screening procedure. The survey program included demographic and social data collection, a survey on smoking habits and alcohol use, a socioeconomic survey, a dietary survey, history of chronic diseases and medication use, Rose questionnaire, anthropometry, triplicate measurement of blood pressure (BP), spirometry, an ECG with findings interpreted according to the Minnesota code, etc.

Waist circumference (WC) was determined with a measuring tape applied horizontally in the middle between the lower edge of the costal arch and the sacral part of the ilium. AO was determined with WC ≥ 94 cm in men and ≥ 80 cm in women [11, 12]. BP was measured three times with an interval of two minutes on the right arm in a sitting position after a 5-minute rest using an automatic digital blood pressure monitor Omron M5-I (Japan). The average value for 3 measurements was registered. Arterial hypertension (AH) was determined at systolic blood pressure (SBP) ≥ 140 mmHg and / or diastolic blood pressure (DBP) ≥ 90 mmHg [12]. The body mass index (BMI) was calculated using the formula $I = m/h2$, where m – body weight (kg), h – height. BMI was considered increased at > 25 kg/m^2 [12]. Individuals who smoked at least one cigarette a day were considered smokers.

The epidemiological diagnosis of coronary artery disease (CAD) was made using validated epidemiological (the Rose Angina Questionnaire) and functional criteria (an ECG with findings classified according to the Minnesota Code (MC)). We used the following ECG determination of CAD based on the mentioned MC classification system for electrocardiographic findings (WHO guidelines):

– ECG changes indicative of new ischemia (new ST-T changes or new left bundle branch block (LBBB) – the Minnesota codes: ST-depression 4.1; 4.2; ST-elevation 9.2; LBBB 7.1);

– development of pathological Q waves in the ECG (the Minnesota codes: 1.1.1–1.2.5, 1.2.7), including the absence of unequivocal pathological Q waves in the first ECG or in the set of ECGs followed by a record with a pathological Q wave, or any Q

wave in leads V2–V3 ≈ 0.2 s, or a QS complex in leads V2 and V3, or Q ≈ 0.03 s and ≈ 0.01 mV deep, or a QS complex in leads I, II, aVL, aVF or V4–V6 in any two leads of a contiguous lead grouping (I, aVL, V6: V4–V6: II, III, aVF).

Diabetes mellitus (DM) was established according to the epidemiological criteria at fasting plasma glucose levels ≥ 7.0 mmol/l [13] and/or normoglycemia in individuals with a medical history of established DM. The glomerular filtration rate (GFR) was calculated using the CKD-EPI formula (Chronic Kidney Disease Epidemiology Collaboration), which takes into account race, sex, age, and serum creatinine [14]. A decrease in the GFR was recorded at GFR < 90 ml/min/1.73 cm^2, GFR was considered normal at GFR ≥ 90 ml/min/1.73 cm^2. Microalbuminuria was not determined.

The WHO Respiratory Diseases questionnaire and the European Community Respiratory Health Survey (ECRHS) were used to detect respiratory symptoms. The epidemiological diagnosis of chronic bronchitis (CB) was established in the presence of cough with mucus for at least three months a year or if there was a medical history of the disease. In addition, the respiratory function was studied with the help of spirometry according to the recommendations for performing spirometry on the Spiro USB Micro spirometer (Medical Limited, Great Britain).

Three reproducible attempts were selected for the analysis. The spirometry results were recorded and processed by the Spida 5 PC-based diagnostic software. We selected the best parameters of forced expiratory volume in 1 second, forced vital capacity, and their ratio to assess the respiratory function. The calculation of the respiratory function indices was carried out using comparative equations of proper values obtained in the Third National Health and Nutrition Examination Survey (NHANES III) [15].

A single blood sampling from the ulnar vein was performed on an empty stomach after 12-hour fasting. Blood parameters of the lipid profile, glucose, and creatinine were measured by the enzymatic method using standard TermoFisher reagents on the automatic biochemistry analyzer KoneLab 30i (Finland). Conversion of serum glucose to plasma glucose was performed according to the formula: plasma glucose (mmol/l) = –0.137 + 1.047 × serum glucose (mmol/l). The values ≥ 5.0 mmol/l were considered increased blood levels of total cholesterol (hypercholesterolemia), the values ≥ 3.0 mmol/l were considered increased blood levels of LDL-C [12].

Statistical processing of the obtained results was performed using the SPSS software package (version 13.0). In the tables and text, the obtained data are presented as absolute and relative values *n* (%) for categorical variables and as *Me* (Q_{25}; Q_{75}) for continuous variables due to non-normal distribution of most variables. The Kolmogorov – Smirnov test was used to check the normality of distribution. The nonparametric Mann – Whitney U-test was used to assess the differences between two independent samples. The Pearson's chi-squared test was used to compare the differences between the sets of data. Associations were evaluated with the help of multiple logistic regression analysis, performed under the following conditions: the dependent variable is dichotomous; independence of observations; absence of multicollinearity, i.e., situations when the independent variables strongly correlate with one other ($r > 0.9$); the linear dependence between each independent variable and the logarithm of the odds ratio (OR) (log odds); independence of the residuals. The results of the multiple logistic regression analysis were presented as OR and 95% confidence interval (95% CI) for OR. The critical significance level of the null hypothesis (p) was equal to 0.05.

RESULTS

1,415 individuals were examined, and AO was determined in 600 people (42.4%). 286 of the 670 examined men had AO (42.7%) and 314 of the 745 examined women had AO (42.1%). The prevalence of AO in the population of Novosibirsk aged 25–44 years was 42.4%: 42.7% in men and 42.1% in women.

Table 1 shows the characteristics of the studied factors depending on the presence of AO in the population of Novosibirsk aged 25–44 years. The individuals with AO, both men and women, demonstrated higher values of SBP, DBP, hip circumference (HC), BMI, age, blood glucose levels, total cholesterol, and LDL-C. Male population did not show any differences in the blood levels of creatinine depending on the presence of AO. Female population with

AO had lower blood creatinine levels than women without AO.

Table 2 shows the prevalence of certain diseases and pathological conditions in people under 45 years of age with AO. In the population of individuals with AO, the prevalence of DM was 2.3 times higher, AH was 2.3 times higher, CB was 1.5 times higher, hypercholesterolemia was 1.3 times higher, and hyper-LDL-cholesterolemia was 1.2 times higher than in individuals without AO. Similar results were found in women. Thus, in women with AO, the prevalence of DM was 13.0 times higher, AH was 4.4 times higher, CB was 1.9 times higher, hypercholesterolemia was 1.2 times higher, and hyper-LDL-cholesterolemia was 1.3 times higher than in individuals without AO. Men showed significant differences only for hypertension and lipid disorders. So, in men with AO, the prevalence of hypertension was 1.8 times higher, hypercholesterolemia – 1.4 times higher, and hyper-LDL-cholesterolemia – 1.2 times higher than in men without AO. No differences were detected in the prevalence of CAD and reduced GFR depending on the presence of AO, both in men and women.

Table 1

Characteristics of the studied factors depending on the presence of abdominal obesity in the population of Novosibirsk aged 25–44 years, *Me* (Q_{25}; Q_{75})

Continuous variables	Population, *n* = 1.415			Men, *n* = 670			Women, *n* = 745		
	AO есть, *n* = 600	AO нет, *n* = 815	*p*	AO есть, *n* = 286	AO нет, *n* = 384	*p*	AO есть, *n* = 314	AO нет, *n* = 431	*p*
SBP	123.0 (114.0; 134.0)	116.5 (107.5; 125.5)	<0.001	128.0 (120.0; 137.5)	122.5 (115.5; 131.5)	<0.001	118.3 (109.5; 129.0)	110.3 (103.5; 118.5)	<0.001
DBP	82.5 (75.0; 90.0)	76.0 (69.5; 82.5)	<0.001	85.5 (79.5; 92.5)	80.0 (74.5; 88.0)	<0.001	79.0 (72.0; 86.0)	72.0 (66.5; 77.9)	<0.001
WC	97.8 (88.8; 103.5)	77.0 (71.0; 85.0)	<0.001	101.1 (98.0; 107.9)	85.9 (81.0; 90.0)	<0.001	89.6 (84.9; 98.0)	72.0 (67.6; 76.0)	<0.001
Glucose	5.83 (5.41; 6.18)	5.62 (5.20; 5.94)	<0.001	5.94 (5.52; 6.25)	5.73 (5.41; 6.15)	<0.001	5.73 (5.41; 6.04)	5.41 (5.10; 5.73)	<0.001
Total cholesterol (TC)	5.14 (4.52; 5.79)	4.83 (4.21; 5.43)	<0.001	5.27 (4.65; 5.50)	4.87 (4.26; 5.50)	<0.001	5.01 (4.39; 5.68)	4.78 (4.19; 5.39)	0.001
LDL-C	3.26 (2.66; 3.83)	3.02 (2.45; 3.60)	<0.001	3.34 (2.82; 4.01)	3.15 (2.55; 3.70)	0.001	3.15 (2.58; 3.72)	2.89 (2.36; 3.50)	<0.001
Creatinine	74.0 (66.5; 82.0)	75.0 (68.0; 82.0)	0.117	81.0 (73.0; 87.0)	79.0 (73.0; 86.0)	0.485	69.0 (64.0; 74.0)	71.0 (66.0; 77.0)	0.002
HC	108.7 (104.0; 114.0)	96.2 (92.9; 100.0)	<0.001	101.1 (104.5; 113.0)	97.1 (94.0; 100.4)	<0.001	109.0 (103.8; 116.0)	95.0 (91.8; 99.0)	<0.001
BMI	29.50 (27.07; 32.43)	22.60 (20.66; 24.66)	<0.001	29.90 (28.02; 32.41)	23.78 (21.85; 25.57)	<0.001	29.0 (25.81; 32.44)	21.78 (20.09; 23.44)	<0.001
Age	39.0 (33.8; 42.8)	35.8 (31.0; 41.1)	<0.001	38.5 (33.0; 42.4)	35.0 (30.4; 40.4)	<0.001	39.5 (34.8; 43.1)	36.4 (31.4; 41.4)	<0.001

Table 2

Prevalence of diseases and pathological conditions depending on the presence of abdominal obesity in the population of Novosibirsk aged 25-44 years, %

Categorical variables	Population, *n* = 1.415			Men, *n* = 670			Women, *n* = 745		
	AO (+), *n* = 600	AO (–), *n* = 815	*p*	AO (+), *n* = 286	AO (–), *n* = 384	*p*	AO (+), *n* = 314	AO (–), *n* = 431	*p*
Detected CAD	3.8	3.0	0.430	2.9	2.6	0.839	4.7	3.4	0.396
DM	3.5	1.5	0.022	4.9	11.0	0.260	2.6	0.2	0.004
AH	28.1	12.3	<0.0001	37.9	21.1	<0.0001	19.2	4.4	<0.001

Table 2 (continued)

Categorical variables	Population, n = 1.415			Men, n = 670			Women, n = 745		
	AO (+), n = 600	AO (–), n = 815	p	AO (+), n = 286	AO (–), n = 384	p	AO (+), n = 314	AO (–), n = 431	p
CB	26.5	17.7	<0.001	31.5	24.4	0.072	21.8	11.7	0.001
Hypercholesterolemia	56.2	43.5	<0.0001	61.4	44.7	<0.0001	51.4	42.5	0.016
Hyper-LDL-cholesterolemia	62.5	50.4	<0.0001	66.3	56.2	0.009	59.1	45.3	0.001
Reduced GFR	21.3	24.4	0.254	9.8	9.9	0.983	30.5	37.3	0.166

At the next stage of the study, a logistic regression analysis was performed to assess the impact of AO on the development of diseases and pathological conditions (Table 3). The categorical variables of CAD, DM, AH, CB, lipid disorders, and reduced GFR were included in individual models as dependent variables, whereas AO, sex, age, and some other parameters were taken as independent variables.

Table 3

Logistic regression analysis of the impact of abdominal obesity on the development of diseases and pathological conditions in the population of Novosibirsk aged 25–44 years

Categorical variables	Population, n = 1,415			Men, n = 670			Women, n = 745		
	OR	95% CI	p	OR	95% CI	p	OR	95% CI	p
Detected CAD	1.158	0.624 - 2.147	0.642	1.048	0.384 – 2.856	0.928	1.194	0.538 – 2.652	0.663
DM	1.971	0.950 – 4.087	0.068	1.255	0.545 – 2.890	0.593	10.765	1.316 – 88.057	0.027
AH	2.550	1.899 – 3.422	0.0001	2.070	1.450 – 2.956	0.0001	4.074	2.343 – 7.082	0.0001
CB	1.830	1.326 – 2.527	0.0001	1.655	1.069 – 2.563	0.024	2.130	1.311 – 3.459	0.002
Hypercholesterolemia	1.486	1.193 – 1.851	0.0001	1.805	1.313 – 2.483	0.0001	1.293	0.957 – 1.746	0.094
Hyper-LDL-cholesterolemia	1.527	1.222 – 1.907	0.0001	1.439	1.040 – 1.990	0.028	1.595	1.180 – 2.156	0.002
Reduced GFR	0.603	0.427 – 0.852	0.004	0.708	0.364 – 1.376	0.309	0.573	0.382 – 0.861	0.007

We found a significant impact of AO on the development of AH in the population, including men and women (Table 3). In the general population, age (OR = 1.089, 95% CI 1.062–1.117, p = 0.0001), male sex (OR = 3.632, 95% CI 2.677–4.928, p = 0.0001), and smoking (OR = 1.689, 95% CI 1.188–2.402, p = 0.003) significantly influenced the development of AH along with AO. In men, only age, along with AO, influenced the development of hypertension (OR = 1.074, 95% CI 1.041–1.107, p = 0.0001). In women, age (OR = 1.128, 95% CI 1.077–1.180, p = 0.0001) and smoking (OR = 2.102, 95% CI 1.166–3.789, p = 0.014), along with AO, had a significant impact on the development of hypertension.

We found a significant impact of AO on the development of CB in the population, including men and women (Table 3). In the general population, age (OR = 1.038, 95% CI 1.010–1.067, p = 0.008), male sex (OR = 1.457, 95% CI 1.062–2.001, p = 0.020), and smoking (OR = 6.284, 95% CI 4.242–9.308, p = 0.0001) significantly influenced the development of CB along with AO. In men, the development of CB was significantly influenced by age (OR = 1.040, 95% CI 1.002–1.080, p = 0.039) and smoking (OR = 7.268, 95% CI 3.981–13.270, p = 0.0001), along with AO. In women, only smoking, along with AO, significantly influenced the development of CB (OR = 5.230, 95% CI 3.062–8.933, p = 0.0001).

A significant effect of AO on the development of hyper-LDL-cholesterolemia in the population, including men, was detected (Table 3). In the general population, age (OR = 1.037, 95% CI 1.019–1.056, p = 0.0001) and male sex (OR = 1.541, 95% CI 1.247–1.905, p = 0.0001) significantly influenced the development of hyper-LDL-cholesterolemia along with AO. In men, only age, along with AO, influenced the development of hyper-LDL-cholesterolemia (OR = 1.032, 95% CI 1.005–1.060,

$p = 0.020$). In women, only age had a significant effect on the development of hyper-LDL-cholesterolemia (OR = 1.042, 95% CI 1.016–1.067, $p = 0.001$).

A significant effect of AO on the development of hypercholesterolemia in the population, including men, was identified (Table 3). In the general population, age (OR = 1.046, 95% CI 1.028–1.065, $p = 0.0001$) and male sex (OR = 1.310, 95% CI 1.064–1.613, $p = 0.011$), along with AO, significantly influenced the development of hypercholesterolemia. In men, only age, along with AO, influenced the development of hypercholesterolemia (OR = 1.048, 95% CI 1.021–1.076, $p = 0.001$). In women, the development of hypercholesterolemia was affected only by age (OR = 1.045, 95% CI 1.021–1.069, $p = 0.0001$).

A significant influence of AO on the development of DM in women was also detected (Table 3). In the general population, the development of DM was influenced by age (OR = 1.113, 95% CI 1.045–1.185, $p = 0.001$) and male sex (OR = 2.976, 95% CI 1.440–6.151, $p = 0.003$); in men – only by age (OR = 1.152, 95% CI 1.065–1.247, $p = 0.0001$) and in women – only by AO.

Finally, a significant reverse effect of AO on the development of reduced GFR in the population, including women, was established (Table 3). In the general population, age (OR = 1.083, 95% CI 1.054–1.13, $p = 0.0001$) and female sex (OR = 0.183, 95% CI 0.125–0.268, $p = 0.0001$), along with AO, significantly influenced the development of reduced GFR. In men, the development of reduced GFR was influenced only by age (OR = 1.099, 95% CI 1.035–1.167, $p = 0.002$). In women, age, along with AO, also had a significant effect on the development of reduced GFR (OR = 1.078, 95% CI 1.046–1.12, $p = 0.0001$). No effect of AO on the CAD development in the population, including men and women, was found.

In the young population under the age of 45, AO had a significant direct effect on the development of AH, CB, hypercholesterolemia, hyper-LDL-cholesterolemia and a reverse effect on the development of reduced GFR. In the male population under 45 years of age, AO had a significant direct effect on the development of AH, CB, hypercholesterolemia, and hyper-LDL-cholesterolemia. In the female population under the age of 45, AO had a significant direct effect on the development of DM, AH, CB, and hyper-LDL-cholesterolemia and a reverse effect on the development of reduced GFR.

DISCUSSION

Our results confirming the direct effect of AO on the AH development in young people under 45 years of age correspond with the known data on the relationship between AO and AH, also as criteria/signs of metabolic syndrome (MS), as well as to data from other studies of recent years. Thus, Y. Zhao et al. in the cohort study of rural Chinese residents of a broad age group showed that AO increased a 6-year risk of developing AH in both men and women [16]. J. B. Almeida et al. found that young women with AO aged 20–59 years demonstrated a two times higher AH prevalence than women without AO [17].

Our results regarding the direct effect of AO on the development of CB in young people under 45 years of age do not contradict the data of other studies. E. Pekkarinen et al. concluded that even mild AO in healthy non-smoking adults was associated with obstructive changes in the lungs and a decrease in the vital capacity of the lungs according to spirometry [18]. Similar data were obtained by A. Vatrella et al. in the cohort study of women in Italy [19]. Discussing a possible mechanism of the association between AO and CB, it is essential to note the etiopathogenetic synergy of proinflammatory biomolecules secreted by visceral adipocytes in AO and factors of chronic inflammation that potentiate the development of chronic inflammatory diseases, including CB [6, 20].

The obtained results regarding the direct effect of AO on the development of lipid disorders (hypercholesterolemia, hyper-LDL-cholesterolemia) in young people under 45 years of age have not come as unexpected, since the data on the relationship between AO and lipid disorders, also as criteria/signs of MS, have also been known for a long time. Z. Hertelyova et al. also found a positive association of non-HDL-C with increased WC and BMI in students. However, unlike us, they did not find an association of WC with the level of total blood cholesterol [21].

MS based on AO and insulin resistance is known to play a potentiating role in the development of type 2 diabetes. In this respect, the expected results regarding the association of AO and DM were not obtained, since this association was identified only

in women. However, it should be noted that our study determined DM only with the help of epidemiological criteria (fasting plasma glucose level) [13] and did not take into account the type of DM. In the 12-year cohort study, F. Salehinia et al. also identified an association of AO with the development of type 2 diabetes only in women over 20 years of age, but not in men [22].

Our results regarding the reverse effect of AO on the development of reduced GFR in young people under 45 years of age, including women, do not correspond with the data of other studies. Several studies demonstrated a direct association of AO with reduced GFR and kidney pathology [23–25]. On the other hand, A. Shahali et al. found no association of AO with an increased risk of kidney failure in either men or women in the cohort study of 7,002 people over the age of 20 [26]. It is important to note that our study considered GFR < 90 ml/min/1.73 cm^2 as reduced GFR, since there were only 4 young people with reduced GFR < 60 ml/min/1.73 cm2 (this criterion for reduced GFR is used in the majority of studies), which was not enough for a correct statistical analysis of the results.

Finally, the expected association of AO with early CAD (according to epidemiological criteria) in young people aged 25–44 years was not identified. The obtained data differ from the known results of other numerous studies showing a direct association of AO with CAD development and its complications. It should be noted that most of these studies were conducted on populations, cohorts or selective clinical groups of people over 45 years of age. Our results presented in Tables 2 and 3 reflect higher prevalence of CAD in individuals with AO and a direct association of CAD with AO, but their statistical significance has not been achieved. This is probably due to the low number of CAD cases in the examined young population under the age of 45.

CONCLUSION

Therefore, it is important to note that AO, including AO in young people, probably causes and triggers the development of not only endocrine and cardiovascular diseases, but also a broad range of other socially sensitive diseases and pathological conditions. These studies will undoubtedly continue, including the search for pathogenetic associations of AO with the development of a wide range of diseases.

REFERENCES

1. Ural D., Kiliçkap M., Göksülük H., Karaaslan D., Kayikçioğlu M., Özer N., Barçin C., Yilmaz M.B., Abaci A., Şengül Ş., Arinsoy T., Erdem Y., Sanisoğlu Y., Şahin M., Tokgözoğlu L. Data on prevalence of obesity and waist circumference in Turkey: Systematic review, meta-analysis and meta-regression of epidemiological studies on cardiovascular risk factors. *Turk Kardiyol. Dern. Ars.* 2018; 46: 577–590. DOI: 10.5543/tkda.2018.62200.
2. Triggiani A.I., Valenzano A., Trimigno V., Di Palma A., Moscatelli F., Cibelli G., Messina G. Heart rate variability reduction is related to a high amount of visceral adiposity in healthy young women. *PLoS One.* 2019; 14. (2019). DOI: 10.1371/journal.pone.0223058.
3. Kim H.Y., Kim J.K., Shin G.G., Han J.A., Kim J.W. Association between Abdominal Obesity and Cardiovascular Risk Factors in Adults with Normal Body Mass Index: Based on the Sixth Korea National Health and Nutrition Examination Survey. *J. Obes. Metab. Syndr.* 2019; 28: 262–270. DOI: 10.7570/jomes.2019.28.4.262.
4. Melin E.O., Thulesius H.O., Hillman M., Landin-Olsson M., Thunander M. Abdominal obesity in type 1 diabetes associated with gender, cardiovascular risk factors and complications, and difficulties achieving treatment targets: A cross sectional study at a secondary care diabetes clinic. *BMC Obes.* 2018; 5. DOI: 10.1186/s40608-018-0193-5.
5. Mohammadi H., Ohm J., Discacciati A., Sundstrom J., Hambraeus K., Jernberg T., Svensson P. Abdominal obesity and the risk of recurrent atherosclerotic cardiovascular disease after myocardial infarction. *Eur. J. Prev. Cardiol.* 2020; 27: 1944–1952. DOI: 10.1177/2047487319898019.
6. Alexopoulos N., Katritsis D., Raggi P. Visceral adipose tissue as a source of inflammation and promoter of atherosclerosis. *Atherosclerosis.* 2014; 233: 104–112. DOI: 10.1016/j.atherosclerosis.2013.12.023.
7. Ragino Y.I., Stakhneva E.M., Polonskaya Y.V., Kashtanova E.V. The role of secretory activity molecules of visceral adipocytes in abdominal obesity in the development of cardiovascular disease: A review. *Biomolecules.* 2020; 10. DOI: 10.3390/biom10030374.
8. Dutheil F., Gordon, B.A. Naughton G., Crendal E., Courteix D., Chaplais E., Thivel D., Lac G., Benson A.C. Cardiovascular risk of adipokines: a review. *J. Int. Med. Res.* 2018; 46: 2082–2095. DOI: 10.1177/0300060517706578.
9. Neeland I.J., Ross R., Després J.P., Matsuzawa Y., Yamashita S., Shai I., Seidell J., Magni P., Santos R.D., Arsenault B., Cuevas A., Hu F.B., Griffin B., Zambon A., Barter P., Fruchart J.C., Eckel R.H. Visceral and ectopic fat, atherosclerosis, and cardiometabolic disease: a position statement. *Lancet Diabetes Endocrinol.* 2019; 7: 715–725. DOI: 0.1016/S2213-8587(19)30084-1.
10. Vikhireva O., Pająk A., Broda G., Malyutina S., Tamosiunas A., Kubinova R., Simonova G., Skodova Z., Bobak M., Pikhart H. SCORE performance in central and eastern Europe and former Soviet Union: MONICA and HAPIEE results. *Eur. Heart J.* 2014; 35. DOI: 10.1093/eurheartj/eht189.

11. Alberti K.G.M.M., Eckel R.H., Grundy S.M., Zimmet P.Z., Cleeman J.I., Donato K.A., Fruchart J.C., James W.P.T., Loria C.M., Smith S.C. Harmonizing the metabolic syndrome: A joint interim statement of the international diabetes federation task force on epidemiology and prevention; National heart, lung, and blood institute; American heart association; World heart federation; International atherosclerosis society; And international association for the study of obesity. *Circulation.* 2009; 120: 1640–1645. DOI: 10.1161/CIRCULATIONAHA.109.192644.
12. Mach F., Baigent C., Catapano A.L., Koskinas K.C., Casula M., Badimon L., Chapman M.J., De Backer G.G., Delgado V., Ference B.A., Graham I.M., Halliday A., Landmesser U., Mihaylova B., Pedersen T.R., Riccardi G., Richter D.J., Sabatine M.S., Taskinen M.R., Tokgozoglu L., Wiklund O., Mueller C., Drexel H., Aboyans V., Corsini A., Doehner W., Farnier M., Gigante B., Kayikcioglu M., Krstacic G., Lambrinou E., Lewis B.S., Masip J., Moulin P., Petersen S., Petronio A.S., Piepoli M.F., Pinto X., Raber L., Ray K.K., Reiner Z., Riesen W.F., Roffi M., Schmid J.P., Shlyakhto E., Simpson I.A., Stroes E., Sudano I., Tselepis A.D., Viigimaa M., Vindis C., Vonbank A., Vrablik M., Vrsalovic M., Gomez J.L.Z., Collet J.P., Windecker S., Dean V., Fitzsimons D., Gale C.P., Grobbee D.E., alvorsen S., Hindricks G., Iung B., Jüni P., Katus H.A., Leclercq C., Lettino M., Merkely B., Sousa-Uva M., Touyz R.M., Nibouche D., Zelveian P.H., Siostrzonek P., Najafov R., Van De Borne P., Pojskic B., Postadzhiyan A., Kypris L., Spinar J., Larsen M.L., Eldin H.S., Strandberg T.E., Ferrières J., Agladze R., Laufs U., Rallidis L., Bajnok L., Gudjonsson T., Maher V., Henkin Y., Gulizia M.M., Mussagaliyeva A., Bajraktari G., Kerimkulova A., Latkovskis G., Hamoui O., Slapikas R., Visser L., Dingli P., Ivanov V., Boskovic A., Nazzi M., Visseren F., Mitevska I., Retterstøl K., Jankowski P., Fontes-Carvalho R., Gaita D., Ezhov M., Foscoli M., Giga V., Pella D., Fras Z., De Isla L.P., Hagström E., Lehmann R., Abid L., Ozdogan O., Mitchenko O., Patel R.S. 2019 ESC/EAS guidelines for the management of dyslipidaemias: Lipid modification to reduce cardiovascular risk. *Eur. Heart J.* 2020; 41: 111–188. DOI: 10.1093/eurheartj/ehz455.
13. Alberti K.G.M.M., Zimmet P.Z. Definition, diagnosis and classification of diabetes mellitus and its complications part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Original articles. 1997. DOI: 10.1002/(SICI)1096-9136(199807)15:7.
14. Levin A., Stevens P.E. Summary of KDIGO 2012 CKD Guideline: Behind the scenes, need for guidance, and a framework for moving forward. *Kidney Int.* 2014; 85: 49–61. DOI: 10.1038/ki.2013.444.
15. Hankinson J.L., Odencrantz J.R., Fedan K.B. Spirometric reference values from a sample of the general U.S. Population. *Am. J. Respir. Crit. Care Med.* 1999; 159: 179–187. DOI: 10.1164/ajrccm.159.1.9712108.
16. Sun H., Liu Y., Liu D., Zhou Q., Guo C., Li Q., Tian G., Wu X., Hu D., Sun X., Zhang M. Metabolically healthy general and abdominal obesity are associated with increased risk of hypertension. *Br. J. Nutr.* 2020; 123: 583–591. DOI: 10.1017/S0007114519003143.
17. Almeida J.B., Kian K.O., Lima R.C., De Souza M.C.C. Total and abdominal adiposity and hypertension in indigenous women in midwest Brazil. *PLoS One.* 2016; 11: 1–12. DOI: 10.1371/journal.pone.0155528.
18. Pekkarinen E., Vanninen E., Länsimies E., Kokkarinen J., Timonen K.L. Relation between body composition, abdominal obesity, and lung function. *Clin. Physiol. Funct. Imaging.* (2012; 32: 83–88. DOI: 10.1111/j.1475-097X.2011.01064.x.
19. Vatrella A., Calabrese C., Mattiello A., Panico C., Costigliola A., Chiodini P., Panico S. Abdominal adiposity is an early marker of pulmonary function impairment: Findings from a Mediterranean Italian female cohort. *Nutr. Metab. Cardiovasc. Dis.* 2016; 26: 643–648. DOI: 10.1016/j.numecd.2015.12.013.
20. Borges M.D., Franca E.L., Fujimori M., Silva S.M.C., de Marchi P.G.F., Deluque A.L., Honorio-Franca A.C., de Abreu L.C. Relationship between proinflammatory cytokines/chemokines and adipokines in serum of young adults with obesity, endocrine, metab. immune disord. *Drug Targets.* 2018; 18: 260–267. DOI: 10.2174/1871530318666180131094733.
21. Hertelyova Z., Salaj R., Chmelarova A., Dombrovsky P., Dvorakova M.C., Kruzliak P. The association between lipid parameters and obesity in university students. *J. Endocrinol. Invest.* 2016; 39: 769–778. DOI: 10.1007/s40618-015-0240-8.
22. Salehinia F., Abdi H., Hadaegh F., Serahati S., Valizadeh M., Azizi F., Hosseinpanah F. Abdominal obesity phenotypes and incident diabetes over 12 years of follow-up: The Tehran Lipid and glucose study. *Diabetes Res. Clin. Pract.* 2018; 144: 17–24. DOI: 10.1016/j.diabres.2018.07.021.
23. Sarathy H., Henriquez G., Abramowitz M.K., Kramer H., Rosas S.E., Johns T., Kumar J., Skversky A., Kaskel F., Melamed M.L. Abdominal obesity, race and chronic kidney disease in young adults: Results from NHANES 1999–2010. *PLoS One.* 2016; 11. DOI: 10.1371/journal.pone.0153588.
24. Dias R.S.C., Calado I.L., De Alencar J.D., Hortegal E.V., Santos E.J.F., De Araújo Brito D.J., Lages J.S., Dos Santos A.M., Filho N.S. Abdominal obesity and reduction of glomerular filtration. *Rev. Assoc. Med. Bras.* 2018; 64: 346–353. DOI: 10.1590/1806-9282.64.04.346.
25. Tsao Y.C., Chen J.Y., Yeh W.C., Li W.C. Gender- and age-specific associations between visceral obesity and renal function impairment. *Obes. Facts.* 2019; 12: 67–77. DOI: 10.1159/000496626.
26. Shahali A., Tasdighi E., Barzin M., Mahdavi M., Valizadeh M., Niroomand M., Azizi F., Hosseinpanah F. Abdominal obesity phenotypes and risk of kidney function decline: Tehran Lipid and Glucose Study. *Obes. Res. Clin. Pract*. 2020;(14):168–175. DOI: 10.1016/j.orcp.2020.03.006.

Authors contribution

Ragino Yu. I. – conception, methodology, visualization, drafting of the manuscript. Khudyakova A.D – carrying out of the research. Striukova E.V. – carrying out of the research, drafting of the manuscript, review and editing of the article. Denisova D.V. – provision of resources. Shcherbakova L.V. – carrying out of formal analysis. All authors have reviewed the article and agreed with its contents.

Authors information

Ragino Yulia I., Dr. Sci. (Med.), Professor, Corresponding Member of the RAS, Head of the IIPM – Branch of IC&G SB RAS, Novosibirsk, Russian Federation. ORCHID 0000-0002-4936-8362.

Khudyakova Alyona D., Cand. Sci (Med.), Head of Laboratory of Genetic and Environmental Determinants of the Human Life Cycle, IIPM – Branch of IC&G SB RAS, Novosibirsk, Russian Federation. ORCHID 0000-0001-7875-1566.

Striukova Evgeniia V., Junior Researcher, Laboratory of Genetic and Environmental Determinants of the Human Life Cycle, IIPM – Branch of IC&G SB RAS, Novosibirsk, Russian Federation. ORCHID 0000-0001-5316-4664.

Denisova Diana V., Dr. Sci. (Med.), Leading Researcher, Laboratory of Preventive Medicine, IIPM – Branch of IC&G SB RAS, Novosibirsk, Russian Federation. ORCID 0000-0002-2470-2133.

Shcherbakova Liliia V., Senior Researcher, Laboratory of Clinical-Populational and Prophylactic Studies on Internal and Endocrine Diseases, IIPM – Branch of IC&G SB RAS, Novosibirsk, Russian Federation. ORCID 0000-0001-9270-9188.

(✉) **Striukova Evgeniia V.,** e-mail: stryukova.j@mail.ru

Received 16.07.2021
Accepted 10.09.2021

УДК 616.149:611.149]-073.756.8
https://doi.org/10.20538/1682-0363-2021-4-49-55

Features of morphometric parameters of vessels in the human portal venous system identified by multislice computed tomography

Russkikh A.N., Shabokha A.D., Tyumentsev N.V., Derevtsova S.N.

V.F. Voino-Yasenetsky Krasnoyarsk State Medical University
1, Partizana Zheleznyaka Str., Krasnoyarsk, 660022, Russian Federation

ABSTRACT

The aim of this study was to identify the morphometric features of the human portal venous system by means of multislice computed tomography (MSCT).

Materials and methods. A contrast X-ray study of the portal vein was carried out in 53 men who were treated in the surgical departments of the Krasnoyarsk Regional Hospital No. 1. The average age of the patients was 54.9 ± 1.7 years (36–71 years). Measurements were performed on 3D models of the vascular bed in the portal venous system (GE Advantage Workstation and Siemens singo.via workstations). Branching patterns, length, diameter, angle of the portal vein formation relative to the midline of the human body, and angles of formation of the vessels forming the portal vein in the frontal plane were evaluated.

Results. Variations in the morphometric parameters of the intrahepatic vessels of the portal vein are obvious, although the branching patterns are not diverse and are reduced to one type – the magistral pattern (according to V.N. Shevkunenko). The veins that form the portal vein are represented by three systems, each of which has a stem and tributaries that differ in branching patterns and other morphological characteristics.

Conclusion. The findings of the study made it possible to supplement the scientific materials regarding branching patterns and morphological characteristics of the portal vein and its tributaries as well as to use the morphometric characteristics of the superior and inferior mesenteric and splenic veins to resolve the issues of surgical intervention on the abdominal organs.

Key words: portal vein, 3D modeling, branching pattern, superior mesenteric vein, inferior mesenteric vein, splenic vein.

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. All patients signed an informed consent to participate in the study. The study was approved by the local Ethics Committee at V.F. Voino-Yasenetsky Krasnoyarsk State Medical University (Protocol No. 84/2018 of 06.06.2018).

For citation: Russkikh A.N., Shabokha A.D., Tyumentsev N.V., Derevtsova S.N. Features of morphometric parameters of vessels in the human portal venous system identified by multislice computed tomography. *Bulletin of Siberian Medicine.* 2021; 20 (4): 49–55. https://doi.org/10.20538/1682-0363-2021-4-49-55.

✉ *Shabokha Anna D.,* e-mail: tat_yak@mail.ru

Особенности морфометрических показателей сосудов воротной системы человека, выявленные посредством мультиспиральной компьютерной томографии

Русских А.Н., Шабоха А.Д., Тюменцев Н.В., Деревцова С.Н.

Красноярский государственный медицинский университет (КрасГМУ) им. проф. В.Ф. Войно-Ясенецкого Россия, 660022, г. Красноярск, ул. Партизана Железняка, 1

РЕЗЮМЕ

Цель – выявление морфометрических особенностей воротной системы человека посредством мультиспиральной компьютерной томографии.

Материалы и методы. Для решения поставленной задачи проведено рентгенконтрастное исследование воротной вены 53 мужчинам, проходившим лечение в хирургических отделениях Красноярской краевой больницы № 1. Средний возраст составил 54,9 ± 1,7 лет (36–71 год). Измерения проводились на мультипланарных реконструкциях сосудистого русла воротной системы (рабочие станции GE Advantage Workstation, Siemens singo.via). Оценивались типы ветвления, длина, диаметр, угол образования воротной вены относительно срединной линии тела человека и углы образования сосудов, образующих воротную вену во фронтальной плоскости.

Результаты. Вариации морфометрических параметров внутрипеченочных сосудов воротной вены очевидны, хотя варианты ветвления неразнообразны и сводятся к одному типу – магистральному (по В.Н. Шевкуненко). Вены, образующие воротную вену, представлены тремя бассейнами, в каждом из которых имеются ствол и притоки, отличающиеся типами ветвления и другими морфологическими характеристиками.

Заключение. Исследование позволило дополнить научные материалы относительно типов ветвления и морфологических параметров воротной вены и ее ветвей, использовать морфометрические характеристики верхней, нижней брыжеечных и селезеночной вен для решения вопросов хирургического вмешательства на органах брюшной полости.

Ключевые слова: воротная вена, 3D-моделирование, тип ветвления, верхняя брыжеечная вена, нижняя брыжеечная вена, селезеночная вена.

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. Все пациенты подписали информированное согласие на участие в исследовании. Исследование одобрено локальным этическим комитетом КрасГМУ (протокол № 84/2018 от 06.06.2018).

Для цитирования: Русских А.Н., Шабоха А.Д., Тюменцев Н.В., Деревцова С.Н. Особенности морфометрических показателей сосудов воротной системы человека, выявленные посредством мультиспиральной компьютерной томографии. *Бюллетень сибирской медицины.* 2021; 20 (4): 49–55. https://doi.org/10.20538/1682-0363-2021-4-49-55.

INTRODUCTION

Variability of the human portal venous system is beyond doubt [1–5]. Features of interposition, branching of veins included in this system, and its stereometric and linear characteristics define the development, course, and methods of surgical intervention for several surgical diseases, which eventually determines the outcome of surgical pathology [6–8]. According to leading gastrointestinal surgeons, enhancement of diagnosis of the structural features in the portal venous system will allow to answer many questions about the therapeutic and diagnostic strategy in the preoperative period [9–12].

Radiographic testing of vessels, organs, and entire systems in various areas of medical practice shows good diagnostic results [13, 14]. A study carried out on a stationary X-ray machine in order to identify the features of rectal vessels of the portal venous system proved high information capacity of the method and allowed to find out postmortem characteristics, such as morphometry, spatial location, and vein branching [15–18].

Variant anatomy of major vessels of the portal venous system has been studied by a number of authors [19–22]. In 2018, I.V. Gaivoronsky et al. presented the main results characterizing variants of portal vein trunk formation and quantitative measurements of the vein length, diameter, and roots, showing a wide range of morphometric characteristics. The results obtained by multislice computed tomography (MSCT) of the abdominal cavity were defined as markers that allowed for planning an optimal surgical strategy and reducing postoperative complications on the part of the mesenteric and portal vein system in acute extensive portal vein or superior mesenteric vein thrombosis [6].

However, the study results can be applied only to operations on abdominal organs, in which the major vessels of the portal venous system are involved. According to A.V. Kolsanov et al. (2017), for a comprehensive study of the portal venous system of living people, it is eligible and competent to use contrast computed tomography with bolus tracking, which is one of the most precise methods to estimate morphometric features of vascular formations. Such a technique is the best for studying variant angioanatomy with visualization of vessels with a diameter of 1 mm and more, which allows to use this method not only in choosing the surgical strategy to treat portal hypertension, but also in all types of liver and pancreatic resection, liver transplantation etc. [9].

The aim of the study was to identify the morphological features of the human portal venous system.

MATERIALS AND METHODS

A contrast X-ray study was carried out on 53 men who were treated in the surgical departments of the Krasnoyarsk Regional Hospital No. 1. The inclusion criterion: patients with surgical diseases of the abdominal organs without circulatory disorders. The average age of the patients was 54.9 ± 1.7 years (36–71 years). All patients signed an informed consent to participate in the study. The study was approved by the local Ethics Committee at V.F. Voino-Yasenetsky Krasnoyarsk State Medical University (Protocol No. 84/2018 of 06.06.2018).

The measurements were performed on 3D reconstructions of the vascular bed of the portal venous system (GE Advantage Workstation and Siemens singo.via working stations) on the basis of MSCT scans of the abdominal cavity using bolus contrasting with Ultravist-370 (Bayer Pharma AG, Germany). The volume of the used contrast medium was 100 ml, the injection rate was 4 ml per second, and the average radiation exposure was 11.3 mSv.

The contrast X-Ray study is applicable to the study of variants of portal venous system formation and their morphometric patterns, as well as branching patterns at different levels of structural organization using classifications by the T. Nakamura (type A – classical anatomy, type B – portal trifurcation, type C – intra-; type D – extrahepatic branching of the anterior branch, and type E – absence of the anterior branch) and V.N. Shevkunenko (magistral, mixed, and distributed patterns) [9, 23, 24].

The length, diameter, and angle of the portal vein formation relative to the midline of the human body as well as angles of formation of the vessels forming the portal vein in the frontal plane were estimated. The measurements were performed by building a central axis of the vessel with further measuring of its linear parameters [25].

Statistical processing was carried out using the SPSS Statistics 17.0 software package. The normality distribution was assessed using the Shapiro – Wilk test. Characteristics of variational series for quantitative features with nonparametric distribution and data with parametric distribution due to their small number were presented using measures of the central tendency (mean (*M*), median (*Me*), mode (*Mo*)) and measures of variance (standard deviation, range, interquartile range [Q_{25}; Q_{75}]). When comparing two independent samples of nonparametric data, the nonparametric Mann – Whitney U-test was used.

RESULTS AND DISCUSSION

3D models of CT scans of the portal venous system among all the examined men were characterized by constant presence of the portal vein, its right branch (with the anterior and posterior branches) and left branch (with the transverse and umbilical portion), as well as splenic, superior and inferior mesenteric veins, and more superior veins, forming the main tributaries. According to X-ray, the portal vein was a cylinder with the diameter of 14.5 [13.0; 14.5] mm, and the

diameter at the place of its formation was similar to the diameter of its origin. The length ranged from 58 to 71 mm and the average length was about 63 mm. The portal vein was formed at the angle of 68 [46; 72]º relative to the midline of the human body, which proves the previously published data on the frequency of the angle (Fig. 1) [6].

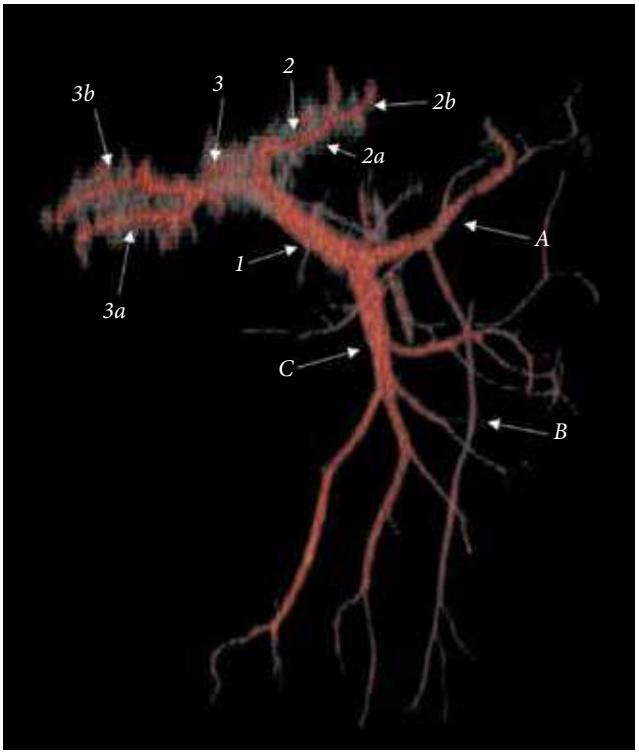


Fig. 1. 3D model of the human portal venous system. 2 – Left branch of the portal vein; 2*a* – Transverse portion; 2*b* – Umbilical portion; 3 – Right branch of the portal vein; 3*a* – Anterior branch; 3*b* – Posterior branch

Morphometric parameters of portal vein branches are shown in Table 1. Formation angles of the main branches of the portal vein were statistically different ($p < 0.05$). If the formation angle of the right main branch made 135.0 [130.0; 141.0]º, the left branch was located at an angle of 53.0 [49.5; 60.0]º relative to the portal vein. In 96% of cases, both branches were formed according to the classical branching pattern (according to the classification by T. Nakamura), and in individual cases, trifurcation and intrahepatic branching of the anterior portion occurred.

The left branch of the portal vein was longer than the right one (82.0 [79.5; 89.0] mm and 46.0 [39.5; 47.5] mm, respectively); the left and right branches had identical diameters (13.0 [10.5; 14.5] mm and 11.0 [10.5; 12.0] mm, respectively). The right branch of the portal vein was dichotomously divided into anterior and posterior branches; the length of the anterior (75.5 [73.0; 77.5] mm) and posterior (80.5 [75.5; 81.0] mm) branches and their diameters (8.0 [7.0; 8.5] mm and 7.0 [6.5; 8.5] mm) did not differ significantly, unlike the angles of their formation. The anterior branch was a kind of continuation of the right branch and departed from it at the angle of 160.0 [145.0; 170.0]º. The posterior branch formed an almost right angle with the right branch (115.0 [100.0; 125.0]º). Portions of the left branch of the portal vein had peculiarities in terms of length. The transverse portion (53.0 [48.0; 61.0] mm) was always longer than the umbilical one (31.0 [28.0; 39.0] mm), while their diameters did not differ significantly.

Table 1

Morphometric parameters of the portal vein branches found by X-ray, *Me* [P_{25}; P_{75}]

Parameter	Length, mm	Diameter, mm	Formation angle, degree*
Portal vein	63.0 [58.0; 71.0]	14.5 [13.0; 14.5]	68.0 [46.0; 72.0]
Right branch of the portal vein:	46.0 [39.5; 47.5]	11.0 [10.5; 12.0]	135.0 [130.0; 141.0]
– anterior branch	75.5 [73.0; 77.5]	8.0 [7.0; 8.5]	160.0 [145.0; 170.0]
– posterior branch	80.5 [75.5; 81.0]	7.0 [6.5; 8.5]	115.0 [100.0; 125.0]
Left branch of the portal vein:	82.0 [79.5; 89.0]		
– transverse portion	53.0 [48.0; 61.0]	13.0 [10.5; 14.5]	53.0 [49.5; 60.0]
– umbilical portion	31.0 [28.0; 39.0]		

*Formation angle of the portal vein relative to the midline of the human body

As a result, variations in the morphometric parameters of intrahepatic vessels of the portal vein are obvious. The vessels that form the portal vein are presented by three systems, each of which has a trunk and tributaries differing in the branching pattern and other morphological characteristics.

The superior mesenteric vein is characterized by the mixed branching pattern [23]. It has one trunk of 93.5 [78.5; 119.5] mm in length with the diameter of 9.5 [6.5; 12.0] mm entering the portal vein at the angle of 170.0 [160.0; 175.0]º and formed by tributary veins of most of the unpaired organs in the upper and lower abdominal cavity (Fig. 2). The tributaries of the superior mesenteric vein have almost the same diameter of 3.5–12 mm, but different length. The shortest tributaries are the jejunal vein (40.0 [38.5; 46.5] mm), the right gastroepiploic vein (45.0 [38.5; 53.5] mm), the

iliac vein (50.0 [48.5; 53.5] mm), the middle colic vein (60.0 [58.5; 63.5] mm), and the ileocolic vein (70.0 [68.5; 78.5] mm). The maximum length is determined in the right colic vein (115.0 [108.5; 120.5] mm), draining the ascending and transverse parts of the colon. The convergence angles of each tributary of the superior mesenteric vein are defined by locations of the internal organs from which the venous drainage is carried out. Since iliac and ileocolic veins are caudal branches, the value of their angles approaches the flat angle and averages 160 (160.0 [155.0; 171.0]º and 160.0 [150.0; 171.0]º, respectively). The given value is the statistical maximum relative to the convergence angles of other veins in this system. The middle (120.0 [110.0; 131.0]º) and right (140.0 [130.0; 145.0]º) colic veins have the average value. The minimal values are typical of the jejunal and right gastroepiploic veins (70.0 [60.0; 81.0]º and 85.0 [80.0; 91.0]º, respectively).

The inferior mesenteric vein system contains fewer veins entering its bed compared with the superior mesenteric vein vasculature. The magistral branching pattern of the inferior mesenteric vein is found in 23% of cases, while in 77% of cases this vein is characterized by the mixed pattern (Fig. 3) [23]. In case of the mixed branching pattern, the inferior mesenteric vein enters the superior mesenteric vein between the right colic and jejunal veins. In most cases, the inferior mesenteric vein of the magistral branching pattern enters the splenic vein (Fig. 1) or is an independent tributary of the portal vein. Its diameter is significantly smaller than that of the superior mesenteric vein and reaches 4.5 [2.0; 6.5] mm. Although length values vary depending on the branching characteristics, they do not significantly differ from the values of this parameter for the superior mesenteric vein. The angle of formation, as in the case of inflow into the superior or splenic vein, ranges from 135 to 151º. Linear parameters and formation angles of the inferior mesenteric vein tributaries do not have statistically significant differences (Table 2).

Table 2

Morphometric parameters of the portal vein roots found by X-ray, *Me* [P_{25}; P_{75}]

Parameter	Length, mm	Diameter, mm	Formation angle, degree
Superior mesenteric vein:	93.5 [78.5; 119.5]	9.5 [6.5; 12.0]	170.0 [160.0; 175.0]
– middle colic vein	60.0 [58.5; 63.5]	9.0 [6.0; 11.0]	120.0 [110.0; 131.0]
– jejunal vein	40.0 [38.5; 46.5]	4.0 [3.5; 6.0]	70.0 [60.0; 81.0]
– iliac vein	50.0 [48.5; 53.5]	5.5 [5.0; 7.0]	160.0 [155.0; 171.0]
– ileocolic vein	70.0 [68.5; 78.5]	5.0 [3.5; 6.5]	160.0 [150.0; 171.0]
– right colic vein	115.0 [108.5; 120.5]	6.0 [6.5; 9.0]	140.0 [130.0; 145.0]
– right gastroepiploic vein	45.0 [38.5; 53.5]	4.0 [3.5; 6.0]	85.0 [80.0; 91.0]
Inferior mesenteric vein:	108.5 [104.0; 111.5]	4.5 [2.0; 6.5]	140.0 [135.0; 151.0]
– left colic vein	40.0 [33.5; 49.5]	3.5 [2.0; 4.5]	175.0 [170.0; 179.0]
– sigmoid vein	50.0 [27.0; 53.5]	3.0 [2.0; 3.5]	165.0 [160.0; 170.0]
– superior rectal vein	30.0 [20.0; 50.0]	3.0 [2.0; 4.0]	160.0 [155.0; 165.0]
Splenic vein:	125.0 [97.5; 129.5]	7.5 [5.5; 8.5]	100.0 [95.0; 111.0]
– left gastroepiploic vein	20.0 [13.5; 29.5]	5.0 [4.0; 6.0]	130.0 [120.0; 135.0]
– short gastric veins	12.0 [7.0; 18.5]	4.0 [3.0; 4.5]	90.0 [90.0; 95.0]
(n = 6–12)			

Unlike the superior and inferior mesenteric vein systems, the splenic vein always has the magistral branching pattern (Fig. 4). The splenic vein has medium diameter (7.5 [5.5; 8.5] mm) and maximum length (125.0 [97.5; 129.5] mm) values and enters the portal vein at a smaller angle (100.0 [95.0; 111.0]º) than in case of the superior and inferior mesenteric veins. Tributaries of the splenic vein are numerous, values of the linear parameters do not differ significantly. The average values for the length, diameter, and convergence angle of the left gastroepiploic vein are 20.0 [13.5; 29.5] mm, 5.0 [4.0; 6.0] mm, and 130.0 [120.0; 135.0]º, respectively. The short gastric veins enter the splenic vein at a right angle (90.0 [90.0; 95.0]º), and the average values for their length and diameter reach 12.0 [7.0; 18.5] mm and 4.0 [3.0; 4.5] mm, respectively.

Radiographic testing of the portal venous system using MSCT with bolus tracking has shown high information capacity, which had been previously demonstrated by A.V. Kolsanov et al. [9].

Estimating the length, diameter, and formation angles of the portal vein and its tributaries, we came to the conclusion that modern diagnostic imaging techniques with the use of contrast agents should be used to study the portal venous system at various levels of its structural organization.

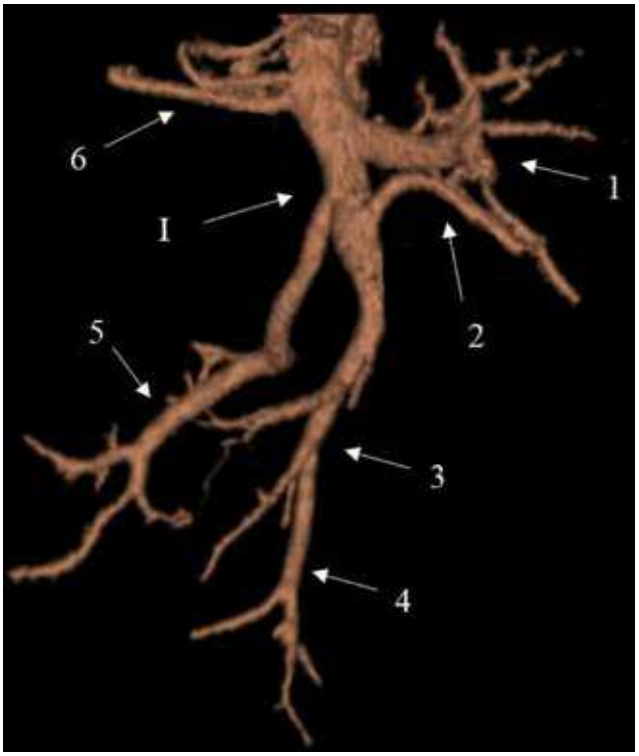


Fig. 2. 3D model of the human superior mesenteric vein: I – superior mesenteric; 1 – middle colic vein; 2 – jejunal vein; 3 – Iliac vein; 4 – Ileocolic vein; 5 – Right colic vein; 6 – Right gastroepiploic vein

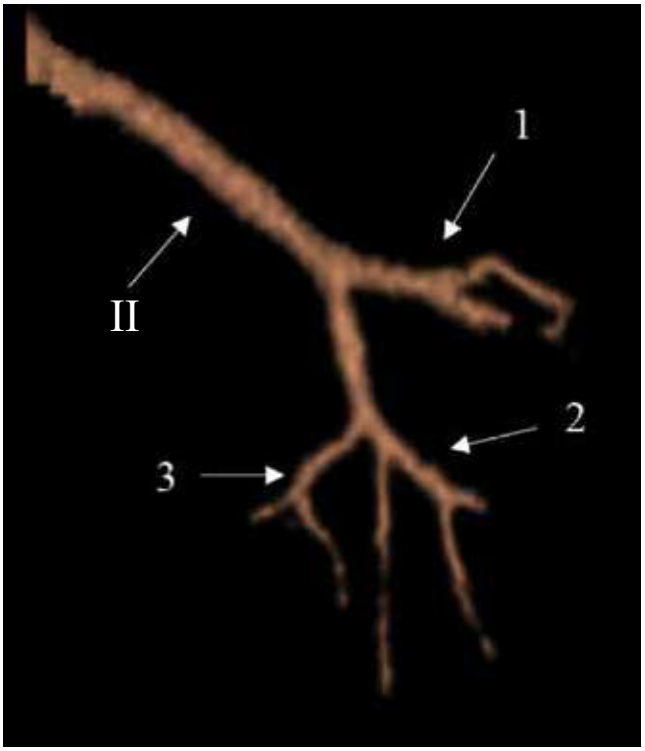


Fig. 3. 3D model of the human inferior mesenteric vein: II – Inferior mesenteric vein; 1 – Left colic vein; 2 – Sigmoid vein; 3 – Superior rectal vein

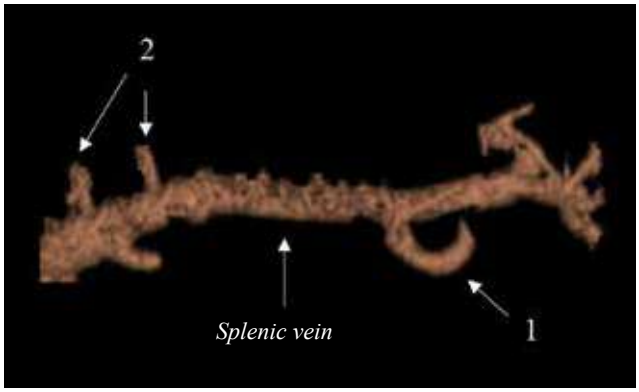


Fig. 4. 3D model of the human splenic vein: 1 – Left gastroepiploic vein; 2 – Short gastric veins

CONCLUSION

The current study provides quantitative landmarks for the major venous structures in the portal venous system. The research results made it possible to supplement scientific materials regarding the branching patterns and morphological parameters of the portal vein and its branches. The obtained data reflecting morphometric characteristics of the superior and inferior mesenteric veins as well as splenic vein prove variability of veins included in the *v. portae* system and a wide range of its structural anatomy and can be used to resolve the issues of surgical intervention on the abdominal organs. Variations typical of each venous system should be taken into account when choosing the strategy of managing patients with portal hypertension or at the preoperative stage.

REFERENCES

1. Khamanarong K., Woraputtaporn W., Amarttayakong P., Ahooja A., Khuntikeo N. Classification of portal vein tributaries in Thai cadavers including a new type V. *Surgical and Radiologic Anatomy.* 2016; 38 (6): 735–739. DOI: 10.1007/S00276-015-1592-7.
2. Sharma V., Chauhan R.S., Sood R.G., Makhaik S., Negi K., Chawla K., Diwan Y., Partap A., Rana S., Gupta A. Study of the normal anatomy and variations of portal vein in North Indian population: a MDCT study. *European Journal of Anatomy*. 2017; 21 (1): 13–18.
3. Cheluvashetty S.B., Rachapalli V. Unusual branching pattern of the portal vein: its importance. *Journal of Clinical and Diagn*ostic Research. 2017; 11 (8): TD01–TD02. DOI: 10.7860/JCDR/2017/29011.10390.
4. Guerra A., De Gaetano A.M., Infante A., Mele C., Marini M.G., Rinninella E., Inchingolo R., Bonomo L. Imaging assessment of portal venous system: pictorial essay of normal anatomy, anatomic variants and congenital anomalies. *European Review for Medical and Pharmacological Sciences.* 2017; 21 (20): 4477–4486.
5. Kovalenko N.A., Gaivoronsky I.V., Kotiv B.N., Tyagun V.S., Novitskaya N.Yu. Variant anatomy of the portal vein and its

application in surgery. *Medline.ru. Russian Biomedical Journal.* 2018; 19 (1): 182–208 (in Russ.).
6. Gaivoronskiy I.V., Kotiv B.N., Kovalenko N.A., Lazarenko V.A. Variant anatomy of the great vessels of the portal vein system and its applied significance. *Kursk Scientific and Practical Bulletin "Humans and Their Health".* 2018; 2: 70–75 (in Russ.). DOI: 10.21626/vestnik/2018-2/11.
7. Russkikh A.N., Shabokha A.D., Gorbunov N.S., Derevtsova S.N. Features of the local constitution in the structure of the veins of the human rectum. *Bulletin of New Medical Technologies.* 2013; 20 (2): 53–57 (in Russ.).
8. Iqbal S., Iqbal R., Iqbal F. Surgical implications of portal vein variations and liver segmentations: a recent update. *Journal of Clinical and Diagnostic Research*. 2017; 11 (2): AE1–AE5. DOI: 10.7860/JCDR/2017/25028.9453.
9. Kolsanov A.V., Zelter P.M., Manukyan A.A., Chaplygin S.S., Ivanova V.D. Studying the anatomy of the portal vein using the preoperative planning system. *Operative Surgery and Clinical Anatomy.* 2017; 1 (2): 3–6 (in Russ.). DOI: 10.17116/operhirurg2017123-6
10. Nakamura T., Tanaka K., Kiuchi T., Kasahara M., Oike F., Ueda M., Kaihara S., Egawa H., Ozden I., Kobayashi N., Uemoto S. Anatomical variations and surgical strategies in right lobe living donor liver transplantation: lessons from 120 cases. *Transplantation.* 2002; 73 (12): 1896–1903. DOI: 10.1097/00007890-200206270-00008.
11. Schmidt S., Demartines N., Soler L., Schnyder P., Denys A. Portal vein normal anatomy and variants: Implication for liver surgery and portal vein embolization. *Semin Intervent. Radiol.* 2008; 25 (2): 86–91. DOI: 10.1055/S-2008-1076688.
12. Sakamoto Yo., Kokudo N., Kawaguchi Yo., Akita K. Clinical Anatomy of the Liver: Review of the 19th Meeting of the Japanese Research Society of Clinical Anatomy. *Liver Cancer.* 2017; 6 (2): 146–160. DOI: 10.1159/000449490.
13. Akagi M., Nakamura Yu., Higaki T., Narita K., Honda Yu., Zhou J., Yu Z., Akino N., Awai K. Deep learning reconstruction improves image quality of abdominal ultra-high-resolution CT. *European Radiology*. 2019; 29 (11): 6163–6171. DOI: 10.1007/S00330-019-06170-3.
14. Karunakaravel K. Utility of cone-beam computed tomography in the assessment of the porto-spleno-mesenteric venous system. *Cardiovascular Diagnosis and Therapy.* 2016; 6 (6): 544–556. DOI: 10.21037/CDT.2016.11.16.
15. Russkikh A.N., Shabokha A.D. X-ray examination methods of veins of the human rectum. *Bulletin of Siberian Medicine*. 2019; 18 (2): 146–156 (in Russ.). DOI: 10.20538/1682-0363-2019-2-146-156
16. Volkova S.N., Stashuk G.A., Chermensky G.V., Naumov E.K. The role of MRI in identifying extramural vascular invasion as an indicator of the presence of regional and distant metastases of the lower ampullary rectum cancer. *Experimental and Clinical Gastroenterology.* 2019; 4 (164): 66–71 (in Russ.). DOI: 10.31146/1682-8658-ECG-164-4-66-71.
17. Takahiro S., Katsu Ya., Jouji T., Yoshiyasu K., Takumi O., Jun A. Diagnosis of rectal varices via color Doppler ultrasonography. *The American Journal of Gastroenterology.* 2007; 102 (10): 2253–2258. DOI: 10.1111/J.1572-0241.2007.01340.X.
18. Cyriac A.Ph., Philip A. Endoscopic ultrasound-guided management of bleeding rectal varices. *ACG Case Reports Journal.* 2017; 4: e101. DOI: 10.14309/CRJ.2017.101.
19. Lang H., de Santibañes E., Schlitt H.J., Malagó M., van Gulik T., Machado M.A., Jovine E., Heinrich S., Ettorre G.M., Chan A., Hernandez-Alejandro R., Campos R.R., Sandström P., Linecker M., Clavien P.-A. 10th Anniversary of ALPPS-lessons learned and quo vadis. *Annals of Surgery*. 2019; 269 (1): 114–119. DOI: 10.1097/SLA.0000000000002797.
20. Durand C.A.L., Rázuri C.B., Valderrama M.W., Arredondo G.M., Ramos D.S. The human liver: vascular anatomy to determine its segments and divisions. *International Journal of Morphology.* 2020; 38 (1): 226–229. DOI: 10.4067/S0717-95022020000100226.
21. Singh H.R., Rymbai M.L., John R., Eapen A., Rabi S. Branching pattern of the portal vein in Indian population. *European Journal of Anatomy.* 2019; 23 (6): 425–433.
22. Carneiro C., Brito Jo., Bilreiro C., Barros M., Bahia C., Santiago I., Caseiro-Alves F. All about portal vein: a pictorial display to anatomy, variants and physiopathology. *Insights into Imaging.* 2019; 10 (1): 38. DOI: 10.1186/S13244-019-0716-8.
23. Shevkunenko V.N. Atlas of peripheral nervous and venous systems. *Moscow: MEDGIZ,* 1949: 345 (in Russ.).
24. Gadzijev E.M. Atlas of applied internal liver anatomy. Foreword by Stig Bengmark. Wien. New York: Springer, 1996; 204.
25. Covey A.M., Brody L.A., Getrajdman G.I., Sofocleous C.T., Brown K.T. Incidence, patterns, and clinical relevance of variant portal vein anatomy. *Am. J. Roentgenol*. 2004; 183 (4): 1055–1064. DOI: 10.2214/AJR.183.4.1831055.

Authors information

Russkikh Andrey N., Cand. Sci. (Med.), Associate Professor, Head of the Department of Operative Surgery and Topographic Anatomy, V.F. Voino-Yasenetsky Krasnoyarsk State Medical University, Krasnoyarsk, Russian Federation.

Shabokha Anna D., Cand. Sci. (Med.), Assistant, Department of Outpatient Pediatrics and Propaedeutics of Childhood Diseases with a Course of Graduate Training, V.F. Voino-Yasenetsky Krasnoyarsk State Medical University, Krasnoyarsk, Russian Federation.

Tyumentsev Nikolay V., Assistant, Department of Radiodiagnostics of the Postgraduate Education Institute, V.F. Voino-Yasenetsky Krasnoyarsk State Medical University, Krasnoyarsk, Russian Federation.

Derevtsova Svetlana N., Dr. Sci. (Med.), Professor, Department of Human Anatomy and Histology, V.F. Voino-Yasenetsky Krasnoyarsk State Medical University, Krasnoyarsk, Russian Federation.

(✉) **Shabokha Anna D.,** e-mail: tat_yak@mail.ru.

Received 02.06.2020
Accepted 28.12.2020

УДК 621.373.8
https://doi.org/10.20538/1682-0363-2021-4-56-62

Comparison of cutting and coagulation properties of 1.56 and 1.94 μm fiber lasers and a 0.98 μm semiconductor laser

Ryabova M.A., Ulupov M.Yu., Shumilova N.A., Portnov G.V., Tikhomirova E.K., Malkova M.E.

Pavlov First Saint Petersburg State Medical University
6-8, L. Tolstogo Str., St. Petersburg, 197022, Russian Federation

ABSTRACT

Aim of the study was to compare the cutting and coagulation properties of 1.56 and 1.94 μm fiber lasers with those of a 0.98 μm semiconductor laser.

Materials and methods. A comparative study of the biological effects of 1.56 and 1.94 μm lasers and a 0.98 μm semiconductor laser used in a constant, continuous mode was carried out. The cutting properties of the lasers were evaluated on the chicken muscle tissue samples by the width and depth of the ablation zone formed via a linear laser incision at a speed of 2 mm/s, while the coagulation properties were assessed by the width of the lateral coagulation zone. The zones were measured using a surgical microscope and a calibration slide. For statistical analysis, power values of 3, 5, 7, 9, and 11 W were chosen for each laser wavelength.

Results. Analysis of the findings confirmed that laser wavelength had a statistically significant effect on the linear dependence between incision parameters and laser power. It was found that the 1.56 μm fiber laser (water absorption) had a greater coagulation ability but a comparable cutting ability compared with the 0.98 μm laser (hemoglobin absorption). When used in the power mode of 7W or higher, the 1.94 μm laser provided superior cutting performance compared with the 0.98 μm semiconductor laser at the same exposure power. Elevating the power in any of the lasers primarily increased the width of the ablation zone, and to a lesser extent – the crater depth and the width of the lateral coagulation zone. Therefore, in comparison with the 0.98 μm semiconductor laser, higher radiation power in the 1.56 and 1.94 μm lasers mainly influences their cutting properties, expanding the width and depth of the ablation zone, and has a smaller effect on their coagulation ability.

Conclusion. The findings of the study showed that the 1.56 and 1.94 μm fiber lasers have better coagulation properties in comparison with the 0.98 μm semiconductor laser. was statistically proven that all incision characteristics (width of the lateral coagulation zone, depth and width of the ablation zone) for the 1.56, 1.94, and 0.98 μm lasers depend on the power of laser radiation. The 1.94 μm laser is superior to the 0.98 μm laser in its cutting properties.

Key words: laser, ablation, coagulation, wavelength, power.

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: Ryabova M.A., Ulupov M.Yu., Shumilova N.A., Portnov G.V., Tikhomirova E.K., Malkova M.E. Comparison of cutting and coagulation properties of 1.56 and 1.94 μm fiber lasers and a 0.98 μm semiconductor laser. *Bulletin of Siberian Medicine.* 2021; 20 (4): 56–62. https://doi.org/10.20538/1682-0363-2021-4-56-62.

✉ *Tikhomirova Ekaterina K.,* e-mail: kt-92@mail.ru

Сравнение режущих и коагуляционных свойств волоконных лазеров с длиной волны 1,56 и 1,94 мкм с полупроводниковым лазером 0,98 мкм

Рябова М.А., Улупов М.Ю., Шумилова Н.А., Портнов Г.В., Тихомирова Е.К., Малкова М.Е.

Первый Санкт-Петербургский государственный медицинский университет имени академика И.П. Павлова (ПСПбГМУ)
Россия, 197022, г. Санкт-Петербург, ул. Льва Толстого, 6-8

РЕЗЮМЕ

Цель – провести сравнительную оценку режущих и коагуляционных свойств волоконных лазеров с длинами волн 1,56 и 1,94 мкм с полупроводниковым лазером 0,98 мкм.

Материалы и методы. Проведено сравнительное исследование биологических свойств волоконных лазеров с длиной волны 1,56 и 1,94 мкм с полупроводниковым лазером 0,98 мкм в постоянном непрерывном режиме. Режущие свойства лазеров оценивались на мышечной ткани курицы по ширине и глубине зоны абляции, формируемой в ходе линейного лазерного разреза со скоростью 2 мм/с, коагуляционные – по ширине боковой зоны коагуляции. Измерение зон проводили в условиях микроскопии с помощью калибровочного предметного стекла. Для статистического анализа выбрали значения мощности 3, 5, 7, 9 и 11 Вт для каждой длины волны лазерного излучения.

Результаты. Анализ полученных результатов измерений подтвердил статистически значимое влияние длины волны лазерного излучения на характер линейной зависимости параметров лазерного разреза от мощности воздействия. Установлено, что волоконный водопоглощаемый лазер с длиной волны 1,56 мкм обладает большей коагулирующей способностью, но сопоставимой способностью к резке тканей по сравнению с гемоглобинпоглощаемым лазером с длиной волны 0,98 мкм. Лазер с длиной волны 1,94 мкм на мощности 7 Вт и выше превосходит по своим режущим свойствам полупроводниковый лазер 0,98 мкм на той же мощности воздействия. Для всех лазеров прирост мощности излучения в большей степени увеличивает ширину зоны абляции, в меньшей степени – глубину кратера и ширину боковой зоны коагуляции. Таким образом, прирост мощности излучения для лазеров с длиной волны 1,56 и 1,94 мкм преимущественно влияет на режущие свойства, увеличивая ширину и глубину формируемой зоны абляции, в меньшей степени – на его коагуляционные способности в сравнении с полупроводниковым лазером с длиной волны 0,98 мкм.

Заключение. По результатам экспериментального исследования обнаружено, что лазеры с длиной волны 1,56 и 1,94 мкм обладают лучшими коагулирующими свойствами в сравнении с полупроводниковым лазером 0,98 мкм. Статистически доказано, что все параметры лазерного разреза (ширины боковой зоны коагуляции, глубины и ширины зоны абляции) для лазеров с длиной волны 1,56; 1,94 и 0,98 мкм зависят от мощности лазерного излучения. Лазер с длиной волны 1,94 мкм превосходит лазер 0,98 мкм по своим режущим свойствам.

Ключевые слова: лазер, абляция, коагуляция, длина волны, мощность.

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

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

Для цитирования: Рябова М.А., Улупов М.Ю., Шумилова Н.А., Портнов Г.В., Тихомирова Е.К., Малкова М.Е. Сравнение режущих и коагуляционных свойств волоконных лазеров с длиной волны 1,56 и 1,94 мкм с полупроводниковым лазером 0,98 мкм. *Бюллетень сибирской медицины.* 2021; 20 (4): 56–62. https://doi.org/10.20538/1682-0363-2021-4-56-62.

INTRODUCTION

In recent years, laser technologies have been rapidly developing with creation of semiconductor and fiber lasers with various wavelengths never used before (0.53, 0.81, 0.97, 1.06, 1.47, 1.56, 1.94 μm) and production of high-power lasers, which expands the scope of their application in different modes (continuous, pulsed, contact, and distance modes). Novel medical equipment expands the range of therapeutic interventions; however, biological effects of the majority of machines rarely receive any empirical evaluation. Therefore, the right operation mode is usually found by trial and error, which makes surgical intervention less predictable and increases the risk of complications. Since the wavelength defines the properties of a laser, a lack of data on the biological effect of different wavelengths prevents practitioners from selecting the right laser device and its operation modes.

In most experimental studies, ablative performance is assessed by measuring the ablation rate, which is the volume of ablated tissue per a unit of time (g/min), while coagulation properties are evaluated by determining the bleeding rate (g/min) and tissue necrosis depth (mm). This method involves the *in vivo* use of living tissue models (in many cases – blood-perfused porcine kidney), though evaluation results provide only circumstantial evidence on the extent of ablation and coagulation properties [1]. Current Russian and foreign studies on the effects of diode and fiber lasers with various wavelengths (0.81, 0.94, 0.97, 1.47, 1.56 μm) use vein models as samples for comparative experimental analysis, which makes it impossible to evaluate the cutting properties of lasers [2–8]. There are few papers on semiconductor lasers being used on the cartilaginous tissue models (0.97; 1.56 μm), but they do not discuss the ablative and coagulation characteristics either [9].

The main aspects of laser performance relevant for clinical practice include cutting properties measured by the width and depth of the ablation zone, as well as coagulation properties, or hemostatic effect of the laser, that manifest themselves through tissue blanching along the incision.

The development of new 1.56 and 1.94 μm fiber lasers makes it relevant to study their biological effects prior to using them in clinical practice. The 0.98 μm lasers have been extensively used for various surgical purposes [1], including ENT surgery [10–13], and, therefore, it is reasonable to use this experience for a comparative analysis of the biological effects of other lasers.

The aim of the study was to compare the cutting and coagulation properties of the 1.56 and 1.94 μm fiber lasers and the 0.98 μm semiconductor laser.

MATERIALS AND METHODS

To assess the biological effects of laser radiation, linear incisions were made on the chicken muscle tissue at a fixed rate. The laser fiber was rigidly fixed with tripods on a support stand at a 60° angle relative to the incision projection. The biological object was placed on a mobile recorder sheet moving uniformly at a speed of 2 mm/s [12]. The width of the ablation crater and the lateral coagulation zone was evaluated using a slide with the graduation of 10 μm and an operating microscope with ×15 magnification. To assess the crater depth, cross sections of the tissue were made relative to the linear incision line, and the parameter was measured using the above-described method. The width and depth of the incision served as indicators of the ablative performance, while the width of the lateral coagulation zone represented the hemostatic properties of the lasers.

The incisions were made with the 1940 and 980/1560 nm lasers (LSP, IRE-Polus, Moscow, Russian Federation) in a continuous contact mode. The procedure was performed with a freshly cleaved optical fiber end with a width of 400 μm after its charring by a short-term contact with a wooden surface (spatula). At power values from 3 to 11 W and interval of 2 W, 3 incisions for each wavelength were made, and the results for each incision were measured in 10 positions (30 measurements in total).

Statistical processing of the data was carried out using the IBM SPSS Statistics software package (version 22). The evaluation methodology included descriptive statistics methods, regression analysis, and multiple regression analysis with a madiating variable. The parameters of the laser incision (width of the lateral coagulation zone, depth and width of the ablation zone) were dependent variables. The regression analysis of each dependent variable was performed separately. The power and wavelength of radiation were regarded as independent variables. Since the experiment involved three lasers with wavelengths of 0.98, 1.56, and 1.94 μm, the qualitative mediating variable "wavelength" was coded with two dummy variables representing the 1.56 and 1.94 μm lasers.

RESULTS AND DISCUSSION

The graphs depicting the dependence of the width of the lateral coagulation zone and the depth and width of the ablation zone on the power and wavelength are shown below (Fig. 1–6).

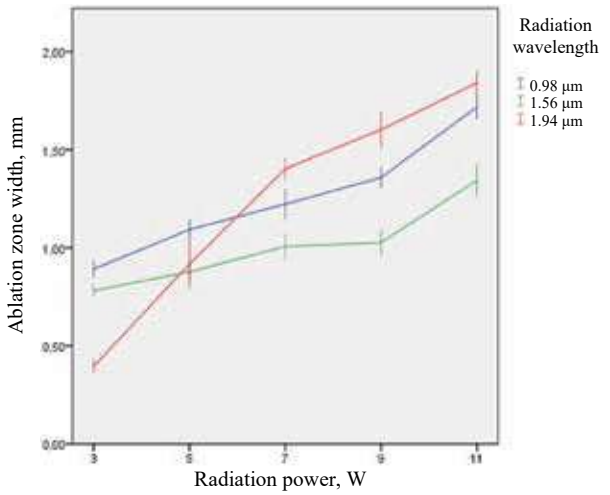


Fig. 1. Dependence of the ablation zone width on laser power (average values and 95 % confidence interval (CI)

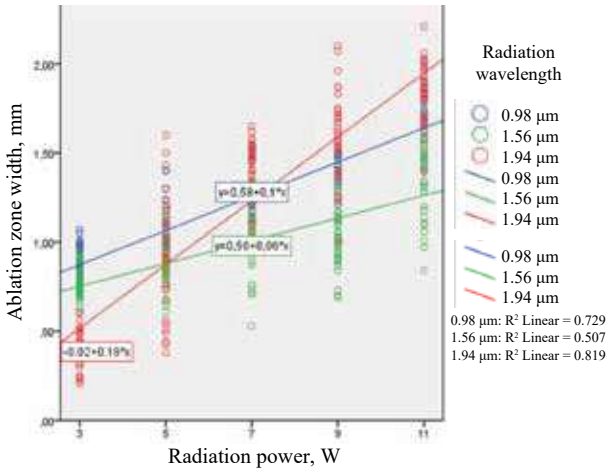


Fig. 2. Dependence of the ablation zone width on laser power (linear regression)

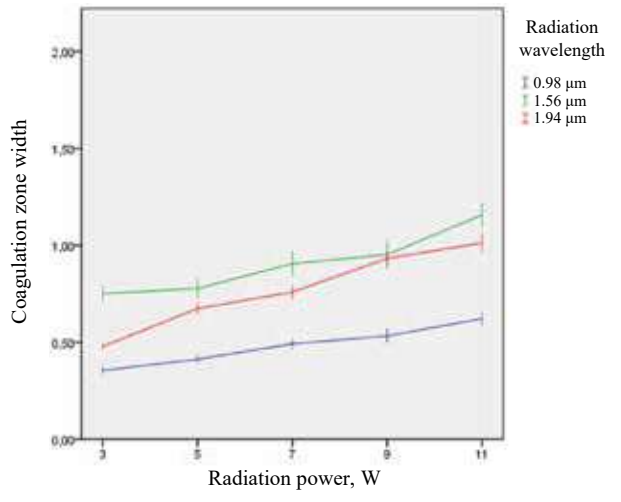


Fig. 3. Dependence of the coagulation zone width on laser power (average values and 95 % CI)

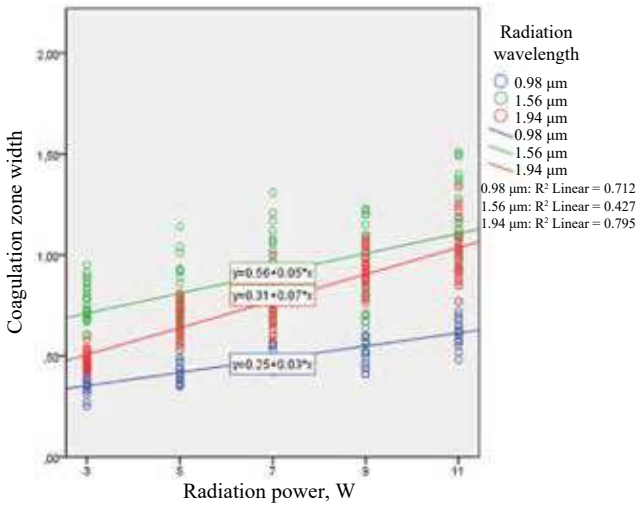


Fig. 4. Dependence of the coagulation zone width on laser power (linear regression)

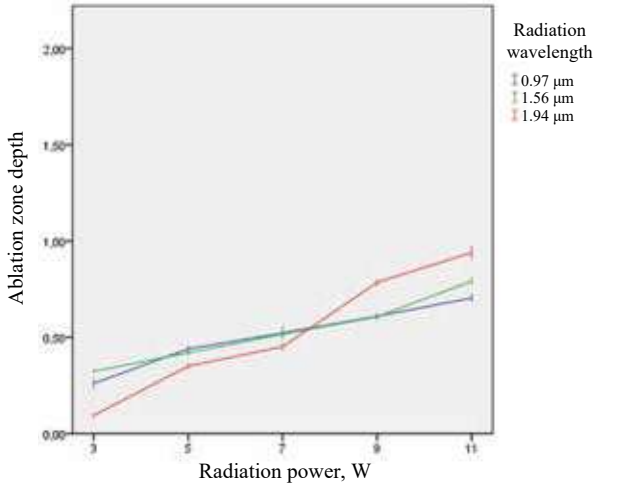


Fig. 5. Dependence of the ablation zone depth on laser power (average values and 95 % CI)

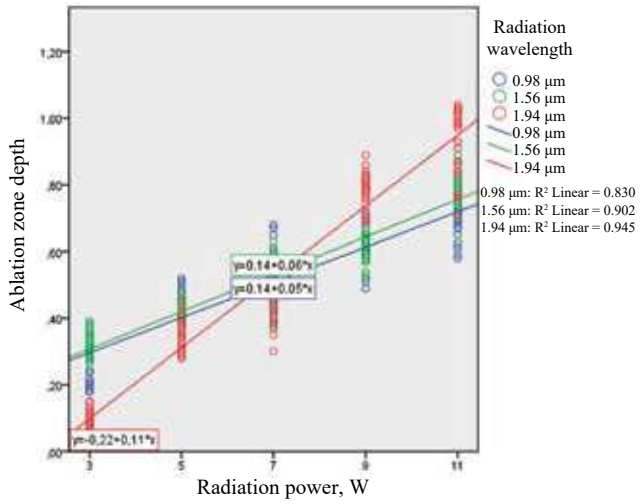


Fig. 6. Dependence of the ablation zone depth on laser power (linear regression)

The statistical analysis of the results was based on multiple regression:

$$Y = \beta_0 + \beta_1 \cdot P + \beta_2 \cdot D_{1,56} + \beta_3 \cdot D_{1,94} + \beta_4 P \cdot D_{1,56} + \\ + \beta_5 \cdot P \cdot D_{1,94} + \varepsilon,$$

where Y is a dependent variable (the coagulation width for the first model; the ablation depth, for the second model; and the ablation width for the third model); P is the laser power; $D_{1,56}$ is a dummy variable that equals 1 for the 1.56 μm laser and 0 in other cases; $D_{1,94}$ is a dummy variable that equals 1 for the 1.94 μm laser and 0 in other cases.

The 0.98 μm laser is a benchmark, and the regression equation for it is $Y = \beta_0 + \beta_1 \cdot P + \varepsilon$. The regression equation for the 1.56 μm laser is $Y = \beta_0 + \beta_2 + (\beta_1 + \beta_4) \cdot P \cdot D_{1.56} + \varepsilon$, while for the 1.94 μm laser, it is $Y = \beta_0 + \beta_3 + (\beta_1 + \beta_5) \cdot P \cdot D_{1.94} + \varepsilon$.

Thus, the significance of the β_2, β_4 coefficients indicates the statistical difference between 0.98 μm and 1.56 μm lasers, and the significance of the β_3, β_5 coefficients indicates the statistical difference between the 0.98 μm and 1.94 μm lasers.

Multiple regression analysis with a mediating variable showed a statistically significant relationship between all the parameters (the width of the coagulation zone, the depth and width of the ablation zone) and the laser power (Table 1, 2). In all but one case, it was established that the laser radiation wavelength (mediating variable) had a statistically significant effect on the linear dependence of laser incision characteristics on the laser power. Only when comparing the 0.98 and 1.56 μm lasers, no statistically significant difference in the effect of the laser power on the ablation depth was revealed.

Table 1

Overview of dependence of incision parameters on power and wavelength

Model	R	R^2	SE	F	df1	df2	*p*
Model 1 (coagulation zone width)	0.88	0.78	0.014	444.7	5	444	<0.001
Model 2 (ablation zone depth)	0.96	0.92	0.004	1107.3	5	444	<0.001
Model 3 (ablation zone width)	0.88	0.78	0.013	378.3	5	444	<0.001

The dependent variable for model *1* – coagulation width; for model *2* – ablation depth; for model *3* – ablation width; *p* – significance level; SE – standard error, df – degrees of freedom.

Table 2

Regression parameters for dependence of laser incision characteristics on radiation power and wavelength

Model	Parameter	Coefficient	SE	p	Lower confidence interval limit	Upper confidence interval limit
Model 1 (coagulation zone width)	Constant	$\beta_0 = 0.483$	0.006	<0.001	0.472	0.495
	Power	$\beta_1 = 0.033$	0.002	<0.001	0.029	0.037
	1.56 μm	$\beta_2 = 0.426$	0.015	<0.001	0.399	0.458
	1.94 μm	$\beta_3 = 0.289$	0.01	<0.001	0.269	0.309
	Power* 1.56 μm	$\beta_4 = 0.017$	0.005	<0.001	0.007	0.027
	Power* 1.94 μm	$\beta_5 = 0.034$	0.004	<0.001	0.027	0.041
Model 2 (ablation zone depth)	Constant	$\beta_0 = 0.508$	0.006	<0.001	0.496	0.519
	Power	$\beta_1 = 0.053$	0.002	<0.001	0.049	0.057
	1.56 μm	$\beta_2 = 0.024$	0.007	<0.001	0.01	0.039
	1.94 μm	$\beta_3 = 0.017$	0.008	0.040	0.001	0.033
	Power* 1.56 μm	$\beta_4 = 0.003$	0.002	0.183	-0.002	0.008
	Power* 1.94 μm	$\beta_5 = 0.054$	0.003	<0.001	0.048	0.059
Model 3 (ablation zone width)	Constant	$\beta_0 = 1.258$	0.014	<0.001	1.23	1.283
	Power	$\beta_1 = 0.096$	0.004	<0.001	0.087	0.105
	1.56 μm	$\beta_2 = -0.25$	0.02	<0.001	-0.289	-0.211
	1.94 μm	$\beta_3 = -0.024$	0.024	0.314	-0.073	0.027
	Power* 1.56 μm	$\beta_4 = -0.032$	0.007	<0.001	-0.045	-0.019
	Power* 1.94 μm	$\beta_5 = 0.083$	0.007	<0.001	0.068	0.097

Statistical analysis of the data obtained showed that the laser power significantly ($p < 0.05$) influenced the cutting and coagulation properties of all lasers. This was confirmed by the statistical significance of the β_1 coefficient in all the three models (width of the coagulation zone, depth and width of the ablation zone).

The 1.56 μm laser radiation (water absorption) did not differ much from the 0.98 μm radiation (he-

moglobin absorption) in its cutting properties (the statistical differences in the ablation zone depths are non-significant, $p > 0.05$), though its coagulation properties were more prominent: compared with the 0.98 μm laser, the 1.56 μm laser created a wider coagulation zone at the same power mode (β_2 differs significantly from 0).

The cutting properties of the 1.94 μm laser followed a different correlation pattern with its power than the 0.98 μm or 1.56 μm lasers (due to a different angle of the regression line, which was confirmed by the fact that β_5 is statistically different from zero in model 2). Unlike the 0.98 μm and 1.56 μm lasers, the power of 3–5 W resulted in a smaller width and depth of the ablation zone, while the power of 9–11 W increased these incision parameters. The 1.94 μm laser was found to have greater coagulation properties than the 0.98 μm laser with hemoglobin absorption.

The research showed that greater laser power primarily increased the ablation zone width (the regression coefficients were 0.1, 0.06, and 0.18 mm/W for the 0.98, 1.56, and 1.94 μm lasers, respectively), to a lesser extent – the ablation crater depth (the regression coefficients were 0.05, 0.06, and 0.11 mm/W, respectively), and to the least extent – the width of the lateral coagulation zone (the regression coefficients were 0.03, 0.05, and 0.07 mm/W, respectively). Thus, the increase in laser power predominantly affected the cutting properties, making the ablation zone deeper and wider, but had a modest effect on the coagulation abilities. Therefore, using greater laser power during surgery will increase the cutting properties of the laser more than the hemostatic ones.

The visual analysis of the regression lines demonstrated that the 1.94 μm laser had less prominent coagulation properties than the 1.56 μm laser, since the former was absorbed by water more easily and had a greater target chromophore absorption rate, as well as a smaller penetration depth. However, the statistical analysis of differences between the 1.94 and 1.56 μm lasers was not performed, as it was not intended by the research design.

Contact laser exposure mainly involves radiation absorption by the area of carbonization and its transmission to the surrounding tissues. Since most of the near-infrared radiation is absorbed by carbon particles, no significant differences in the cutting properties of the tested wavelengths were recorded. All the lasers showed good cutting properties. The difference in the coagulation properties appears to depend on the amount of remaining radiation (unabsorbed by carbon) penetrating the tissue and can be explained by differences in the wavelength characteristics (different chromophores and tissue absorption coefficient).

CONCLUSION

The research and the resulting data analysis confirmed the statistically significant dependence of all laser incision characteristics (width of the lateral coagulation zone, depth and width of the ablation zone) on the radiation power for the 0.98, 1.56, and 1.94 μm lasers. The 1.56 and 1.94 μm lasers have better coagulation properties compared with the 0.98 μm semiconductor laser. The 1.94 μm laser is superior to the 0.98 μm laser in its cutting properties.

REFERENCES

1. Mooseburger A.S., Herrmann T.R., Liatsikos E., Nagele U., Traxer O. Lasers and laser technologies. Clinical recommendations of the European Association of Urology. Translation: Siromolot Yu.S. Moscow: 2011; 59 p (in Russ.). URL: https://uroweb.org/wp-content/uploads/21_Lazer.pdf
2. Choy D.S. History of lasers in medicine. *Photomed. Laser Surg*. 2014; 32(3): 119–120. DOI: 10.1089/pho.2014.9864.
3. Goldman L., Blaney D.J., Kindel D.J. Jr., Franke E.K. Effect of the laser beam on the skin. *J. Invest. Dermatol.* 1963; 40: 121–122. DOI: 10.1038/jid.1963.21.
4. Tyurin D.S., Dibirov M.D., Shimanko A.I., Tebenikhin V.S., Arefiev M.N., Volkov A.S., Tsuranov S.V., Shvydko V.S., Magdiev A.Kh. Assessment of morphological changes in the venous wall after endovasal laser and radiofrequency obliteration. *Phlebology.* 2016; 10 (4): 164–170 (in Russ.). DOI: 10.17116/flebo2016104164-170
5. Shaydakov E.V., Bulatov V.L., Ilyukhin E.A., Sonkin I.N., Grigoryan A.G., Galchenko M.I. Optimal regimes of endovenous laser obliteration at the wavelengths of 970, 1470, and 1560 nm: the multicenter retrospective longitudinal cohort study. *Phlebology*. 2013; 1: 22–29 (in Russ.).
6. Yanushko V.A., Rogovoy N.A., Turlyuk D.V., Klimchuk I.P., Kalinin S.S. Endovenous laser coagulation of the saphenous veins of the lower extremities (1470 nm and 1560 nm) in the complex treatment of varicose veins. *Military Medicine.* 2017; 47: 74–78 (in Russ.).
7. Shakhrai S.V., Gain M.Yu., Gain Yu.M., Ryabtseva S.N. Experimental ground for high-intensity laser wavelength choice for chronic hemorrhoids low-invasive surgical treatment. *Kazan Medical Journal.* 2013; 2 (94): 271–277 (in Russ.). DOI: 10.17816/KMJ1604.
8. Kang K. et al. The feasibility of 1940-nm diode laser in tonsillectomy. *Med. Laser.* 2016; 5 (2): 77–82. DOI: 10.25289/ml.2016.5.2.77.
9. Bagratashvili V.N., Bagratashvili N.V., Gapontsev V.P., Makhmutovats G.Sh., Minaev V.P., Omelchenko A.I., Samartsev I.E., Sviridov A.P., Sobol E.N., Tsypina S.I. Change in the optical properties of hyaline cartilage heated by the near-IR laser radiation. *Quantum Electronics.*

2001; 31 (6): 534–538 (in Russ.). DOI: 10.1070/QE2001v031n06ABEH001996.
10. Shumilova N.A. Experience in the use of high-energy lasers in otorhinolaryngology. *Folia Otorhinolaryngologiae et Pathologiae Respiratoriae.* 2016; 1: 73–81 (in Russ.).
11. Karpishchenko S.A., Ryabova M.A., Ulupov M.Yu. Laser surgery in otorhinolaryngology. *Consilium Medicum.* 2014; 16 (11): 73–76 (in Russ.).
12. Ryabova M.A., Ulupov M.Yu., Shumilova N.A. Features of laser effects on tissues with the wavelength of 1470 nm. *Folia Otorhinolaryngologiae et Pathologiae Respiratoriae*. 2016; 22 (3): 36–42 (in Russ.).
13. Shumilova N.A., Fedotova Y.S., Ryabova M.A. Biological effects of contact action of 1470 vs. 810 nm semiconductor lasers *in vitro*. *Modern Technologies in Medicine.* 2014; 6 (4): 62–67 (in Russ.).

Acknowledgements

The authors of the article are thankful to A.V. Radionov for his help in writing this paper.

Authors contribution

Ryabova M.A. – design. Ulupov M.Yu. – collection of data for analysis, analysis of the data. Shumilova N.A. – review of publications on the topic of the article. Tikhomirova E.K., Portnov G.V. – carrying out of the experimental part of the study. Malkova M.E. – statistical analysis of the results.

Authors information

Ryabova Marina A., Dr. Sci. (Med.), Professor of the ENT Department, Pavlov First Saint Petersburg State Medical University, Saint Petersburg, Russian Federation. ORCID 0000-0002-6714-9454.

Ulupov Mikhail Yu., Cand. Sci. (Med.), Associate Professor of the ENT Department, Pavlov First Saint Petersburg State Medical University, Saint Petersburg, Russian Federation., ORCID 0000-0002-8460-9889.

Shumilova Natalia A., Cand. Sci. (Med.), Assistant of the ENT Department, Pavlov First Saint Petersburg State Medical University, Saint Petersburg, Russian Federation. ORCID 0000-0001-5191-9154.

Portnov Gleb V., Cand. Sci. (Med.), Assistant of the ENT Department, Pavlov First Saint Petersburg State Medical University, Saint Petersburg, Russian Federation. ORCID 0000-0002-4117-140X.

Tikhomirova Ekaterina K., Post-Graduate Student, ENT Department, Pavlov First Saint Petersburg State Medical University, Saint Petersburg, Russian Federation. ORCID 0000-0001-6952-6543.

Malkova Mariya E., Post-Graduate Student, ENT Department, Pavlov First Saint Petersburg State Medical University, Saint Petersburg, Russian Federation. ORCID 0000-0001-9579-1017.

(✉) **Tikhomirova Ekaterina K.,** e-mail: kt-92@mail.ru

Received 04.06.2020
Accepted 28.12.2020

УДК 616.33-002.27:616.33-006.6]:577.125:577.15
https://doi.org/10.20538/1682-0363-2021-4-63-70

Characteristics of lipid peroxidation processes and factors of the antioxidant defense system in chronic atrophic gastritis and gastric cancer

Smirnova O.V., Tsukanov V.V., Sinyakov A.A., Moskalenko O.L., Elmanova N.G., Ovcharenko E.S., Kasparov E.V.

Federal Research Center "Krasnoyarsk Science Center of the Siberian Branch of the Russian Academy of Sciences", Research Institute of Medical Problems of the North
3G, Partizana Zheleznyaka Str., 660022, Krasnoyarsk, Russian Federation

ABSTRACT

Background. The problem of gastric cancer remains unresolved throughout the world, while chronic atrophic gastritis (CAG) increases the likelihood of its development by 15 times. In the Russian Federation, the incidence of gastric cancer (GC) is among the highest, with it prevailing among males. One of the leading mechanisms in molecular pathology of membranes is lipid peroxidation (LPO). The severity of oxidative membrane damage depends on concomitant diseases, contributing to emergence and progression of pathological processes and development of cancer. Currently, the problem of LPO is unsolved in biological systems.

The aim of this study was to investigate the state of LPO and antioxidant defense system in CAG and GC.

Materials and methods. The parameters were studied in 45 patients with CAG and 50 patients with GC. The control group included 50 practically healthy volunteers without gastrointestinal complaints, who did not have changes in the gastric mucosa according to the fibroesophagogastroduodenoscopy (FEGDS) findings.

Results. In patients with CAG, an increase in malondialdehyde, superoxide dismutase, catalase, glutathione S-transferase, and glutathione peroxidase was found in the blood plasma compared with the control group. In patients with CAG, lipid peroxidation was activated, and the malondialdehyde level increased by 3.5 times relative to normal values. At the same time, the body fought against oxidative stress by increasing the activity of antioxidant enzymes, such as superoxide dismutase, catalase, glutathione S-transferase, and glutathione peroxidase. All patients with GC showed pronounced oxidative stress in the blood plasma in the form of a 45-fold increase in malondialdehyde. The activity of the main antioxidant enzyme superoxide dismutase was reduced in GC. Catalase was activated, which indicated pronounced oxidative stress, significant damage to blood vessels, and massive cell death. Glutathione-related enzymes (glutathione S-transferase and glutathione peroxidase) and the antioxidant protein ceruloplasmin were activated, which also indicated significant oxidative stress and severe intoxication in patients with GC.

Conclusion. Depending on the stage and type of cancer, an in-depth study of lipid peroxidation and factors of the antioxidant defense system can be used to correct therapy and prevent cancer and can serve as markers of progression and prognosis in gastric cancer.

Key words: chronic gastritis, gastric cancer, chemiluminescent activity of neutrophil granulocytes, Eastern Siberia.

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 project "Development and implementation of a software package for screening and early diagnosis of gastric cancer in terms of immune, prooxidant, and antioxidant systems to reduce mortality and disability rates among the population" was supported by the Krasnoyarsk Regional Science Foundation.

Conformity with the principles of ethics. All individuals signed an informed consent to participate in the study. The study was approved by the local Ethics Committee at the Federal Research Center "Krasnoyarsk Science Center of the Siberian Branch of the Russian Academy of Sciences" (Protocol No. 4 of 02.08.2019).

✉ *Smirnova Olga V.,* e-mail: ovsmirnova71@mail.ru

For citation: Smirnova O.V., Tsukanov V.V., Sinyakov A.A., Moskalenko O.L., Elmanova N.G., Ovcharenko E.S., Kasparov E.V. Characteristics of lipid peroxidation processes and factors of the antioxidant defense system in chronic atrophic gastritis and gastric cancer. *Bulletin of Siberian Medicine.* 2021; 20 (4): 63–70. https://doi.org/10.20538/1682-0363-2021-4-63-70.

Характеристика процессов липопероксидации и факторов системы антиоксидантной защиты при хроническом атрофическом гастрите и раке желудка

Смирнова О.В., Цуканов В.В., Синяков А.А., Москаленко О.Л., Елманова Н.Г., Овчаренко Е.С., Каспаров Э.В.

Красноярский научный центр Сибирского отделения Российской академии наук (КНЦ СО РАН), Научно-исследовательский институт медицинских проблем Севера (НИИ МПС)
Россия, 660022, г. Красноярск, ул. Партизана Железняка, 3г

РЕЗЮМЕ

Актуальность. Проблема рака желудка является нерешенной во всем мире, при этом хронический атрофический гастрит (ХАГ) повышает вероятность его развития в 15 раз. В России показатели заболеваемости раком желудка (РЖ) – одни из самых высоких, мужская заболеваемость здесь лидирует. Одним из ведущих механизмов молекулярной патологии мембран является перекисное окисление липидов (ПОЛ). Выраженность окислительной деструкции мембран зависит от сопутствующих заболеваний, способствуя возникновению и прогрессированию патологических процессов, развитию онкологического заболевания. В настоящее время проблема ПОЛ является нерешенной в биологических системах.

Целью настоящего исследования явилось изучение состояния ПОЛ и антиоксидантной защиты при ХАГ и РЖ.

Материалы и методы. Изучены показатели у 45 пациентов с ХАГ и 50 больных РЖ. Контрольная группа представлена 50 практически здоровыми добровольцами, не имеющими гастроэнтерологических жалоб, у которых отсутствовали изменения слизистой оболочки желудка по результатам фиброэзофагогастродуоденоскопии.

Результаты. У больных ХАГ в плазме крови обнаруживалось увеличение малонового диальдегида, активности супероксиддисмутазы, каталазы, глутатион-S-трансферазы, глутатионпероксидазы относительно контрольной группы. У больных ХАГ происходит активация перекисного окисления липидов, увеличение малонового диальдегида в 3,5 раза относительно нормальных величин. При этом сам организм борется с окислительным стрессом, увеличивая активность антиоксидантных ферментов (супероксиддисмутазы, каталазы, глутатион-S-трансферазы, глутатионпероксидазы). У всех больных РЖ в плазме крови выявлялся выраженный окислительный стресс в виде повышения в 45 раз малонового диальдегида. Активность основного фермента антиоксидантной защиты (супероксиддисмутазы) снижена при РЖ. Активирована каталаза, которая свидетельствует о выраженном окислительном стрессе и значительном повреждении сосудов, о массовом клеточном распаде. Активны ферменты глутатионового звена (глутатион-S-трансфераза и глутатионпероксидаза), антиоксидантный белок (церулоплазмин), которые также указывают на значительный окислительный стресс и выраженный интоксикационный синдром у больных РЖ.

Заключение. Углубленное изучение процессов перекисного окисления липидов и факторов системы антиоксидантной защиты в зависимости от стадии онкопроцесса и типов рака может использоваться для коррекции терапии и профилактики онкозаболеваний, а также в качестве маркеров прогрессирования и прогноза рака желудка.

Ключевые слова: хронический гастрит, рак желудка, хемилюминесцентная активность нейтрофильных гранулоцитов, Восточная Сибирь.

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

Источник финансирования. Проект «Разработка и внедрение программного комплекса скрининга и ранней диагностики рака желудка по показателям иммунной, прооксидантной и антиоксидантной систем для снижения показателей смертности и инвалидизации населения» проведен при поддержке Красноярского краевого фонда науки.

Соответствие принципам этики. Все участники подписали информированное согласие. Исследование одобрено локальным этическим комитетом ФИЦ КНЦ СО РАН (протокол № 4 от 02.08.2019).

Для цитирования: Смирнова О.В., Цуканов В.В., Синяков А.А., Москаленко О.Л., Елманова Н.Г., Овчаренко Е.С., Каспаров Э.В. Характеристика процессов липопероксидации и факторов системы антиоксидантной защиты при хроническом атрофическом гастрите и раке желудка. *Бюллетень сибирской медицины.* 2021; 20 (4): 63–70. https://doi.org/10.20538/1682-0363-2021-4-63-70.

INTRODUCTION

The problem of gastric cancer (GC) remains unresolved all over the world, while chronic atrophic gastritis increases the likelihood of its development by 15 times. In the Russian Federation, the incidence rate of GC is among the highest, with it prevailing among men [1–3]. Low survival rates are due to late diagnosis of a malignant disease and low treatment efficiency at the end stages of the disease. GC is characterized by regional variability; the disease is 2 times more common in the Far East, Eastern Siberia, and the North of the European part of Russia [4, 5]. One of the leading mechanisms in the molecular pathology of membranes is lipid peroxidation (LPO). The severity of oxidative damage to membranes depends on concomitant diseases, contributing to the emergence and progression of pathological processes and development of cancer. Currently, the problem of LPO is unsolved in biological systems. According to the Correa cascade, a precancerous condition of the stomach (chronic atrophic gastritis) associated with *Helicobacter pylori* infection can advance to GC (adenocarcinoma) [6–8]. Atrophic changes in the gastric mucosa trigger membrane destruction, and produced reactive oxygen species (ROS) contribute to oxidative damage to tissues, enhancing lipid peroxidation in cell membranes [9–11]. The role of toxic products of LPO and free radicals in the development of chronic oxidative stress and advancement of the disease to GC is not excluded. Progression of the malignant disease aggravates disorders in LPO and antioxidant defense (AOD) systems, reduces tumor resistance in patients with GC, and causes the emergence of histotoxic hypoxia, disorders of tissue respiration, and an increase in LPO products, closing the vicious pathogenetic circle [12–14].

The aim of this study was to research the state of lipid peroxidation and antioxidant defense systems in chronic atrophic gastritis and gastric cancer.

MATERIALS AND METHODS

The parameters were studied in 45 patients with chronic atrophic gastritis (CAG) and 50 patients with gastric cancer (GC). The control group encompassed 50 practically healthy volunteers who did not have gastroenterological complaints or changes in the gastric mucosa (GM) according to the results of fibroesophagogastroduodenoscopy. The study was approved by the local Ethics Committee at the Federal Research Center of the KSC SB RAS (Protocol No. 4 of 02.08.2019). All ethical principles imposed by Art. 24 of the Constitution of the Russian Federation and the Declaration of Helsinki by the World Medical Association were observed in the study. Each participant confirmed that they take part in the study voluntarily by signing a voluntary informed consent to participate in the study.

The diagnosis of CAG was verified according to the clinical data, medical history data, fibroesophagogastroduodenoscopy, and morphological examination of the mucous membrane of the greater and lesser curvature of the stomach using the updated Sydney System. Diagnosis of GC was carried out by oncologists at Krasnoyarsk Regional Oncology Dispensary, taking into account the full range of instrumental and morphological examination. This study included patients with GC associated with *Helicobacter pylori* infection and adenocarcinoma as a histological variant of the tumor.

The material of the study was venous blood which was drawn from the cubital vein in the morning, from 8 to 9 o'clock, on an empty stomach, upon admission of the patient to the hospital before

the start of pathogen-specific therapy. The presence of *H. pylori* was detected in all patients included in the study by ELISA to determine the titer of specific IgG antibodies to the *H. pylori* CagA antigen. If the titer of antibodies to *H. pylori* corresponded to 30 EIU or more, it was assessed as a positive result. If the antibody titer was less than 30 EIU, it was assessed as a negative result.

In addition, for chronic atrophic gastritis, serological diagnosis was performed to determine pepsinogens in the blood serum. The diagnosis of severe CAG of the gastric mucosa was made when the level of pepsinogen-1 was less than 25 μg/l and the value of the pepsinogen-1/pepsinogen-2 ratio was less than 3. The final diagnosis was always verified by the results of a morphological examination of the gastric mucosa (GM).

In the blood serum, spectrophotometric methods were used to determine the LPO-AOD parameters: malondialdehyde, activity of glutathione S-transferase, glutathione peroxidase, superoxide dismutase, catalase, and ceruloplasmin. The ratio of pro- and antioxidant factors was used to calculate an integral coefficient of individual oxidative stress assessment (coefficient of oxidative stress – COS).

$$COS = \frac{(DC_i/DC_n) \times (KD \text{ and } CT_i/KD \text{ and } CT_n) \times (TBA - AP_i/TBA - AP_n)}{(SOD_i/SOD_n \times (GSH_i/GSH_n) \times (\alpha - \text{tocopherol}_i/\alpha - \text{tocopherol}_n/) \times (\text{retinol}_i/\text{retinol}_n)}$$

where *i* – the levels of the parameter in the examined patients; *n* – the level of the parameter in the control group. With COS> 1, the development of oxidative stress was recorded.

Statistical analysis of the results was carried out using the Statistica for Windows 8.0 (StatSoft Inc., USA, 2008) and Microsoft Excel, 2007 (Microsoft, USA) software packages [15]. Nonparametric data were determined: the median and the interquartile range *Me* (C_{25} – C_{75}). Statistically significant differences were established using the Mann – Whitney test. The critical level of statistical significance when testing scientific hypotheses was considered equal to $p < 0.05$.

RESULTS

We studied the features of LPO and AOD systems in the blood plasma of patients with CAG and GC. The level of malondialdehyde indicated the severity of oxidative stress in the blood plasma. The products of LPO are opposed by the activity of antioxidant enzymes (superoxide dismutase (SOD), catalase, glutathione S-transferase, glutathione peroxidase) and the effect of the antioxidant protein ceruloplasmin.

The median plasma concentration of malondialdehyde in patients with CAG and GC increased relative to the control group (Table). An increase in the median concentration of malondialdehyde in the plasma was found in patients with GC (adenocarcinoma) compared with patients with CAG. Malondialdehyde is considered the end product of LPO and is a parameter of LPO processes triggered in cells by free radicals and ROS. Malondialdehyde, being an active compound, can react with proteins, carbohydrates, and nucleic acids, and the formed complexes reduce their biological activity.

Lipoprotein particles (very-low-density lipoproteins (VLDL), low-density lipoproteins (LDL), high-density lipoproteins (HDL)) are a necessary component of LPO in the blood. Altered lipoproteins damage the endothelial lining of blood vessels, contributing to the development of atherosclerosis. A significant increase in MDA in the blood plasma of patients with CAG and GC indicates excessive formation of ROS, which become an altering factor in the vascular endothelium.

The median plasma superoxide dismutase concentration increased in CAG patients in comparison with the control group. In contrast, in the patients with GC, a decrease in the median concentration of superoxide dismutase was found compared with the CAG. Superoxide dismutase is the most important enzyme of the antioxidant defense system; at the stage of one-electron oxygen reduction, it interrupts the chain of free-radical processes at its inception with the formation of a superoxide anion radical. The extracellular SOD isoform is active in the blood plasma. As a rule, an increase in its activity indicates an increase in the number of free radicals and reactive oxygen species in the intercellular fluid. Excessive production of this enzyme is due to increased activity of glial cells and fibroblasts.

The median concentration of catalase in the blood plasma increased in the patients with CAG and GC compared with the control group. Significant catalase activity indicated damage to the endothelium of blood vessels following oxidative stress.

Table

Parameters of pro- and antioxidant systems in the blood plasma in patients with chronic atrophic gastritis and gastric cancer compared with the control group, *Me* (C_{25}–C_{75})

Parameter	Control, *n* = 50 (1)	CAG, *n* = 45 (2)	GC, *n* = 50 (3)
MDA, μmol / 1 g protein	1.6 (0.96–2.24)	5.24 (4.38–5.88) $p_{1-2} < 0.001$	56.35(32.46–101.74) $p_{1-3} < 0.001$; $p_{2-3} < 0.001$
SOD, units / min / 1 g protein	204.41 (151.05–250.32)	570.5 (314–670.8) $p_{1-2} < 0.001$	235.2 (133.7–462.27) $p_{2-3} < 0.001$
CAT, μmol / s / 1 g protein	0.27 (0.16–0.39)	0.66 (0.42–0.71) $p_{1-2} = 0.03$	0.87 (0.67–1.01) $p_{1-3} = 0.02$
GST, mmol / min / 1 g protein	41.3 (37.7–42.64)	70.6 (63.5–105.7) $p_{1-2} < 0.001$	83.5 (79.3–110.6) $p_{1-3} < 0.001$
GPO, μmol / 1 g protein	105.9 (81.19–162.38)	177.5 (150.1–236.05) $p_{1-2} = 0.007$	168.6 (158.7–211.5) $p_{1-3} = 0.05$
CP, mg / l	192.5 (157.5–227.0)	149.6 (113.7–189.8)	375.8 (282.9–826.06) $p_{1-3} < 0.001$; $p_{2-3} < 0.001$

Note: statistically significant differences between the CAG patients and the control group – $p_{1-2,}$ between the GC patients and the control group – $p_{1-3,}$ between the CAG patients and the GC patients – $p_{2-3.}$

Catalase lacks an extracellular isoform, therefore, its high activity in the blood plasma is due to massive cell death, which proves histodestruction in CAG and GC.

Glutathione in the antioxidant defense system acts against endotoxicosis. The median concentration of glutathione S-transferase in the plasma in the patients with CAG and GC increased compared with the control group. In the patients with CAG and GC, the median concentration of glutathione S-transferase in the plasma was significantly higher than in the control group, as was the median concentration of glutathione peroxidase. Probably, the increased activity of these enzymes indicates the severity of intoxication, the presence of oxidative stress, and insufficient effectiveness of the antioxidant defense system in patients with CAG and GC.

The median ceruloplasmin level in the patients with GC was significantly elevated compared with all other studied groups, which proves an increase in oxidative stress in GC following the combined effect of various pathogenetic factors. Ceruloplasmin is an essential antioxidant copper-containing glycoprotein with ferroxidase and superoxide-removing activity. The protein ceruloplasmin inhibits superoxide and ferritin-dependent lipid peroxidation in lipoprotein particles of the blood plasma. According to the ratio of pro- and antioxidant components, COS was calculated for chronic atrophic gastritis (3.5) and for gastric cancer (45).

DISCUSSION

In the patients with CAG, an increase in malondialdehyde, superoxide dismutase, catalase, glutathione S-transferase, and glutathione peroxidase activity was found in the blood plasma compared with the control group. Thus, in the patients with CAG, lipid peroxidation was activated, and malondialdehyde increased by 3.5 times compared with normal values. At the same time, the body fought against oxidative stress by increasing the activity of antioxidant enzymes (superoxide dismutase, catalase, glutathione S-transferase, and glutathione peroxidase). Parameters of LPO and AOD in CAG prove the presence of cell destruction in the gastric mucosa infected with *Helicobacter pylori*.

All patients with GC showed pronounced oxidative stress in the blood plasma in the form of a 45-fold increase in the content of malondialdehyde. The activity of superoxide dismutase was reduced in GC. Activated catalase indicated massive cell death and significant vascular damage. Glutathione-related enzymes (glutathione S-transferase and glutathione peroxidase) and the antioxidant protein ceruloplasmin, which also combat oxidative stress and severe intoxication in GC patients, were activated. In patients with GC, multidirectional shifts in the activity of the antioxidant system enzymes were revealed. Compared with CAG, the activity of the main enzyme superoxide dismutase was reduced, which reflects the classic version of the peroxide theory of

carcinogenesis [16] with inhibition of antioxidant enzymes.

The effect of antioxidant defense enzymes in GC has ambiguous changes: the activity of superoxide dismutase decreases, while the activity of the second line of defense (glutathione S-transferase and glutathione peroxidase) increases. Most likely, these processes are associated with the fact that an increase in the ROS level causes depletion of the enzymatic activity of AOD. ROS attack thiol proteins through interaction with the protein SH-group, changing their structural modification. These proteins are the key enzymes in the metabolism of nucleotides, carbohydrates, and the antiradical defense system (glutathione-related enzymes). This modification enhances the formation of the superoxide anion radical, therefore, ROS formation only increases.

In GC, hypoxia enhancing nitrate reductase activity is observed in the cell. Increased NO synthesis reacts with excess amount of superoxide anion radical to form peroxynitrite, causing the formation of carcinogenic nitrosamines. The resulting products interfere with apoptosis of tumor cells and enhance their metastasis. The tumor process in GC is associated with increased production of ROS, which at high concentrations can irreversibly damage tumor cells, while they themselves contribute to tumor progression. Strengthening the processes of free radical oxidation of membrane lipids and their interaction with LPO products lead to changes in lipid and protein domains. All this contributes to malignant transformation, invasiveness, uncontrolled tumor growth and metastasis [17] and affects the state of the antioxidant system enzymes, which are crucial for the malignant process [18, 19]. Therefore, in patients with GC, an imbalance of antiradical defense system is found, which contributes to better survival of tumor cells and tumor progression.

The activation of free radical oxidation is proved by the obtained results on the increase in LPO in the blood plasma of patients with CAG and GC. At the same time, the content of LPO products and pronounced disorders of the combined functioning of the antioxidant enzymes in the blood plasma increased in patients with GC [20]. Depending on the stage of cancer and its types, an in-depth study of LPO and AOD can be used to correct therapy and prevent cancer and can serve as markers of progression and prognosis in GC [21, 22].

CONCLUSION

The study of lipid peroxidation and antioxidant defense parameters in CAG and GC associated with *Helicobacter pylori* infection proved the importance of these biochemical processes in the pathogenesis of the diseases. In CAG, there is an increase in lipid peroxidation, which the body tries to compensate by activating the enzymes of AOD. The coefficient of oxidative stress (COS) in CAG is 3.5, which implies that the level of the end products of lipid peroxidation in CAG patients is 3.5 times higher than in healthy people. The increase in lipid peroxidation in CAG is probably due to morphological changes in the cells of the gastric mucosa. The higher the COS, the more structural changes in the gastric mucosa. Consequently, early detection of LPO and AOD parameters makes it possible to identify a risk group among CAG patients who need a more effective pathogen-specific therapy aimed at eliminating atrophic changes in the gastric mucosa.

In GC, tumor growth, intoxication, progressive destruction of healthy gastric mucosa, unresponsiveness of the immune system, etc. are revealed. All this causes a drastic rise in lipid peroxidation, and the coefficient of oxidative stress is 45. AOD is not effective; LPO, destroying membranes, enhances the decay of cells and tissues and complicates the clinical course of GC, hence, resulting in ineffective therapy in late stages of cancer. The established disturbances in the function of AOD have a significant impact on the viability and functions of cancer cells. The imbalance between lipid peroxidation and factors of the antioxidant defense system is closely related to enzymatic changes in the exchange of nucleotides, these processes regulating each other according to the feedback principle. The development of oxidative stress is accompanied by structural modification of biological membranes, enzymes, and nucleotides. The intensity of metabolic processes and rearrangements in the pathogenesis at the cellular level depend on the severity of all these disorders. Therefore, the key issue in the development of GC is the balance between prooxidants and antioxidants. Early diagnosis of patients with CAG and their complex pathogen-specific therapy will reduce the number of patients with advancement of the disease to gastric cancer and reduce the overall mortality and disability rates among the Russian population.

REFERENCES

1. Lazebnik L.B., Vasiliev Yu.V., Shcherbakov P.L. et al. *Helicobacter pylori*: prevalence, diagnosis, treatment. *Experimental and Clinical Gastroenterology.* 2010; 2: 3–7 (in Russ.).
2. Bordin D.S., Masharova A.A., Khomeriki S.G. Chronic gastritis: a modern perspective on an old problem. *Modern Gastroenterology*. 2013; 1(69): 72–79 (in Russ.).
3. Vernigorodsky S.V. Features of structural changes of the gastric mucosa of different age groups patients in chronic gastritis. *The Journal of Clinical and Experimental Morphology*. 2014; 1(9): 4–7 (in Russ.).
4. Volkova N.N. Risk factors for the development of chronic atrophic gastritis (literature review). *Russian Medical Journal*. 2013; 21 (31): 1617–1620 (in Russ.).
5. Denisov N.L., Ivashkin V.T., Lobzin Yu.V. Chronic gastritis from the standpoint of the interaction of immune, infectious and morphological factors. *Russian Journal of Gastroenterology, Hepatology, Coloproctology.*2008; 6 (18): 22–27 (in Russ.).
6. Bakulin I.G., Pirogov S.S., Bakulina N.V., Stadnik E.A., Golubev N.N. Prophylaxis and early diagnosis of stomach cancer. *Russian Journal of Evidence-Based Gastroenterology.* 2018; 7 (2): 44–58 (in Russ.).
7. Stepanov Yu.M., Simonova E.V. Increase of informativeness of endoscopic diagnosis for precancerous changes and gastric cancer in patients with atrophic gastritis. *Gastroenterology*. 2013; 4 (50): 23–33 (in Russ.).
8. Skvortsov V.V., Skvortsova E.M. Topical issues of diagnosis and treatment of type B antral gastritis. *Poliklinika*. 2012; 1 (1): 102–106 (in Russ.).
9. Adamu M.A., Weck M.N., Rothenbacher D. et.al. Incidence and risk factors for the development of chronic atrophic gastritis: five year follow up of a population based cohort study. *Int. J. Cancer.* 2011; 128: 1652–1658.
10. Correa P. Gastric cancer: overview. *Gastroenterol. Clin. North. Am.* 2013; 42 (2): 211–217.
11. Graham D.Y. Helicobacter pylori update: gastric cancer, reliable therapy, and possible benefits. *Gastroenterology*. 2015; 148 (4): 719–731.
12. Watari J., Chen N., Amenta P.S. et.al. Helicobacter pylori associated chronic gastritis, clinical syndromes, precancerous lesions, and pathogenesis of gastric cancer development. *J. Watari*. *World J. Gastroenterol*. 2014; 20 (18): 5461–5473.
13. Yoshida T. et al. Cancer development based on chronic active gastritis and resulting gastric atrophy as assessed by serum levels of pepsinogen and *Helicobacter pylori* antibody titer. *Int*. *J. Cancer*. 2014; 134 (6): 1445–1457.
14. Smirnova O.V., Titova N.M., Manchuk V.T., Elmanova N.G. Features of cytokine regulation in patients with mechanical jaundice of malignant genesis. *Modern Problems of Science and Education.* 2015; 4: 425 (in Russ.).
15. Pavlovich I.M., Gordienko A.V., Batskov S.S. et al. Effect of *Helicobacter pylori* on the morphological status of the gastric mucosa in chronic gastritis. *Medico-Biological and Socio-Psychological Problems of Safety in Emergency Situations*. 2013; 2: 32–35 (in Russ.).
16. Lyu B.N. Aging, age-related pathologies and carcinogenesis (oxygen-peroxide concept). Almaty: KazNTU, 2003: 706 (in Russ.).
17. Hryniewicz-Jankowska A., Augoff K., Biernatowska A., Podkalicka J., Sikorski A.F. Membrane rafts as a novel target in cancer therapy. *Biochim. Biophys. Acta Rev. Cancer.* 2014; 1845 (2): 155–165. DOI: 10.1016/j.bbcan.2014.01.006.
18. Rosenko L.Ya., Sidorenko Yu.S., Frantsiyants E.M. Does the volume of the tumor affect the state of the antioxidant defense of the body? *Problems in Oncology.* 1999; 45 (5): 538–541 (in Russ.).
19. Sidorenko Yu.S., Musienko N.V., Frantsiyants E.M. Some indices of activity of proteolytic system in tissue of malignant tumor and perifocal zone at different cancer localizations. *Bulletin of the SSC RAS.* 2008; 4 (2): 93–98 (in Russ.).
20. Goroshinskaya I.A., Medvedeva D.E., Surikova E.I., Nemashkalova L.A., Kachesova P.S., Malinin S.A., Kaminsky G.V., Maslov A.A., Kit O.I. State of oxidative metabolism in the blood of gastric cancer patients with different tumor histotypes. *Modern Problems of Science and Education.*2019; 1 (in Russ.). URL: http://www.science-education.ru/ru/article/view?id=28440.
21. Bandu R., Mok H.J., Kim K.P. Phospholipids as cancer biomarkers: Mass spectrometry-based analysis. *Mass Spectrom. Rev*. 2016; 37 (2): 107–138. DOI: 10.1002/mas.21510.
22. Yan G., Li L., Zhu B., Li Y. Lipidome in colorectal cancer. *Oncotarget*. 2016; 7 (22): 33429–33439. DOI: 10.18632/oncotarget. 7960.

Authors contribution

Smirnova O.V. – conception and design, analysis and interpretation of data, drafting of the manuscript. Tsukanov V.V. – editing of the manuscript. Sinyakov A.A. – collection and processing of clinical material, statistical processing of data. Moskalenko O.L. – collection and processing of clinical material, statistical processing of data. Elmanova N.G. – collection and processing of clinical material. Ovcharenko E.S. – collection and processing of clinical material. Kasparov E.V. – editing of the manuscript.

Authors information

Smirnova Olga V., Dr. Sci. (Med.), Head of the Laboratory of Clinical Pathophysiology, Research Institute of Medical Problems of the North, Krasnoyarsk Science Center of the SB RAS, Krasnoyarsk, Russian Federation. ORCID 0000-0003-3992-9207.

Tsukanov Vladislav V., Dr. Sci. (Med.), Professor, Head of the Clinical Department of Digestive System Pathology in Adults and Children, Research Institute of Medical Problems of the North, Krasnoyarsk Science Center of the SB RAS, Krasnoyarsk, Russian Federation. ORCID 0000-0002-9980-2294.

Sinyakov Aleksandr A., Cand. Sci. (Biology), Junior Researcher, Laboratory of Clinical Pathophysiology, Research Institute of Medical Problems of the North, Krasnoyarsk Science Center of the SB RAS, Krasnoyarsk, Russian Federation. ORCID 0000-0002-4474-1893.

Moskalenko Olga L., Cand. Sci. (Biology), Senior Researcher, Laboratory of Clinical Pathophysiology, Research Institute of Medical Problems of the North, Krasnoyarsk Science Center of the SB RAS, Krasnoyarsk, Russian Federation. ORCID 0000-0003-4268-6568.

Elmanova Nina G., Junior Researcher, Laboratory of Clinical Pathophysiology, Research Institute of Medical Problems of the North, Krasnoyarsk Science Center of the SB RAS, Krasnoyarsk, Russian Federation. ORCID 0000-0001-6073-0601.

Ovcharenko Elizaveta S., Junior Researcher, Laboratory of Clinical Pathophysiology, Research Institute of Medical Problems of the North, Krasnoyarsk Science Center of the SB RAS, Krasnoyarsk, Russian Federation. ORCID 0000-0001-6884-7871.

Kasparov Eduard V., Dr. Sci. (Med.), Professor, Director of Research Institute of Medical Problems of the North, Deputy Director of Krasnoyarsk Science Center of the SB RAS, Krasnoyarsk, Russian Federation. ORCID 0000-0002-5988-1688.

(✉) **Smirnova Olga V.,** e-mail: ovsmirnova71@mail.ru

Received 05.10.2020
Accepted 28.12.2020

УДК 577.122.34.084.1:547.992.2:546.57-022.532
https://doi.org/10.20538/1682-0363-2021-4-71-78

The effect of coal-derived humic substances and their silver-containing bionanocomposites on arginine balance in peritoneal macrophages of intact mice

Trofimova E.S.[1, 2], Zykova M.V.[2], Danilets M.G.[1], Ligacheva A.A.[1], Sherstoboev E.Yu.[1], Grigorieva I.O.[3], Mikhalev D.A.[2], Tsupko A.V.[2], Logvinova L.A.[2], Perminova I.V.[3], Belousov M.V.[2]

[1] *Goldberg Research Institute of Pharmacology and Regenerative Medicine, Tomsk National Research Medical Center (NRMC)*
3, Lenina Av., Tomsk, 634028, Russian Federation

[2] *Siberian State Medical University (SSMU)*
2, Moscow Tract, Tomsk, 634050, Russian Federation

[3] *Lomonosov Moscow State University*
1, Leninskie Gory, Moscow, 119991, Russian Federation

ABSTRACT

Background. Antigen-presenting cells (APCs), especially macrophages, play an important role in the body defense against various pathogens. Their dysfunction and polarization are associated with most inflammatory and autoimmune diseases. The inflammatory process is regulated by activation and / or inhibition of genes differentially expressed by macrophages. Successful correction of inflammation leads firstly to elimination of inflammatory stimuli and then to remodeling and restoration of tissues and organs. It was experimentally confirmed that silver-containing bionanocomposites based on natural humic substances (HS) obtained from coal of different origin, as well as initial matrices of these HS, are capable of activating pro- and anti-inflammatory properties of macrophages.

Aim. To study cytotoxic, pyrogenic, and immunomodulatory properties (arginine balance) of initial HS samples and samples of silver nanoparticles ultradispersed in these HS matrices (HS-AgNPs) in the cell culture of peritoneal macrophages, as well as their effect on pro- and anti-inflammatory properties of APCs.

Materials and methods. Cultural and biochemical methods were used in the study.

Results. The study showed that the samples CHE-K, CHE-AgNPs, CHS-K, and CHP-K increased M1 macrophage polarization due to stimulation of the NO-synthase activity and inhibition of arginase. The samples CHI-K, CHI-AgNPs, CHP-AgNPs, and CHS-AgNPs modulated an alternative M2 or M2-like state of macrophage activation. At the same time, HS are not cytotoxic at effective concentrations, and three out of four studied samples did not contain pyrogenic impurities.

Conclusion. The use of HS and their silver-containing bionanocomposites, which have the ability to greatly affect the polarization of antigen-presenting cells, is a promising research area in correction of the inflammatory response for solving an important social and medical problem of treating chronic wounds.

Key words: coal-derived humic substances, silver nanoparticles, macrophage polarization, arginine balance.

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 work was carried out with the financial support of the Russian Science Foundation (Grant No. 20-65-47052).

Conformity with the principles of ethics. Experiments using laboratory animals were carried out in accordance with the principles of humanity set out in the directives of the European Community (86/609/EEC) and the Decla-

✉ *Trofimova Evgeniya S.,* e-mail: trofimova_es@pharmso.ru

ration of Helsinki. The study was approved by the Bioethics Committee at Goldberg Research Institute of Pharmacology and Regenerative Medicine (Protocol No. 171052020 of 18.05.2020).

For citation: Trofimova E.S., Zykova M.V., Danilets M.G., Ligacheva A.A., Sherstoboev E.Yu., Grigorieva I.O., Mikhalev D.A., Tsupko A.V., Logvinova L.A., Perminova I.V., Belousov M.V. The effect of coal-derived humic substances and their silver-containing bionanocomposites on arginine balance in peritoneal macrophages of intact mice. *Bulletin of Siberian Medicine.* 2021; 20 (4): 71–78. https://doi.org/10.20538/1682-0363-2021-4-71-78.

Влияние гуминовых веществ угля и биокомпозиций с наночастицами серебра на их основе на баланс аргинина в перитонеальных макрофагах интактных мышей

Трофимова Е.С.[1, 2], Зыкова М.В.[2], Данилец М.Г.[1], Лигачева А.А.[1], Шерстобоев Е.Ю.[1], Григорьева И.О.[3], Михалёв Д.А.[2], Цупко А.В.[2], Логвинова Л.А.[2], Перминова И.В.[3], Белоусов М.В.[2]

[1] *Научно-исследовательский институт фармакологии и регенеративной медицины (НИИФиРМ) имени Е.Д. Гольдберга, Томский научный исследовательский медицинский центр (НИМЦ) Российской академии наук Россия, 634028, г. Томск, пр. Ленина, 3*

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

[3] *Московский государственный университет имени М.В. Ломоносова Россия, 119991, г. Москва, Ленинские Горы, 1*

РЕЗЮМЕ

Введение. Антигенпрезентирующие клетки, особенно макрофаги, играют важную роль в защите организма от различных патогенов, их дисфункции, и поляризация связана с большинством воспалительных и аутоиммунных заболеваний. Воспалительный процесс жестко регулируется активацией и (или) ингибированием дифференциально экспрессируемых макрофагами генов. Успешная коррекция воспалительного процесса приводит к устранению воспалительных стимулов и далее ремоделированию и восстановлению тканей и органов. Экспериментально доказано, что биокомпозиции с наночастицами серебра на основе природных гуминовых веществ (ГВ) угля различного генеза, а также исходные матрицы данных ГВ способны активировать про- и противовоспалительные свойства макрофагов.

Цель. Исследование в культуре клеток перитонеальных макрофагов цитотоксических, пирогенных и иммуномодулирующих свойств (баланс аргинина) исходных образцов ГВ и образцов наночастиц серебра, ультрадиспергированных в данных матрицах гуминовых веществ (ГВ-AgNPs),а также их влияния на про- и противовоспалительные свойства антигенпрезентирующих клеток.

Материалы и методы. Использовались культуральные и биохимические методы.

Результаты. Показано, что образцы CHE-K, CHE-AgNPs, CHS-K, CHP-K за счет усиления активности NO-синтазы и ингибиции аргиназы способствуют поляризации перитонеальных макрофагов по классическому типу (M1). Образцы CHI-K, CHI-AgNPs, CHP-AgNPs и CHS-AgNPs модулируют альтернативный M2 или M2-подобный тип (M2-like state) активации макрофагов. При этом ГВ не цитотоксичны в эффективных концентрациях, а также три из четырех исследуемых образцов не содержат пирогенных примесей.

Заключение. Применение ГВ и серебросодержащих бионанокомпозиций на основе ГВ, обладающих способностью широко влиять на поляризацию антигенпрезентирующих клеток, является перспективным направлением исследований коррекции воспалительной реакции и, в частности, для решения острой социальной и медицинской проблемы – лечения хронических ран.

Ключевые слова: гуминовые вещества угля, наночастицы серебра, поляризация макрофагов, баланс аргинина.

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

Источник финансирования. Работа выполнена при финансовой поддержке РНФ (грант № 20-65-47052).

Соответствие принципам этики. Эксперименты с использованием лабораторных животных выполнялись с соблюдением принципов гуманности, изложенными в директивах Европейского сообщества (86/609/EEC) и Хельсинкской декларации. Исследование одобрено биоэтическим комитетом НИИФиРМ им. Е.Д. Гольдберга (протокол № 171052020 от 18.05.2020).

Для цитирования: Трофимова Е.С., Зыкова М.В., Данилец М.Г., Лигачева А.А., Шерстобоев Е.Ю., Григорьева И.О., Михалёв Д.А., Цупко А.В., Логвинова Л.А., Перминова И.В., Белоусов М.В. Влияние гуминовых веществ угля и биокомпозиций с наночастицами серебра на их основе на баланс аргинина в перитонеальных макрофагах интактных мышей. *Бюллетень сибирской медицины.* 2021; 20 (4): 71–78. https://doi.org/10.20538/1682-0363-2021-4-71-78.

INTRODUCTION

It is known that antigen-presenting cells (APCs), especially macrophages (MPs), play an important role in the initiation of inflammation and pathogenesis of chronic (Crohn's disease, ulcerative colitis, asthma, allergies, atopic dermatitis, periodontosis) and autoimmune (rheumatoid arthritis, multiple sclerosis, diabetes mellitus, cardiovascular diseases, neurodegenerative disorders) diseases and cancer [1]. Macrophages are the first line of defense and, depending on the nature of the antigen (bacteria, viruses) or changes in the microenvironment (ischemia, necrosis, and apoptosis of cells), they are activated and take on various types of phenotypic and functional polarization [2].

The type of MP polarization determines the development of a specific immune response and activation of Th1, Th2, Th17, and Treg cells, which are further responsible for the pathological process and inflammatory response, systemic metabolism, hematopoiesis, vasculogenesis, and tissue homeostasis [3, 4]. Macrophages mainly exist in two different phenotypes: 1) M1 macrophages (classically activated or proinflammatory) through the expression of transcription factors, mainly nuclear factor-κB (NF-κB), produce proinflammatory cytokines, such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), IL-6, IL-12, and IL-23; and 2) M2 (alternatively activated, anti-inflammatory) which are immunoregulatory cells producing anti-inflammatory cytokines IL-10 and transforming growth factor-β (TGF-β) [1, 5–8].

The functions of M1 macrophages are traditionally associated with microorganism phagocytosis, microbicidal activity, induction of inflammation, and antitumor activity. M2 marcophages suppress inflammation and promote tissue repair and remodeling, homeostasis, and vasculogenesis. The M1/M2 balance determines the fate of the organ in conditions of inflammation or injury. In this case, MPs are very plastic, and one phenotype can repolarize into another [3]. During infection (inflammation), tissue-resident MPs initially exhibit the M1 phenotype; however, long-term development of this phase causes chronic inflammation, tissue damage, and loss of organ function. Under these conditions, suppression of inflammation and activation of M2 macrophages are extremely necessary [6, 9]. Thus, control and modulation of macrophage functions, their polarization, and a relationship between pro- and anti-inflammatory responses determine the outcome of inflammation and are necessary for managing inflammation and restoring organ functions [10].

Effective anti-inflammatory drugs are already available and a number of drugs of various origins are under development. The study of biocomposites based on humic substances (HS) and their silver-containing bionanocomposites possessing antimicrobial, anti-inflammatory, and wound healing properties for treatment of purulent, chronic non-healing wounds is innovative and has not been described in the world literature. First of all, the effect of these substances on the mechanisms of the anti-inflammatory response and formation of the immune response is unknown. The relevance of this kind of research is beyond doubt, since a lack of effective methods for treating chronic non-healing wounds leads to infection, and against the background of the existing problem of antibiotic resistance – to sepsis and death. Therefore, potentially effective and safe substances with antimicrobial and wound-healing properties should be carefully studied and introduced into clinical practice following confirmation of the therapeutic efficacy.

The aim of this study was to investigate the cytotoxic, pyrogenic, and immunomodulatory properties (arginine balance) of initial HS samples and samples of silver nanoparticles ultradispersed in these HS matrices (HS-AgNPs) in the cell culture of peritoneal macrophages, as well as their effect on pro- and anti-inflammatory properties of APCs.

MATERIALS AND METHODS

Test systems. In the experiments, we used conventional C57BL/6 mice (total of 80 heads) of both sexes at the age of 8–10 weeks, obtained from the Department of Experimental Biomodels at the Goldberg Research Institute of Pharmacology and Regenerative Medicine, the structural unit of Tomsk NRMC.

The substances under study were humic substances and silver-containing bionanocomposites based on them (HS-AgNPs), synthesized at the Laboratory of Natural Humic Systems of the Lomonosov Moscow State University, Chemistry Department. They were dissolved immediately before use in the culture medium. The synthesis of HS-AgNPs biomaterials was carried out by reducing silver ions in HS solutions (at a concentration of 15 g/l) using an $AgNO_3$ solution until the final concentration of silver nanoparticles was 20 mmol/l. The characteristics of the research objects are presented in Table 1.

Table 1

Experimental samples of coal-derived humic substances and silver-containing bionanocomposites based on them

Names of commercial samples of coal-derived HS	Sample code	
	HS (basic matrix)	HS with Ag nanoparticles (HS-AgNPs)
"Powhumus" (Humintech, Germany)	CHP-K	CHP-AgNPs
"Sakhalin humates", Russian Federation	CHS-K	CHS-AgNPs
"Irkutsk humates", Russian Federation	CHI-K	CHI-AgNPs
Humic substances, Genesis, Russian Federation	CHE-K	CHE-AgNPs

Cell preparation. From the cell suspension obtained by washing the abdominal cavity of mice with ice-cold sodium chloride solution, mature peritoneal MPs were isolated using the EasySep™Biotin Positive Selection Kit and antibodies specific to macrophage receptors, Anti-Mouse F4/80 Antibody (both Stem Cell, USA).

Cultivation conditions. MPs ($2.5–3 \times 10^6$) were cultured for 48 hours (37°C, 5% CO_2, 100% humidity) in a complete culture medium (RPMI 1640 (Sigma, USA) with the addition of 10% fetal bovine serum (FBS) (Hyclone, UK), 20 mM of HEPES (Sigma, USA), 0.05 mM of 2-mercaptoethanol (Sigma, USA), 50 μg/ml of gentamicin (Sigma, USA), and 2 mM of L-glutamine (Sigma, USA) in 96-well plates in the presence of various concentrations of the studied samples or 0.1 μg/ml lipopolysaccharide (LPS) (Sigma, USA).

Study of the arginine balance and cytotoxicity. According to the attached protocols, the content of nitrites in the production of nitric oxide (NO) was determined in the supernatant by mixing the supernatant with the Griess reagent (Sigma-Aldrich, USA) in equivalent volumes, and the activity of arginase was measured in the cell lysate by the concentration of urea using a test system Urea-450 (Erba Lachema, Czech Republic). Cell proliferation was also assessed in the MP lysate, for which, 4 h before the end of cultivation, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium-bromide (MTT, Sigma, USA) was added to the wells at the final concentration of 200 μg/ml. The precipitate was dissolved with dimethyl sulfoxide (Sigma, USA). The absorption of solutions (units of optical density) was measured on a UNIPLAN PIKON enzyme immunoassay analyzer (AIFR-01, Pikon LLC, Russian Federation) at a wavelength of 540 nm. The nitrite concentration (μM) was calculated with a calibration curve constructed using standard sodium nitrite solutions. The amount of the enzyme catalyzing the formation of 1 μM urea per minute was taken as 1 unit of activity (U.A.) of arginase.

Determination of pyrogenicity. To determine the impurity of LPS – endotoxin – HS were treated for 1 h with the antibiotic polymyxin B (50 μg/ml), then the cells were added and incubated as described above.

Statistical processing was performed using Statistica 13.3 software, using one-way ANOVA, Dunnett's test, and Student's *t*-test, after checking for normality of distribution using the Shapiro – Wilk test (data distribution corresponds to normal), where M is the sample mean; m is the error of the mean; level of statistical significance of differences $p < 0.05$, and sample size $n \geq 5$ depending on the research method.

RESULTS AND DISCUSSION

It is known that humic substances are dark-colored nitrogen-containing organic compounds, the color intensity of which is directly proportional to the concentration of the sample [11]. Based on this, the effect of a native dark color of the HS samples on the spectrophotometric parameters of aqueous solutions was preliminary evaluated. It was shown that all HS samples at a concentration of 1 μg/ml did not affect the spectrophotometric parameters of MP supernatants even without the addition of the Griess reagent (Table 2). With an increase in the concentration to 10 μg/ml, only the CHS sample had an optical density 2.6 times higher than the control values, but at the concentration of 100 μg/ml, all samples were 2–15 times darker than the control.

It was found that at the same concentration of the studied substances, the optical density of the CHS-AgNPs sample was lower than the initial matrix of HS, while that of the samples CHP-AgNPs and CHI-AgNPs,

on the contrary, was higher. Further, in the study of cell proliferation, it was shown that all the studied basic HS samples did not exhibit toxic effects in any of the concentrations used (Table 3). MP cultivation with the samples CHP-AgNPs, CHS-AgNPs, CHE-AgNPs, and CHP-AgNPs, starting from a concentration of 10 μg/ml, led to inhibition of cell proliferation by 2–3 times. Therefore, in the further work, in order to avoid false-positive results and pronounced toxic effects, when assessing the activating properties of the studied substances, concentrations of 1 and 10 μg/ml were used, and for the CHS sample – only 1 μg/ml.

Table 2

Effect of coal-derived humic substances and silver-containing bionanocomposites based on them on the optical density of macrophage supernatants (without Griess reagent), units of optical density, *M* ± *m*

Studied substance	Control (MP + medium)	MP + HS, μg/ml		
		1	10	100
CHP-K	119 ± 3	130 ± 4	135 ± 3	260 ± 8*♦
CHP-AgNPs		121 ± 3	128 ± 3	372 ± 3*♦■
CHS-K		132 ± 1	307 ± 6*●	1,777 ± 9*♦
CHS-AgNPs		130 ± 3	132 ± 4■	336 ± 3*♦■
CHI-K	127 ± 4	127 ± 2	135 ± 2	222 ± 2*♦
CHI-AgNPs		125 ± 1	133 ± 3	321 ± 5*♦■
CHE-K		139 ± 5	137 ± 1*	266 ± 4*♦
CHE-AgNPs		134 ± 2	137 ± 2	251 ± 2*♦

* differences compared with the control.

Note: ● – differences between concentrations of 1 and 10 μg/ml, ♦ – differences between concentrations of 10 and 100 μg/ml, ■ – differences between HS-AgNPs samples and basic HS matrices at the same concentration; level of statistical significance of differences $p < 0.05$, $n = 6$.

Table 3

Effect of various concentrations of coal-derived humic substances and silver-containing bionanocomposites based on them on the peritoneal macrophage proliferation of intact C57BL/6 mice, *M* ± *m*

Studied substance	Control 1 (MP + medium)	Control 2 (MP + LPS)	MP + HS		
			1	10	100
CHP-K	460 ± 9	425 ± 3	500 ± 8●	467 ± 8	512 ± 10●
CHP-AgNPs			494 ± 11	154 ± 6*●■	169 ± 6*●■
CHS-K	510 ± 4	451 ± 3	541 ± 11●	594 ± 9●	536 ± 8●
CHS-AgNPs			496 ± 9	169 ± 7*●■	164 ± 7*●■
CHI-K	282 ± 12	279 ± 7	291 ± 9●	308 ± 6●	340 ± 17*●
CHI-AgNPs			298 ± 13	197 ± 2*●■	208 ± 11*●■
CHE-K			264 ± 8●	260 ± 5	556 ± 10*●
CHE-AgNPs			294 ± 9	255 ± 2	273 ±10■

* – differences compared with Control 1.

Note: ● – differences between the parameter and Control 2, ■ – differences between HS-AgNPs samples and basic HS matrices at the same concentration; level of statistical significance of differences $p < 0.05$. The LPS concentration – 0.1 μg/ml, $n = 6$.

The study of the NO-activating properties of the initial HS matrices showed that the cultivation of MPs with the CHP-K and CHE-K samples led to an increase in the concentration of nitrites in comparison with the intact control by 1.2 (CHP-K) and 17 (CHE-K) times at the concentration of 1 μg/ml, and by 13 times – at the concentration of 10 μg/ml (Table 4). The basic matrices of the CHS-K and CHI-K samples, as well as none of the AgNPs samples, did not affect the secretory properties of MPs. The NO-activating effect of all substances was significantly lower than that of the mitogen-stimulated control.

It is known that consumption of L-arginine increases drastically in activated MPs. Molecular markers of M1 are NO synthase (iNOS), CD16, CD32, CD40, CD80, and CD86, while M2 macrophages are characterized by

the activation of arginase-1 (Arg-1) and transglutaminase 2, macrophage surface marker CD36, receptor of transferrin CD71, CD163, mannose (MMR or CD206), CCL-22, and E-cadherin [1, 3, 7]. Classically activated MPs convert arginine into nitric oxide and citrulline with iNOS; alternatively activated MPs convert arginine into urea and ornithine by means of arginase. The study of the effect of the analyzed substances on the arginine balance in peritoneal MPs showed that an increase in the production of nitrites during the MP cultivation with the CHE-K (by 29 times) and CHE-AgNPs (by 1.5 times) samples was accompanied by a decrease in the arginase activity in cell lysates by 1.2 and 4 times, respectively, compared with the intact control (Table 5).

Table 4

Effect of different concentrations of coal-derived humic substances and silver-containing bionanocomposites based on them on the nitric oxide production by peritoneal macrophages of intact C57BL/6 mice, *M* ± *m*

Studied substance	Concentration	HS	Control 1 (MP + medium)	Control 2 (MP + LPS)
CHP-K	1	3.30 ± 0.13*●	2.66 ± 0.14	69.70 ± 0.18*
	10	34.63 ± 0.43*●		
CHP-AgNPs	1	2.77 ± 0.11●■		
	10	2.59 ± 0.07●■		
CHS-K	1	2.93 ± 0.06●	2.51 ± 0.15	65.93 ± 0.37*
CHS-AgNPs	1	2.77 ± 0.11●		
CHI-K	1	2.41 ± 0.06●		
	10	2.58 ± 0.13●		
CHI-AgNPs	1	2.81 ± 0.16●		
	10	2.87 ± 0.10●		
CHE-K	1	41.61 ± 0.28*	2.44 ± 0.17	37.89 ± 0.71*
	10	33.91 ± 0.71*		
CHE-AgNPs	1	2.53 ± 0.07●■		
	10	2.41 ± 0.07●■		

* differences compared with Control 1.

Note: ● – differences between the parameter and Control 2, ■ – differences between HS-AgNPs samples and basic HS matrices at the same concentration; level of statistical significance of differences $p < 0.05$. The LPS concentration – 0.1 μg/ml, $n = 6$.

Table 5

Effect of various concentrations of coal-derived humic substances and silver-containing bionanocomposites based on them on the activity of NO-synthase (nitrite production) and arginase (urea fermentation) in peritoneal macrophages of intact C57BL/6 mice, *M* ± *m*

Studied substance	Concentration, μg/ml	Nitrite concentration, μM	Urea fermentation, U.A.
Control 1 (MP + medium)	–	2.20 ± 0.22	53.64 ± 0.40
Control 2 (MP + LPS)	0.1	64.56 ± 0.67*	42.47 ± 0.46*
CHP-K	10	28.74 ± 0.72*●	52.85 ± 0.35●
CHP-AgNPs	10	2.49 ± 0.03●■	5.29 ± 0.82*●■
CHS-K	1	2.88 ± 0.19*●	33.81 ± 0.46*●
CHS-AgNPs	1	2.52 ± 0.05●	20.71 ± 0.56*●■
CHI-K	10	2.51 ± 0.07●	60.16 ± 0.45●
CHI-AgNPs	10	2.42 ± 0.04●	56.81 ± 0.74●
CHE-K	10	63.48 ± 0.30*	43.13 ± 0.35*
CHE-AgNPs	10	3.26 ± 0.11*●■	12.91 ± 0.51*●■

* – differences compared with Control 1.

Note: ● – differences between the parameter and Control 2, ■ – differences between HS-AgNPs samples and basic HS matrices at the same concentration; level of statistical significance of differences $p < 0.05$; $n = 5$ (nitrites) and $n = 10$ (arginase).

The CHP-K sample, against the background of a 13-fold increase in the nitrite concentration, did not affect the arginase activity, and the CHS-K, CHS-AgNPs, and CHP-AgNPs samples, not showing NO-activating properties, significantly increased urea fermentation relative to Control 1. The CHI-K and CHI-AgNPs samples did not affect the studied parameters.

The literature shows that extracts of plant origin may contain an admixture of endotoxin (LPS), which also causes an increase in the production of nitric oxide [12].

In order to assess the degree of purification of the studied substances from LPS, experiments were carried out using polymyxin B, which binds directly to endotoxin and, thus, blocks its stimulating effect. Table 6 shows that the antibiotic did not affect the NO-stimulating properties of the CHS-K and CHS-AgNPs, CHI-K and CHI-AgNPs, and CHE-K and CHE-AgNPs samples. During cultivation of the CHP-K and CHP-AgNPs samples with the antibiotic, the concentration of nitrites decreased by 1.3–1.7 times. The results obtained indicate the absence of endotoxin impurity and pyrogenic properties in the CHS-K and CHS-AgNPs, CHI-K and CHI-AgNPs, and CHE-K and CHE-AgNPs samples, and their presence in the CHP-K and CHP-AgNPs samples.

Table 6

The effect of polymyxin B on the NO-producing properties of coal-derived humic substances and silver-containing bionanocomposites based on them in peritoneal macrophages of intact C57BL/6 mice, *M* ± *m*

Studied substance, μg/ml		Control 1 (MP + medium)		Control 2 (MP + LPS)		MP + HS	
		polymyxin B		polymyxin B		polymyxin B	
		–	+	–	+	–	+
CHP-K	10	2.34 ± 0.03	2.36 ± 0.11	32.31 ± 0.41*	3.56 ± 0.11▲■	25.43 ± 0.02*●	14.93 ± 0.16▲■♦
CHP-AgNPs	10					2.64 ± 0.08●	1.91 ± 0.05▲■♦
CHS-K	1					3.63 ± 0.15*●	4.08 ± 0.09■
CHS-AgNPs	1					2.34 ± 0.08●	2.71 ± 0.06♦
CHI-K	10	2.48 ± 0.07	2.16 ± 0.13	36.63 ± 0.62*	2.72 ± 0.1▲■	2.78 ± 0.15●	2.53 ± 0.10
CHI-AgNPs	10					2.76 ± 0.07●	2.40 ± 0.08
CHE-K	10					45.30 ± 0.51*●	41.02 ± 0.54■♦
CHE-AgNPs	10					2.66 ± 0.06●	2.29 ± 0.06

* – differences compared with Control 1 without polymyxin.

Note: ▲ – differences between the parameter and incubation of each substance without polymyxin; ● – differences between the parameter and Control 2 without polymyxin; ■ – differences between the parameter and Control 1 with polymyxin; ♦ – differences between the parameter and Control 2 with polymyxin; level of statistical significance of differences $p < 0.05$. The polymyxin B concentration – 50 μM, LPS – 0.1 μg/ml, $n = 5$.

CONCLUSION

The studies have shown that the CHE-K, CHE-AgNPs, and CHS-K samples contribute to the polarization of antigen-presenting cells according to the classical type (M1) by increasing the activity of NO synthase and inhibition of arginase. The basic CHP-K matrix, which significantly enhances the NO-stimulating properties of cells against the background of stable arginase, can also be attributed to this type of substance. The functions of proinflammatory macrophages M1 are associated with phagocytosis, microbicidal activity, induction of inflammation and adaptive immune response, and antitumor activity and are accompanied by the secretion of Th1 cytokines.

On the contrary, the CHI-K and CHI-AgNPs samples did not affect the activity of NO-synthase and arginase of peritoneal MPs, which allows to consider these substances as activators of alternative, anti-inflammatory properties of M2 macrophages. The latter are aimed at formation of the extracellular matrix, repair and remodeling of tissues, suppression of inflammation, stimulation of vascular formation, apoptotic cell phagocytosis, and synthesis of anti-inflammatory cytokines (IL-10, TGF-β, IL-4, IL-1ra). The CHP-AgNPs and CHS-AgNPs samples that inhibit arginase activity but do not affect nitrite production can be attributed to the M2-like state polarization which has some, but not all, characteristics of M2 cells [3]. At the same time, HS are not cytotoxic at effective concentrations, and three out of four studied samples do not contain pyrogenic impurities.

Therefore, macrophages undergo various dynamic changes at each stage of wound healing. Firstly, M1 macrophages mediate tissue damage and initiate inflammatory reactions. Secondly, at the early stages of repair, infiltrating MPs exhibit the M2 phenotype to suppress acute inflammation, and then their depletion inhibits the formation of excessively vascularized and scar tissue. The use of silver-containing bionanocomposites based on HS, which have the ability to greatly affect the polarization of APCs, is a promising research area for solving an acute social and medical problem of treating chronic wounds.

REFERENCES

1. Shapouri-Moghaddam A., Mohammadian S., Vazini H., Taghadosi M., Esmaeili S.A., Mardani F., Seifi B., Mohammadi A., Afshari J.T., Sahebkar A. Macrophage plasticity, polarization, and function in health and disease. *J. Cell. Physiol*. 2018; 233 (9): 6425–6440. DOI: 10.1002/jcp.26429.

2. Nathan C., Ding A. Nonresolving inflammation. *Cell*. 2010; 140 (6): 871–882. DOI: 10.1016/j.cell.2010.02.029.
3. Mantovani A., Biswas S. K, Galdiero M.R., Sica A.,Locati M. Macrophage plasticity and polarization in tissue repairand remodeling. *J. Pathol.* 2013; 229: 176–185. DOI: 10.1002/path.4133.
4. Patel U., Rajasingh S., Samanta S., Cao T., Dawn B., Rajasingh J. Macrophage polarization in response to epigenetic modifiers during infection and inflammation. *Drug Discov. Today*. 2017; 22 (1): 186–193. DOI: 10.1016/j.drudis.2016.08.006.
5. Mantovani A, Sica A, Sozzani S, Allavena P, Vecchi A, Locati M. The chemokine system in diverse forms of macrophage activation and polarization. *Trends Immunol.* 2004; 25: 677–686. DOI: 10.1016/j.it.2004.09.015.
6. Medzhitov R. Inflammation 2010: new adventures of an old flame. *Cell*. 2010; 140 (6): 771–776. DOI: 10.1016/j.cell.2010.03.006.
7. Tugal D., Liao X., Jain M.K. Transcriptional control of macrophage polarization Arterioscler. *Thromb. Vasc. Biol*., 2013; 33: 1135–1144. DOI: 10.1161/ATVBAHA.113.301453.
8. Pandolfi F., Altamura S., Frosali S., Conti P. Key role of DAMP in inflammation, cancer, and tissue repair. *Clin. Ther*. 2016; 38 (5): 1017–1028. DOI: 10.1016/j.clinthera.2016.02.0280149-2918/$.
9. Nathan C. Points of control in inflammation. *Nature.* 2002; 420 (6917); 846–852. DOI: 10.1038/nature01320.
10. Mohammadi A., Sharifi A., Pourpaknia R., Moghaddam S., Sahebkarf A. Manipulating macrophage polarization and function using classical HDAC inhibitors: Implications for autoimmunity and inflammation. *Critical Reviews in Oncology/Hematology*. 2018; 128: 1–18. DOI: 10.1016/j.critrevonc.2018.05.009.
11. Trofimova E.S., Zykova M.V., Ligacheva A.A., Sherstoboev E.Yu., Zhdanov V.V., Belousov M.V., Yusubov M.S., Krivoshchekov S.V., Danilets M.G., Dygai A.M. Effects of humic acids isolated from peat of various origin on *in vitro* production of nitric oxide: a screening study. *Bulletin of Experimental Biology and Medicine, First Online*: 2016; 161 (5): 687–692. DOI: 10.1007/s10517-016-3486-z.
12. Schepetkin I.A., Xie G., Kirpotina L.N., Jutila M.A., Quinn M.T., Klein R.A. Macrophage immunomodulatory activity of polysaccharides isolated from Opuntiapolyacantha. *International Immunopharmacology.* 2008; 8 (10): 1455–1466. DOI: 10.1016/j.intimp.2005.10.005.

Authors contribution

Zykova M.V., Belousov M.V., and Sherstoboev E.Yu. substantiated the relevance of this work. Perminova I.V., Grigorieva I.O., Tsupko A.V., Mikhalev D.A., Logvinova L.A. and Zykova M.V. synthesized samples and carried out their standardization. Trofimova E.S., Danilets M.G., and Ligacheva A.A. developed an experiment, assessed the biological activity of the studied substances, and carried out cell culture studies. Danilets M.G. and Ligacheva A.A. carried out data processing. Trofimova E.S. and Danilets M.G. performed theoretical calculations. Danilets M.G., Trofimova E.S., Zykova M.V., and Belousov M.V. were involved in writing the text of the article. All authors participated in the discussion of the results.

Authors information

Trofimova Evgeniya S., Cand. Sci. (Med.), Senior Researcher, Goldberg Research Institute of Pharmacology and Regenerative Medicine, Tomsk NRMC, Tomsk, Russian Federation. ORCID 0000-0002-5367-715X.

Zykova Maria V., Dr. Sci. (Pharmacy), Associate Professor, Head of the Department of Chemistry, Siberian State Medical University, Tomsk, Russian Federation. ORCID 0000-0002-1973-8983.

Danilets Marina G., Dr. Sci. (Biology), Principal Researcher, Goldberg Research Institute of Pharmacology and Regenerative Medicine, Tomsk NRMC, Tomsk, Russian Federation. ORCID 0000-0001-7862-4778.

Ligacheva Anastasia A., Cand. Sci. (Biology), Researcher, Goldberg Research Institute of Pharmacology and Regenerative Medicine, Tomsk NRMC, Tomsk, Russian Federation. ORCID 0000-0002-3337-1516.

Sherstoboev Evgeny Yu., Dr. Sci. (Med.), Professor, Principal Researcher, Head of the Department of Immunopharmacology, Goldberg Research Institute of Pharmacology and Regenerative Medicine, Tomsk NRMC, Tomsk, Russian Federation. ORCID 0000-0002-6178-5329.

Grigorieva Irina O., Post-Graduate Student, Department of Chemistry, Lomonosov Moscow State University, Moscow, Russian Federation. ORCID 0000-0002-7978-9774.

Mikhalev Dmitry A., Laboratory Assistant, Department of Chemistry, Siberian State Medical University, Tomsk, Russian Federation. ORCID 0000-00002-5292-1368.

Tsupko Andrey V., Laboratory Assistant, Department of Chemistry, Siberian State Medical University, Tomsk, Russian Federation. ORCID 0000-0001-7169-8846.

Logvinova Lyudmila A., Assistant, Department of Chemistry, Siberian State Medical University, Tomsk, Russian Federation. ORCID 0000-0002-0167-7043.

Perminova Irina V., Dr. Sci. (Chemistry), Professor, Department of Medicinal Chemistry, Head of the Laboratory of Natural Humic Systems, Lomonosov Moscow State University, Moscow, Russian Federation. ORCID 0000-0001-9084-7851.

Belousov Mikhail V., Dr. Sci. (Pharmacy), Professor, Head of the Department of Pharmaceutical Analysis, Siberian State Medical University, Tomsk, Russian Federation. ORCID 0000-0002-2153-7945.

(✉) **Trofimova Evgeniya S.,** e-mail: trofimova_es@pharmso.ru

Received 01.09.2021
Accepted 05.10.2021

УДК 616.24-002-085.33-036.8
https://doi.org/10.20538/1682-0363-2021-4-79-85

Effectiveness of antimicrobial therapy for community-acquired pneumonia in real clinical practice

Uryasev O.M., Shakhanov A.V., Korshunova L.V.

Ryazan State Medical University
9, Vysokovoltnaya Str., Ryazan, 390026, Russian Federation

ABSTRACT

Background. Community-acquired pneumonia (CAP) remains one of the most common infectious diseases, occupying an important place in the structure of mortality worldwide.

Aim. To evaluate the effectiveness of antimicrobial therapy for community-acquired pneumonia in hospitalized patients in real clinical practice.

Materials and methods. A retrospective, observational study was conducted, which included 236 patients hospitalized for community-acquired pneumonia at the Regional Clinical Hospital in Ryazan in 2019. Based on these case histories, an analysis of the effectiveness of the initial empiric antimicrobial therapy was performed.

Results. The initial empiric antimicrobial therapy in 73% of cases included administration of ceftriaxone, in 45% of cases – levofloxacin, in 14% of cases – azithromycin. It was found that initial antimicrobial therapy was effective in 58% of patients who did not require replacement for the antibiotic. A need for a change in the treatment regimen was significantly associated with an increase in the length of hospitalization ($p < 0.001$), heart rate upon admission ($p = 0.032$), myelocyte count in the complete blood count ($p < 0.001$), and urea and blood creatinine levels ($p = 0.004$ and $p = 0.044$, respectively). The selected antimicrobial therapy regimen was significantly associated with the expected treatment effectiveness ($p = 0.039$). The choice of levofloxacin in monotherapy or in combination with ceftriaxone was accompanied by a decrease in the relative risk of replacing the antimicrobial, compared with other treatment regimens (odds ratio (OR) = 0.86 (95% confidence interval (CI): 0.55–1.34) and OR = 0.57 (95% CI: 0.37–0.87), respectively).

Conclusion. Empiric antimicrobial therapy for community-acquired pneumonia in real clinical practice complies with current recommendations, however, at the same time, its ineffectiveness persists. Respiratory fluoroquinolones are most effective in treating pneumonia in hospitalized patients.

Key words: pneumonia, antibiotics, fluoroquinolones.

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: Uryasev O.M., Shakhanov A.V., Korshunova L.V. Effectiveness of antimicrobial therapy for community-acquired pneumonia in real clinical practice. *Bulletin of Siberian Medicine.* 2021; 20 (4): 79–85. https://doi.org/10.20538/1682-0363-2021-4-79-85.

✉ *Shakhanov Anton V.,* e-mail: shakhanovav@gmail.com

Эффективность антибактериальной терапии внебольничной пневмонии в условиях реальной клинической практики

Урясьев О.М., Шаханов А.В., Коршунова Л.В.

Рязанский государственный медицинский университет (РязГМУ) имени академика И.П. Павлова
Россия, 390026, г. Рязань, ул. Высоковольтная, 9

РЕЗЮМЕ

Актуальность. Внебольничная пневмония остается одним из наиболее распространенных инфекционных заболеваний, занимающим важное место в структуре смертности населения во всем мире.

Цель – оценить эффективность антибактериальной терапии внебольничной пневмонии у госпитализированных пациентов в условиях реальной клинической практики.

Материалы и методы. Проведено ретроспективное наблюдательное исследование, в которое включены 236 больных, госпитализированных по поводу внебольничной пневмонии в ГБУ РО «Областная клиническая больница» (г. Рязань) в течение 2019 г. На основании данных историй болезни проведен анализ эффективности стартовой эмпирической антибактериальной терапии.

Результаты. Стартовая эмпирическая антимикробная терапия в 73% случаев включала назначение цефтриаксона, 45% – левофлоксацина, 14% – азитромицина. Установлено, что стартовая антибактериальная терапия была эффективной у 58% пациентов, которым не потребовалась замена антибиотика. Потребность в смене схемы терапии была значимо ассоциирована с увеличением сроков госпитализации ($p < 0,001$), частотой сердечных сокращений при поступлении ($p = 0,032$), уровнем миелоцитов в общем анализе крови ($p < 0,001$), уровнем мочевины и креатинина крови ($p = 0,004$ и $p = 0,044$ соответственно). Выбранная схема стартовой антибактериальной терапии значимо ассоциирована с ожидаемой эффективностью лечения ($p = 0,039$). Выбор левофлоксацина в монотерапии или в комбинации с цефтриаксоном сопровождался снижением относительного риска замены антибактериального препарата по сравнению с иными вариантами терапии (OR = 0,86 (95% CI: 0,55–1,34) и OR = 0,57 (95% CI: 0,37–0,87) соответственно).

Заключение. Эмпирическая антибактериальная терапия внебольничной пневмонии в целом соответствует действующим рекомендациям, однако при этом сохраняется крайне высокая частота ее неэффективности. Наибольшую эффективность в лечении пневмонии у госпитализированных пациентов имеют респираторные фторхинолоны.

Ключевые слова: пневмония, антибиотики, фторхинолоны.

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: Урясьев О.М., Шаханов А.В., Коршунова Л.В. Эффективность антибактериальной терапии внебольничной пневмонии в условиях реальной клинической практики. *Бюллетень сибирской медицины.* 2021; 20 (4): 79–85. https://doi.org/10.20538/1682-0363-2021-4-79-85.

INTRODUCTION

Pneumonia is a group of acute infectious diseases (mainly bacterial ones) with different etiology, pathogenesis, and morphological characteristics. They are characterized by focal lesions in the lungs with the obligatory presence of intraalveolar exudate. Pneumonia is considered community-acquired, if it develops outside the hospital or is diagnosed in the first 48 hours of hospitalization.

Community-acquired pneumonia (CAP) remains one of the most common infectious diseases, occupying an important place in the structure of mortality worldwide. Among adults in Europe and North America, pneumonia occurs in 5–10 people per 1,000 population [1]. In the Russian Federation, where the population exceeds 140 million people, it can be stated that every year, more than 1.5 million people develop pneumonia among adult population [2]. In general, the Russian Federation demonstrates persistent

pneumonia morbidity, but in some regions, including the Ryazan region, a steady increase is registered [3]. Every year, about 400 thousand people with pneumonia are hospitalized in the Russian Federation, while the mortality rate for severe forms of the disease in the inpatient setting is approaching 10% [2].

High mortality rate from pneumonia is observed not only in the inpatient setting. Pneumonia is ranked 1st among the causes of mortality from infectious diseases and 6th among all causes in the overall structure of mortality [4, 5]. Despite advances in modern methods of diagnosis and treatment, CAP remains one of the topical problems in the Russian healthcare. In recent years, to improve the effectiveness of pneumonia patient management, international and national guidelines have been implemented, which help doctors choose the most rational treatment strategy in a specific clinical situation. However, in real clinical practice, the recommended approaches to the choice of empiric antimicrobial therapy are often neglected, which leads to an increase in the number of unfavorable outcomes in patients with pneumonia [6]. Therefore, the aim of the study was to evaluate the effectiveness of antimicrobial therapy for CAP in real clinical practice.

MATERIALS AND METHODS

A retrospective observational study was conducted to assess the comparative effectiveness of empiric antimicrobial therapy for CAP in real clinical practice. To carry out the study, a register of all CAP cases was formed that were registered at the Regional Clinical Hospital (Ryazan) from January to December of 2019. At the first stage of the study, an analysis of 236 case histories was carried out, including a clinical and demographic analysis of disease cases. At the second stage, two study groups were identified, depending on the need to change the empiric antimicrobial therapy prescribed at the time of hospitalization, in order to determine the most effective treatment regimens. When forming the study groups, the effectiveness of treatment was understood as achievement of the criteria for an effective response to antimicrobial therapy and absence of the need to revise the antimicrobial therapy regimen following the current guidelines [7]. At the second stage of the study, 3 case histories were excluded from further analysis due to the impossibility to definitely determine the applied initial antimicrobial therapy regimen.

Patient records were used as primary documentation for the study, from which the following information was obtained:

– demographic and medical and statistical information, including age, gender, date and duration of hospitalization, the outcome of hospitalization, information on concomitant diseases;

– information about the course of the disease, including the period from the onset of symptoms to the start of antimicrobial therapy, complaints upon admission, data of clinical examinations and additional research methods, including the severity of pneumonia, the presence of complications, the volume and localization of the lesion according to chest X-ray, findings of complete blood count and blood biochemistry test, results of sputum test.

Statistical analysis was performed using the StatSoft Statistica 10 software package. Normal distribution of variables was assessed using the Shapiro – Wilk test. The results obtained are presented as *Me [Q25; Q75*], where *Me* is the median, and *Q25* and *Q75* are lower and upper quartiles, respectively. The Kruskal – Wallis and Mann – Whitney tests were used to compare the groups by quantitative criteria. Relative parameters of qualitative variables (frequencies and proportions) were compared using Fisher's exact test with Yates' correction. The differences were considered statistically significant at $p < 0.05$.

RESULTS

Cases of hospitalizations for CAP were recorded all year round, with peaks, according to the register, in the periods from April to May and from October to December. A significant decrease in the number of hospitalizations was noted only in summer months.

Among those included in the study, there were 122 (52%) men and 114 (48%) women, which indicates that there is no associated between gender and the development of pneumonia. The average age of those hospitalized for pneumonia was 61 years. The average hospital stay was 12 days. Most often, according to medical records, patients complained of cough, fever, shortness of breath, and generalized weakness. Less commonly, the complaints included sputum production, sweating, and chest pain (Figure).

Severe CAP was observed in 39 patients (17%). In 97 (41%) patients, the disease was complicated by the development of respiratory failure, in 7 (3%) patients – by exudative pleuritis. Other complications, including abscess, pleural empyema, and toxic shock syndrome, were isolated.

According to chest X-ray, the lesion volume in 55 (23%) cases corresponded to focal pneumonia, in 13 (6%) cases – to segmental pneumonia, in 130 (55%)

cases – to multilobar pneumonia, and in 36 (15%) cases – to lobar pneumonia. In 2 (1%) patients, chest X-ray revealed interstitial pneumonia. Most often, 104 (44%) patients had right-sided pneumonia, 74 (31%) patients had left-sided pneumonia, and 58 (25%) patients had bilateral pneumonia. The inflammatory process in 49% of cases was localized in the lower lobe, in 4% of cases – in the middle lobe, in 15% of cases – in the upper lobe; multiple localization was determined in 32% of patients.

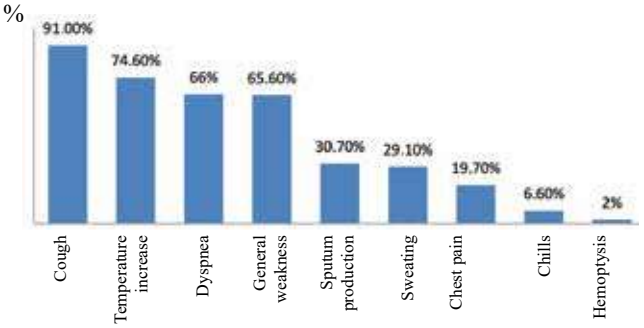


Figure. Complaints of patients hospitalized for pneumonia, %

The outcome of hospitalization in 92 (39%) patients was recovery, in 133 (56%) patients – improvement of the condition and discharge for outpatient treatment, in 11 (5%) cases – death of the patient. It was found that the outcome of hospitalization depended on the patient's age ($p = 0.0140$), respiratory rate ($p = 0.0012$), blood urea level ($p = 0.0118$), and blood oxygen saturation ($p = 0.0268$) and did not depend on the duration of the onset of the disease before hospitalization ($p = 0.8216$) and the level of systolic and diastolic blood pressure ($p = 0.6043$ and $p = 0.4468$, respectively). Analyzing the relationship between the complete blood count parameters and the outcome of hospitalization, it was found that the outcome of hospitalization was associated with the level of neutrophils ($p = 0.0248$), but did not depend on the level of erythrocytes, leukocytes, and hemoglobin ($p = 0.2428$, $p = 0.1083$, and $p = 0.9250$, respectively).

The results of the sputum culture test in the primary documentation were present only in 26 (11%) patients: of them, in 65% of cases the causative agent of pneumonia was *Streptococcus pneumoniae*, in 15% of cases – *Escherichia coli*, in 11% of cases – *Klebsiella pneumoniae*, and in 4% of cases – *Staphylococcus aureus* and *Pseudomonas aeruginosa*. In 31% of cases, the sputum culture test detected fungi of the *Candida* genus.

Analyzing the prescribed treatment, we obtained data that the initial empiric antimicrobial therapy in 73% of cases included prescription of ceftriaxone, in 45% of cases – levofloxacin, in 14% of cases – azithromycin; in some cases the therapy regimen included the use of amikacin, amoxicillin, vancomycin, gentamicin, josamycin, clarithromycin, co-trimoxazole, meropenem, metronidazole, cefotaxime, and ertapenem. It was found that initial antimicrobial therapy was effective only in 136 (58%) patients who did not require replacement of the antibacterial drug. The need to change the therapy regimen was significantly associated with an increase in hospitalization time ($p < 0.001$), heart rate upon admission ($p = 0.032$), the level of myelocytes in the complete blood count ($p < 0.001$), and the level of urea and blood creatinine ($p = 0.004$ and $p = 0.044$, respectively) and was not associated with other clinical and laboratory parameters (Table 1).

Table 1

Clinical and demographic analysis of disease cases, *Me* [Q_{25}; Q_{75}]

Parameter	Total, $n = 236$	Replacement of antibacterial treatment was not required, $n = 136$	Replacement of antibacterial treatment was required, $n = 100$	p
Age, years	61 [41.5; 75.5]	65.5 [51; 79]	56 [37; 69]	< 0.001
Gender, male/female	122/114	64/72	58/42	$\chi 2 = 2.95$; $p = 0.085$
Length of hospitalization (days)	12 [9; 16]	10 [8; 14]	15 [11; 17]	<0.001
Time from the onset of symptoms to hospitalization (days)	6 [4; 10]	7 [4; 8,5]	6 [4; 11]	0.719
Respiratory rate (per minute)	20 [18; 22]	20 [18; 22]	20 [18; 21]	0.359
Systolic blood pressure (mmHg)	130 [120; 140]	130 [116; 140]	125 [120; 140]	0.760
Diastolic blood pressure (mmHg)	80 [70; 80]	80 [70; 80]	80 [70; 80]	0.442
Heart rate (beats per minute)	84 [78; 92]	82 [74; 92]	84,5 [80; 97]	0.032
Blood oxygen saturation (%)	95 [92; 97]	95 [91; 97]	95 [92; 97]	0.766
Time from the onset of symptoms to the start of antimicrobial therapy, days	6 [4; 9]	7 [4; 9]	6 [4; 11]	0.654
Duration of initial antimicrobial therapy, days	7 [5; 8]	7 [6; 8]	6 [3; 7]	<0.001
Duration of prescribed antimicrobial therapy, days	9 [7; 12]	7 [6; 8]	12 [10; 15.5]	<0.001

Table 1 (continued)

Parameter	Total, $n = 236$	Replacement of antibacterial treatment was not required, $n = 136$	Replacement of antibacterial treatment was required, $n = 100$	p
Complete blood count				
Erythrocytes, $\times 10^{12}$	4.6 [4.2; 4.9]	4.6 [4.2; 4.9]	4.6 [4.2; 5]	0.456
Hemoglobin, g/l	132 [119; 143]	130 [116; 141]	133 [122; 146]	0.082
Platelets, $\times 10^9$	229 [169; 329]	233 [178; 349]	211.5 [129; 294]	0.398
Leukocytes, $\times 10^9$	9.6 [7.3; 13.9]	9.7 [7.5; 14.1]	9.6 [6.5; 13.9]	0.381
Basophils, %	1 [1; 1]	1 [1; 1]	1 [1; 1]	1.000
Eosinophils, %	2 [1; 4]	2 [1; 4]	2 [1; 3]	0.907
Myelocytes, %	8 [5; 11]	8 [5; 11]	0 [0; 0]	<0.001
Immature neutrophils, %	2 [1; 2]	2 [1; 4]	1.5 [1; 2]	0.648
Band neutrophils, %	2 [1; 4]	2 [1; 4.5]	2 [1; 4.5]	0.837
Segmented neutrophils, %	65 [54; 74]	65 [54; 74]	65 [57; 75]	0.778
Lymphocytes, %	21 [11.55; 29]	21 [14.6; 29]	20 [10; 28]	0.348
Monocytes, %	7 [6; 10]	8 [6; 10]	7 [5.5; 10]	0.384
Blood biochemistry test				
Urea, mmol / l	5.7 [4.4; 8.8]	6.35 [4.8; 10.35]	5.4 [4; 7.4]	0.004
Creatinine, mmol / l	0.102 [0.081; 0.121]	0.107 [0.086; 0.135]	0.099 [0.08; 0.116]	0.044

It was found that the chosen regime of initial antimicrobial therapy was significantly associated with the expected effects of treatment and the need to replace the antibacterial drugs ($p = 0.039$). At the same time, only the regimens with levofloxacin were accompanied by a decrease in the relative risk of antibiotic replacement compared with any other treatment options. Monotherapy with levofloxacin reduced the relative risk of antibiotic replacement in etiotropic therapy by 14% compared with other treatment options. The most effective combination of ceftriaxone and levofloxacin reduced the risk of antibiotic replacement by 43%. Any other treatment options that did not include respiratory fluoroquinolones demonstrated an increased relative risk of antibiotic replacement.

Table 2

Effect of initial antimicrobial therapy on the need for further change of the antibacterial drug

Antimacrobial therapy regimen	Replacement of the antibiotic was required	Replacement of the antibiotic was not required	Relative risk of replacing the antibiotic
Ceftriaxone	42	42	OR = 1.28 (95% CI: 0.96–1.72)
Azithromycin	7	6	OR = 1.27 (95% CI: 0.75–2.16)
Levofloxacin	14	23	OR = 0.86 (95% CI: 0.55–1.34)
Azithromycin + ceftriaxone	9	10	OR = 1.11 (95% CI: 0.68–1.83)
Ceftriaxone + levofloxacin	17	45	OR = 0.57 (95% CI: 0.37–0.87)
Other options for antimicrobial therapy	11	7	OR = 1.48 (95% CI: 0.99–2.21)

Note: OR – odds ratio, CI – confidence interval.

DISCUSSION

Many studies are devoted to assessing the effectiveness and safety of antimicrobial drugs in pneumonia. The most commonly prescribed antibiotic in the treatment of pneumonia in the inpatient setting in the Russian Federation is ceftriaxone. According to the pharmacoeconomic analysis carried out in Nizhny Novgorod, the drug load of 1 CAD patient with ceftriaxone per day is 3.2 g, which exceeds the load with ampicillin / sulbactam by 2.3 times, levofloxacin and azithromycin – by 5 times, moxifloxacin – by 19 times [8]. Our results also confirm high frequency of ceftriaxone use for initial antimicrobial therapy for pneumonia, but the effectiveness of this prescription is doubtful.

According to the study conducted by Professor A.I. Sinopalnikov in 2012 in real clinical practice, in hospitalized patients, initial antimicrobial therapy was effective and did not require replacement of the antibacterial drug in only 45% of patients, which is consistent with our data [6]. In this case, a single antibiotic replacement was sufficient to obtain the necessary clinical effect only in 39% of cases, in 16% of cases,

a two- or three-time change of etiotropic therapy was required. In 2012, the main reason for the ineffectiveness of the initial therapy, according to data obtained by Professor A.I. Sinopalnikov, was low adherence to recommendations for CAP treatment, including widespread use of drugs with insufficient antimicrobial activity against the key pathogens (primarily cefazolin and ciprofloxacin). However, in our work, similar treatment failure was noted with the use of antibiotics included in the current clinical guidelines, which may indicate other mechanisms, for example, an increase in the drug resistance of typical pathogens causing pneumonia observed in recent years [7].

It has now been shown that monotherapy with β-lactam antibiotics is often insufficiently effective due to an increase in the number of CAP cases associated with atypical pathogens, such as *Mycoplasma pneumoniae* and *Chlamydophila pneumoniae*. The study by K. Eljaaly, conducted in the USA, showed that inclusion of drugs fighting atypical pathogens in empiric antimicrobial therapy for CAP can significantly reduce the risk of clinical treatment failure (RR = 0.851; 95% CI, 0.732–0.99; $p = 0.037$), while not affecting the overall mortality and development of adverse drug reactions requiring antibiotic withdrawal (RR = 0.549; 95% CI, 0.259–1.165, $p = 0.118$ and RR = 0.83; 95% CI, 0.542–1.270, $p = 0.39$, respectively) [9].

Many studies were devoted to comparing regimens of etiotropic therapy for pneumonia. In the work by Wei Nie et al. [10], 2,946 publications on this topic were analyzed, of which 16 articles were included in a further meta-analysis devoted to comparing the effectiveness of monotherapy with β-lactam antibiotics and their combination with macrolides. The meta-analysis showed that two-component therapy was associated with higher treatment effectiveness and a reduced risk of death due to pneumonia. Similarly, our results indicate that the combination of ceftriaxone and azithromycin is associated with a lower relative risk of failure compared with both ceftriaxone monotherapy and azithromycin monotherapy (Table 2). At the same time, even the combination ceftriaxone + azithromycin is less effective in comparison with monotherapy with levofloxacin and especially in comparison with the combination ceftriaxone + levofloxacin.

The advantages of monotherapy with respiratory fluoroquinolones in patients with CAP are shown in a meta-analysis by A. Raz-Pasteur et al., including the results of inpatient treatment of 4,809 patients. It was found that monotherapy with fluoroquinolones reduced the relative risk of treatment ineffectiveness and antibiotic withdrawal (RR = 0.72 (0.57–0.91) and RR = 0.65 (0.54–0.78), respectively). Additionally, it was accompanied by a smaller number of diarrhea cases compared with the combination macrolide + β-lactam antibiotic (RR = 0.13 (0.05–0.34)) [11].

Similar results were obtained by K. Skalsky et al. in a systematic review on the comparative effectiveness of macrolides and fluoroquinolones in patients with pneumonia [12]. The study involved 83 publications, of which 16 randomized controlled trials conducted from 1993 to 2005 were included in a further analysis. According to the meta-analysis results, it was found that mortality from pneumonia did not differ in the groups of fluoroquinolones and macrolides, however, the use of fluoroquinolones was accompanied by a lower risk of clinical and microbiological treatment failure (RR 0.63 (95% CI 0.49–0.81)). The authors of the meta-analysis indicated that in therapy with fluoroquinolones, the clinical effect occurred faster and the cure time was shorter. However, not all results on the benefits of respiratory fluoroquinolones are so clear-cut.

In a prospective, randomized clinical study conducted by M. Izadi from December 2016 to June 2017 in Iran, it was shown that the effectiveness of monotherapy with oral levofloxacin at a dose of 750 mg per day did not differ from that of the combination of ceftriaxone at a dose of 1,000 mg per day and oral azithromycin at a dose of 250 mg per day. Although it is worth noting that the doses of drugs used in the study are lower than those traditionally used in Russian clinical practice [13]. The benefits of respiratory fluoroquinolones were shown in a systematic review by J.H. Lee, comparing the combinations fluoroquinolone + β-lactam antibiotic versus macrolide + β-lactam antibiotic in patients with severe CAP. According to the review, the combination macrolide + β-lactam antibiotic resulted in a lower percentage of deaths than the combination fluoroquinolone + β-lactam antibiotic (19.4% versus 26.8%) and was accompanied by a decrease in the duration of hospital stay by 3.05 days [14]. Therefore, the issue of recommending monotherapy with fluoroquinolones as the first line therapy in patients hospitalized for CAP requires further study, at the same time, their high effectiveness in combination therapy is beyond doubt.

CONCLUSION

Our analysis of CAP in hospitalized patients showed that the applied empiric antimicrobial therapy

for CAP generally complies with the current guidelines. However, extremely high frequency of its ineffective outcome remains. Among the groups of drugs used in clinical practice, respiratory fluoroquinolones have the greatest effectiveness in the treatment of pneumonia in hospitalized patients, which can be recommended as one of the key drugs in the treatment of this cohort of patients.

REFERENCES

1. Rozenbaum M.H., Pechlivanoglou P., Werf T.S. et al. The role of *Streptococcus pneumoniae* in community-acquired pneumonia among adults in Europe: a meta-analysis. *Eur. J. Clin. Microbiol. Infect. Dis*. 2013; 32 (3): 305–316. DOI: 10.1007/s10096-012-1778-4.
2. Chuchalin A.G. Pneumonia: The urgent problem of the 21st century medicine. *Terapevticheskiy arkhiv*. 2016; 88 (3): 4–12 (in Russ.). DOI: 10.17116/terarkh20168834-12.
3. Belykh N.A., Fokicheva N.N., Piskunova M.A. et al. Clinical and epidemiological features of mycoplasma infection in children in the Ryazan region. *I.P. Pavlov Russian Medical Biological Herald*. 2018; 26 (2): 258–267 (in Russ.). DOI: 10.23888/PAVLOVJ2018262258-267.
4. Borodulin B.E., Chernogaeva G.Yu., Borodulina E.A. et al. Mortality from community-acquired pneumonia in a multidisciplinary hospital during 10 years. *Medical Almanac.* 2012; 2 (21): 34–36 (in Russ.).
5. Mironova A.A., Narkevich A.N., Kurbanismailov R.B. Mortality rate of population of the Krasnoyarsk region in 2016. *Eruditio Juvenium*. 2019; 7 (2): 223–231 (in Russ.). DOI: 10.23088/HMJ201972223-231.
6. Sinopal'nikov A.I., Astaf'ev A.V., Styrt E.A. Antibacterial therapy of community-acquired pneumonia in a hospital: real clinical practice. *Clinical Medicine.* 2012; 90 (12): 21–26.
7. Chuchalin A.G., Sinopal'nikov A.I., Kozlov R.S. et al. Clinical guidelines on diagnosis, treatment, and prevention of severe community-acquired pneumonia in adults. *Pulmonology.* 2014; (4): 13–48 (in Russ.).
8. Zhukova O.V., Ruina O.V., Kononova S.V., Konyshkina T.M. Analysis of the efficiency of antimicrobial treatment for community-acquired pneumonia in clinical practice. *Terapevticheskiy Arkhiv*. 2017; 89 (8): 17–21 (in Russ.). DOI: 10.17116/terarkh201789817-21.
9. Eljaaly K., Alshehri S., Aljabri A. et al. Clinical failure with and without empiric atypical bacteria coverage in hospitalized adults with community-acquired pneumonia: a systematic review and meta-analysis. *BMC Infect. Dis*. 2017; 17 (1): 385. DOI: 10.1186/s12879-017-2495-5.
10. Nie W., Li B., Xiu Q. β-Lactam/macrolide dual therapy versus β-lactam monotherapy for the treatment of community-acquired pneumonia in adults: a systematic review and meta-analysis. *J. Antimicrob. Chemother*. 2014; 69 (6):1441–1446. DOI: 10.1093/jac/dku033.
11. Raz-Pasteur A., Shasha D., Paul M. Fluoroquinolones or macrolides alone versus combined with β-lactams for adults with community-acquired pneumonia: Systematic review and meta-analysis. *Int. J. Antimicrob. Agents*. 2015; 46 (3): 242–248. DOI: 10.1016/j.ijantimicag.2015.04.010.
12. Skalsky K., Yahav D., Lador A. et al. Macrolides vs. quinolones for community-acquired pneumonia: meta-analysis of randomized controlled trials. *Clin. Microbiol. Infect*. 2013; 19 (4): 370–378. DOI: 10.1111/j.1469-0691.2012.03838.x.
13. Izadi M., Dadsetan B., Najafi Z. et al. Levofloxacin versus ceftriaxone and azithromycin combination in the treatment of community acquired pneumonia in hospitalized patients. *Recent. Pat. Antiinfect. Drug Discov*. 2018; 13 (3): 228–239. DOI: 10.2174/1574891X13666181024154526.
14. Lee J.H., Kim H.J., Kim Y.H. Is β-lactam plus macrolide more effective than β-lactam plus fluoroquinolone among patients with severe community-acquired pneumonia?: a systemic review and meta-Analysis. *J. Korean Med. Sci*. 2017; 32 (1): 77–84. DOI: 10.3346/jkms.2017.32.1.77.

Authors information

Uryasev Oleg M., Dr. Sci. (Med.), Professor, Head of the Department of Intermediate-Level Therapy, Ryazan State Medical University, Ryazan, Russian Federation. ORCID 0000-0001-8693-4696.

Shakhanov Anton V., Cand. Sci. (Med.), Associate Professor, Department of Intermediate-Level Therapy, Ryazan State Medical University, Ryazan, Russian Federation. ORCID 0000-0002-5706-9418.

Korshunova Lyudmila V., Cand. Sci. (Med.), Department of Intermediate-Level Therapy, Ryazan State Medical University, Ryazan, Russian Federation. ORCID 0000-0003-0945-0772.

(✉) **Shakhanov Anton V.,** e-mail: shakhanovav@gmail.com

Received 21.10.2020
Accepted 28.12.2020

УДК 617.7-007.681:617.749:577.112.854
https://doi.org/10.20538/1682-0363-2021-4-86-92

Features of the level of matrix metalloproteinase-2, -3, -9 and tissue inhibitors of metalloproteinases-1, -2, -3, -4 in the aqueous humor of patients with primary open-angle glaucoma

Chernykh V.V.[1], Konenkov V.I.[2], Ermakova O.V.[1], Orlov N.B.[2], Trunov A.N.[1, 3]

[1] S. Fyodorov Eye Microsurgery Federal State Institution, Novosibirsk Branch
10, Kolkhidskaya Str., Novosibirsk, 630096, Russian Federation

[2] Research Institute of Clinical and Experimental Lymphology – a branch of the Federal Research Center "Institute of Cytology and Genetics of the Siberian Branch of the Russian Academy of Sciences"
2, Timakova Str., Novosibirsk, 630117, Russian Federation

[3] Federal Research Center for Fundamental and Translational Medicine of the Siberian Branch of the Russian Academy of Sciences
2, Timakova Str., Novosibirsk, 630117, Russian Federation

ABSTRACT

Aim. To study the content of matrix metalloproteinase (MMP)-2, -3, -9 and tissue inhibitors of metalloproteinases (TIMPs) -1, -2, -3, -4 in the aqueous humor of patients with moderate primary open-angle glaucoma (POAG).

Materials and methods. The experimental group included 47 patients with verified moderate primary open-angle glaucoma. The control group consisted of 26 patients with uncomplicated cataract. The levels of MMP-2, -3, -9 were determined with Luminex Performance Human MMP Magnetic Panel 3-plex kit (R&D Systems, USA), the concentration of TIMPs-1, -2, -3, - 4 was determined with the Human TIMP Magnetic Luminex Performance Assay 4-plex kit (R&D Systems, USA). The study was carried out using flow-through field fluorometry on a Bio-Plex 200 double-beam laser analyzer (Bio-Rad, USA).

Results. The study showed a statistically significant increase in the levels of matrix metalloproteinase-2 and tissue inhibitors of matrix metalloproteinases-1, -2, -3, -4 in the aqueous humor of patients with moderate POAG compared with patients with uncomplicated cataract.

Conclusion. The obtained data on high concentrations and imbalance in the levels of matrix metalloproteinases and their tissue inhibitors in the aqueous humor of patients with moderate POAG confirm the role of local inflammation, as well as impairments in the structure of the extracellular matrix and its remodeling in the mechanisms of development of this pathology.

Key words: primary open-angle glaucoma, pathogenesis, matrix metalloproteinases, tissue inhibitors of matrix metalloproteinases, aqueous humor.

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

Source of financing. The study was carried out at the expense of the state assignment, the research topic "Study of the pathogenesis of open-angle glaucoma based on the assessment of the imbalance of cytokines and growth factors" (No. AAAA-A18-118082290059-3) and within the agreements on research and practice cooperation between S. Fyodorov Eye Microsurgery Federal State Institution and Research Institute of Clinical and Experimental Lymphology – a branch of the Federal Research Center "Institute of Cytology and Genetics of the Siberian Branch of the Russian Academy of Sciences", and between Research Institute of Clinical and Experimental Lymphology – a branch of the Federal Research Center "Institute of Cytology and Genetics of the Siberian Branch of the Russian Academy of Sciences" and Federal Research Center for Fundamental and Translational Medicine of the Siberian Branch of the Russian Academy of Sciences.

✉ *Trunov Aleksander N.,* e-mail: trunov1963@yandex.ru

Conformity with the principles of ethics. All patients signed an informed consent to surgery, collection of the aqueous humor, as well as the use of the study data for scientific purposes. The study was approved by the Biomedical Ethics Committee at the Novosibirsk Branch of S. Fyodorov Eye Microsurgery Federal State Institution (Protocol No. 2 of 02.09.2018).

For citation: Chernykh V.V., Konenkov V.I., Ermakova O.V., Orlov N.B., Trunov A.N. Features of the level of matrix metalloproteinase-2, -3, -9 and tissue inhibitors of metalloproteinases-1, -2, -3, -4 in the aqueous humor of patients with primary open-angle glaucoma. *Bulletin of Siberian Medicine.* 2021; 20 (4): 86–92. https://doi.org/10.20538/1682-0363-2021-4-86-92.

Особенности содержания матриксных металлопротеиназ 2, 3, 9 и тканевых ингибиторов матриксных металлопротеиназ 1, 2, 3, 4 во внутриглазной жидкости пациентов с первичной открытоугольной глаукомой

Черных В.В.[1], Коненков В.И.[2], Ермакова О.В.[1], Орлов Н.Б.[2], Трунов А.Н.[1, 3]

[1] Национальный медицинский исследовательский центр (НМИЦ), Межотраслевой научно-технический комплекс (МНТК) «Микрохирургия глаза» им. акад. С.Н. Федорова, Новосибирский филиал Россия, 630096, г. Новосибирск, ул. Колхидская, 10

[2] Научно-исследовательский институт клинической и экспериментальной лимфологии – филиал Федерального исследовательского центра «Институт цитологии и генетики СО РАН» (НИИКиЭЛ ФИЦ ИЦиГ СО РАН) Россия, 630117, г. Новосибирск, ул. Тимакова, 2

[3] Федеральный исследовательский центр фундаментальной и трансляционной медицины СО РАН Россия, 630117, г. Новосибирск, ул. Тимакова, 2

РЕЗЮМЕ

Цель – изучить содержание матриксных металлопротеиназ (MMP) 2, 3, 9 и тканевых ингибиторов матриксных металлопротеиназ (TIMP) 1, 2, 3, 4 во внутриглазной жидкости пациентов с развитой стадией первичной открытоугольной глаукомы.

Материалы и методы. Обследованы 47 пациентов с верифицированным, на основании офтальмологического обследования, диагнозом развитой стадии первичной открытоугольной глаукомы, которые составили основную группу. Контрольную группу составили 26 пациентов с диагнозом «неосложненная катаракта».

Концентрацию MMP-2, MMP-3, MMP-9 определяли с использованием набора Luminex Performance Human MMP Magnetic Panel (3-Plex) (R&D Systems, США), определение концентрации TIMP-1, TIMP-2, TIMP-3, TIMP-4 проводили с помощью набора Human TIMP Magnetic Luminex Performance Assay 4-plex (R&D Systems, США). Исследование проводилось методом проточной флуориметрии на двухлучевом лазерном анализаторе Bio-Plex 200 (Bio-Rad, США).

Результаты. Установлена статистически значимо высокая концентрация матриксной металлопротеиназы-2 и тканевых ингибиторов матриксных металлопротеиназ 1, 2, 3, 4 во внутриглазной жидкости пациентов с развитой стадией первичной открытоугольной глаукомы относительно данных, полученных при исследовании внутриглазной жидкости лиц с неосложненной катарактой.

Заключение. Полученные в исследовании данные о высоких концентрациях и нарушении баланса содержания матриксных металлопротеиназ и их тканевых ингибиторов в внутриглазной жидкости пациентов с развитой стадией первичной открытоугольной глаукомы подтверждают роль местного воспалительного процесса, а также нарушений структуры внеклеточного матрикса и его ремоделирования в механизмах развития патологического процесса.

Ключевые слова: первичная открытоугольная глаукома, патогенез, матриксные металлопротеиназы, тканевые ингибиторы матриксных металлопротеиназ, внутриглазная жидкость.

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

Источник финансирования. Исследование проводилось за счет средств государственного задания, тема НИР «Изучение патогенеза открытоугольной глаукомы на основе оценки дисбаланса цитокинов и факторов роста» (№ АААА-А18-118082290059-3), а также в рамках договоров о научно-практическом сотрудничестве между НМИЦ «МНТК "Микрохирургия глаза" им. акад. С.Н. Федорова» и НИИКЭЛ – филиал ИЦиГ СО РАН, НИИКЭЛ и Федеральным исследовательским центром фундаментальной и трансляционной медицины СО РАН.

Соответствие принципам этики. Все пациенты подписали информированное согласие на проведение операции, забор внутриглазной жидкости, а также использование данных исследования в научных целях. Исследование одобрено комитетом по биомедицинской этике Новосибирского филиала НМИЦ «МНТК "Микрохирургия глаза" им. акад. С.Н. Федорова» (протокол № 2 от 02.09.2018).

Для цитирования: Черных В.В., Коненков В.И., Ермакова О.В., Орлов Н.Б., Трунов А.Н. Особенности содержания матриксных металлопротеиназ 2, 3, 9 и тканевых ингибиторов матриксных металлопротеиназ 1, 2, 3, 4 во внутриглазной жидкости пациентов с первичной открытоугольной глаукомой. *Бюллетень сибирской медицины.* 2021; 20 (4): 86–92. https://doi.org/10.20538/1682-0363-2021-4-86-92.

INTRODUCTION

Primary open-angle glaucoma (POAG) is widely spread in all countries of the world, and its increasing prevalence has significant medical and social consequences for patients and the community [1–4]. The ophthalmological community is to a certain extent unsatisfied with results of treatment for this pathology, which makes POAG a pressing issue of modern ophthalmology. A thorough study of the development of pathological mechanisms in this disease is a significant research task which would allow to elaborate pathogen-specific treatment.

According to the analysis of publications devoted to POAG pathogenesis, morphological and structural changes in the eye drainage system leading to impaired aqueous humor outflow and an increase in the intraocular pressure (IOP) are some of the main causes of POAG [5–9].

Some researchers suggest that these morphological and structural changes in the eye drainage zone may be manifestations of the local aseptic destructive and inflammatory process in patients with POAG, the development of which depends on the imbalance and content change of various biologically active molecules with proinflammatory and fibrotic activity (cytokines, growth factors, etc.), as evidenced by publications of recent years [10–14]. In order to understand the role of local destructive and inflammatory process in POAG development, it is important to determine changes in the local content and balance of matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMP), which play an essential role in degradation of extracellular matrix proteins degradation and tissue remodeling [15–20], as well as in ophthalmic disorders [21].

In addition, the publications provide data on the ability of a number of cytokines to affect MMP and TIMP production and participate in regulation of MMP and TIMP synthesis [22–24].

Currently, few publications contain data on high concentrations of various MMPs and TIMPs in both the aqueous humor and tear and tissue structures of the eye affected by POAG. Based on the conducted studies, the authors of the publications suggest that excessive production of MMPs and TIMPs is associated with the active synthesis of various classes of cytokines, and the resulting imbalance in the studied biologically active molecules can lead to disturbances of the extracellular matrix and remodeling of the trabecular meshwork and lamina cribrosa of the sclera with impaired aqueous humor outflow and development of glaucomatous process. The authors also suggest possible role of MMP in the mechanisms of apoptosis in retinal ganglion cells and glaucomatous optic neuropathy [25–30].

However, it is not always possible to interpret the data presented in the literature unequivocally, which allows to conclude on the relevance of further study of the content and balance of MMP and TIMP in the aqueous humor of POAG patients to understand the molecular mechanisms of POAG development, as well as to formulate the aim of this study.

The aim of the study was to investigate the content of MMP-2, 3, 9 and TIMPs-1, 2, 3, 4 in the aqueous humor of patients with moderate POAG.

MATERIALS AND METHODS

The study was conducted in accordance with the principles of the Declaration of Helsinki of the World Medical Association “Ethical principles for medical research involving human subjects”, the Federal Law

of the Russian Federation of November 21, 2011 No. 323 FL "On the basics of protecting the health of citizens in the Russian Federation", as well as the requirements of the Federal Law of July 27, 2006 N 152-FL (as amended on July 21, 2014) "On Personal Data" (as amended and supplemented, entered into force on September 1, 2015).

The study examined 73 patients. The experimental group consisted of 47 patients who underwent clinical and instrumental examination, including best visual acuity, binocular ophthalmoscopy, spheroperimetry, echoopthalmography, optical coherence tomography (OCT), and intraocular pressure (IOP) measurement. Moderate POAG and uncomplicated cataract were revealed. There were 16 (34.0%) men and 31 (66.0%) women in the experimental group. The average age of patients was 64.3 ± 5.9 years. The control group included 26 patients with uncomplicated cataract. There were 8 (30.8%) men and 18 (69.2%) women in the group, the average age of patients was 67.1±3.2 years. Thus, the studied groups did not differ in terms of age and gender.

Exclusion criteria from both groups were acute and chronic eye disorders, diabetic retinopathy, neovascular glaucoma, uveitis of various etiology and localization, hemophthalmus, and autoimmune diseases and cancer of any localization. The study excluded patients who, in order to normalize intraocular pressure, took medications containing prostaglandin analogs, capable of affecting the activity of the local destructive and inflammatory process.

Samples of the aqueous humor (AH) (100–150 μL) were collected from all patients at the initial stages of surgical treatment. The samples were frozen and stored at –70 °C until the study was conducted.

Determination of the concentration of matrix metalloproteinases (MMPs) and tissue inhibitors of matrix metalloproteinases (TIMPs) in AH

Once frozen, AH was defrosted to room temperature before the study. To remove the sediment, it was centrifuged at 4°C, 10,000 rpm, for 10 min. The concentrations of MMP-2, MMP-3, and MMP-9 were determined using the Luminex Performance Human MMP Magnetic Panel 3-plex kit (R&D Systems, USA). The levels of TIMP-1, TIMP-2, TIMP-3, and TIMP-4 were identified using the Human TIMP Magnetic Luminex Performance Assay 4-plex kit (R&D Systems, USA). The study was conducted using flow-through field fluorometry on a two-beam laser analyzer Bio-Plex 200 (Bio-Rad, USA).

Bio-Plex manager Software version 4.1 was used for data processing. Data analysis was performed using the Statistica 10 software package (StatSoft Inc., USA). The concentrations were measured in ng/ml. The use of the Kolmogorov – Smirnov and Lilliefors tests for normality allowed to establish the absence of normal distribution in the obtained samples. In this regard, the study used the methods of non-parametric statistics. The significance of differences in variation series in unrelated samples was assessed using the Mann–Whitney *U*-test. Data were presented as the median and upper (75%) and lower (25%) quartiles, *Me* (*Q1–Q3*). The differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

As a result of this study, we identified the presence of a significantly higher concentration of MMP-2 in the AH of patients with moderate POAG compared with the data obtained in the AH of patients with uncomplicated cataract ($p = 0.001$, Table).

At the same time, when determining the content of MMP-3 and MMP-9 in the AH of patients in the examined groups, it was found that their levels did not differ statistically significantly ($p = 0.08$ and $p = 1$, respectively), and the samples in which the concentration of the studied biologically active molecules was higher than the lower limits of the sensitivity were isolated.

Table

Content of matrix metalloproteinases and tissue inhibitors of matrix metalloproteinases in the aqueous humor of patients with moderate POAG and uncomplicated cataract, ng/ml, *Me* (Q_1–Q_3)

Parameter	Patients with POAG, *n* = 47	Patients with uncomplicated cataract, *n* = 26	*p*
MMP-2	1.87 [1.43; 2.31] *	1.43 [1.09; 1.81]	0.001
MMP-3	0.06 [0.00; 0.09]	0.02 [0.00; 0.01]	0.08
MMP-9	0.00 [0.00; 0.00]	0.00 [0.00; 0.00]	1
TIMP-1	30,822.74 [23,320; 44,298] *	18,254.11 [14,058; 25,685]	0.001
TIMP-2	40,570.62 [33,690; 49,381] *	27,520.26 [24,159; 31,400]	0.001
TIMP-3	11,771.36 [10,788; 14,086] *	10,364.90 [8,097; 11,333]	0.012
TIMP-4	110.41 [80.03; 129.05] *	77.57 [69.83; 93.86]	0.001

* statistically significant differences compared with the group of patients with uncomplicated cataract.

When comparing the obtained data on the MMP content in the AH of patients in the examined groups with the results of other studies presented in the literature, the following can be noted.

Our data on significantly higher concentrations of MMP-2 in the AH of patients with POAG are similar to the results presented in the study by A.D. Nga et al., where comparable data were obtained on the content of this matrix metalloproteinase in the AH [26]. Our data are also consistent with the data presented by P. Sahay et al., in which high concentrations of MMP-2 were found in the lacrimal fluid of patients with POAG [27].

At the same time, we did not reveal significantly higher concentrations of MMP-3 in the AH of patients with moderate POAG, data on which were presented by A.D. Nga et al. [26]. We also could not confirm the conclusion made in the study by L. Markiewicz et al., who found high concentration of MMP-9 in the AH of POAG patients and regarded it as a marker of inflammation development [29].

The analysis of the data obtained in the study made it possible to state the following facts reflecting the content of TIMPs in the AH of patients in the examined groups. The concentrations of TIMP-1 in the AH of patients with moderate POAG were found to be significantly higher than in patients with uncomplicated cataract ($p = 0.001$). Similar results were obtained when examining the levels of TIMP-2 in the AH of the patients. The patients with moderate POAG had significantly higher values of the studied parameter compared with the data in the control group ($p = 0.001$).

The study also showed the presence of a significantly higher concentration of TIMP-3 and TIMP-4 in the AH of patients with moderate POAG relative to the values of the studied parameters in the AH of patients with uncomplicated cataract ($p = 0.012$ and $p = 0.001$, respectively).

When comparing the data on the content of TIMPs in the AH of the patients obtained in this work with the results of other studies presented in the literature, the following can be noted. Our data on statistically significantly higher concentrations of TIMP-1, TIMP-2, and TIMP-4 in the AH of patients with POAG are similar to the results presented in the study by E.L. Ashworth Briggs et al. There, comparable data were presented, and it was concluded that an imbalance between MMP and TIMP with a shift towards their increased levels was found in the AH samples, which can lead to inhibition of MMP activity with changes in the composition of the extracellular matrix in the trabecular meshwork with a subsequent increase in resistance to the AH outflow, as well as to increased intraocular pressure [31].

Additionally, higher concentrations of TIMP-1 and TIMP-2 in the AH of POAG patients were identified in the study by A.D. Nga et al. [26]. In the study by N. Fountoulakis et al., it was concluded that the most significant changes were established when TIMP-4 was determined in the AH; it was also established that this biologically active molecule was crucial in the pathogenesis of POAG [32]. The analysis of the data obtained in this study and research results presented in the literature on the content of TIMPs in the AH of POAG patients made it possible to state fewer discrepancies between them than in the analysis of data on the content of MMPs. However, a relatively small number of publications devoted to the study of MMPs and TIMPs indicates the need for further research in this area.

CONCLUSION

The conducted study allowed to establish that in patients with moderate POAG, significantly high levels of MMP-2 and TIMPs-1, -2, -3, -4 were identified. The study also confirms the importance of local inflammatory process and impairment of the extracellular matrix structure and its remodeling in the mechanisms of POAG development.

REFERENCES

1. Egorov E.A., Alekseev V.N. Pathogenesis and treatment of primary open-angle glaucoma. M.: GEOTAR-Media, 2017: 224 (in Russ.).
2. Stevens G.A., White R.A., Flaxman S.R., Price H., Jonas J.B., Keeffe J., Leasher J., Naidoo K., Pesudovs K., Resnikoff S., Taylor H., Bourne R.R.; Vision Loss Expert Group. Global prevalence of visual impairment and blindness: magnitude and temporal trends, 1990–2010. *Ophthalmology.* 2013; 120 (12): 2377–2384. DOI: 10.1016/j.ophtha.2013.05.025.
3. Tham Y.C., Li X., Wong T.Y., Quigley H.A., Aung T., Cheng C.Y. Global prevalence of glaucoma and projections of glaucoma burden through 2040: a systematic review and meta-analysis. *Ophthalmology*. 2014; 121: 2081–2090. DOI: 10.1016/j.ophtha.2014.05.013.
4. Bourne R.R., Taylor H.R., Flaxman S.R., Keeffe J., Leasher J., Naidoo K., Pesudovs K., White R.A., Wong T. Y., Resnikoff S., Jonas J.B. Vision loss expert group of the global burden of disease study. Number of people blind or visually impaired by glaucoma worldwide and in world regions 1990–2010: A meta-analysis. *PLoS One.* 2016; 11(10): e0162229. DOI: 10.1371/journal.pone.0162229.
5. Sihota R., Goyal A., Kaur J., Gupta V., Nag T.C. Scanning electron microscopy of the trabecular meshwork: understanding the pathogenesis of primary angle closure glaucoma. *Indi-*

an. J. Ophthalmol. 2012; 60.(3): 183–188. DOI: 10.4103/0301-4738.95868.
6. Huang A.S., Mohindroo C., Weinreb R.N. Aqueous humor outflow structure and function imaging. At the bench and bedside: A Review. *J. Clin. Exp. Ophthalmol*. 2016; 7 (4): 578. DOI: 10.4172/2155-9570.1000578.
7. Song M.M., Lei Y., Wu J.H., Sun X.H. The progress of studies on aqueous humor dynamics abnormality induced by trabecular meshwork and Schlemm canal endothelial cell senescence and its relation with glaucoma. *Zhonghua Yan Ke Za Zhi*. 2017; 53 (11): 868–873. DOI: 10.3760/cma.j.issn.0412-4081.2017.11.014.
8. Wang K., Read A.T., Sulchek T., Ethier C.R. Trabecular meshwork stiffness in glaucoma. *Exp. Eye. Res*. 2017; 158: 3–12. DOI: 10.1016/j.exer.2016.07.011.
9. Chernykh V.V., Bgatova N.P., Orlov N.B., Ermakova O.V., Trunov A.N. Local inflammatory process as a possible manifestation of aqueous humor uveolymphatic outflow defects in glaucoma. Part 2. *National Journal Glaucoma*. 2018; 17 (2): 311 (in Russ.). DOI: 10.25700/NJG.2018.02.01.
10. Kokubun T., Tsuda S., Kunikata H., Yasuda M., Himori N., Kunimatsu-Sanuki S., Maruyama K., Nakazawa T. Characteristic profiles of inflammatory cytokines in the aqueous humor of glaucomatous eyes. *Ocul. Immunol. Inflamm*. 2018; 26 (8): 1177–1188. DOI: 10.1080/09273948.2017.1327605.
11. Khalef N., Labib H., Helmy H., El Hamid M.A., Moemen L, Fahmy I. Levels of cytokines in the aqueous humor of eyes with primary open angle glaucoma, pseudoexfoliation glaucoma and cataract. *Electron Physician*. 2017; 9 (2): 3833–3837. DOI: 10.19082/3833.
12. Pantalon A., Obadă O., Constantinescu D., Feraru C., Chiseliţă D. Inflammatory model in patients with primary open angle glaucoma and diabetes. *Int. J. Ophthalmol*. 2019; 12 (5): 795–801. DOI: 10.18240/ijo.2019.05.15.
13. 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 aqueous humor 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.
14. Ten Berge J.C., Fazil Z., Van Den Born I., Wolfs R.C.W., Schreurs M.W.J., Dik W.A., Rothova A. Intraocular cytokine profile and autoimmune reactions in retinitis pigmentosa, age-related macular degeneration, glaucoma and cataract. *Acta Ophthalmol*. 2019; 97 (2): 185–192. DOI: 10.1111/aos.13899.
15. Cui N., Hu M., Khalil R.A. Biochemical and biological attributes of matrix metalloproteinases. *Prog. Mol. Biol. Transl. Sci.* 2017; 147: 173. DOI: 10.1016/bs.pmbts.2017.02.005.
16. Chen Q., Jin M., Yang F., Zhu J., Xiao Q., Zhang L. Matrix metalloproteinases: inflammatory regulators of cell behaviors in vascular formation and remodeling. *Mediators Inflamm*. 2013; 2013: 928315. DOI: 0.1155/2013/928315.
17. Nissinen L., Kähäri V.M. Matrix metalloproteinases in inflammation. *Biochim. Biophys. Acta.* 2014; 1840 (8): 2571–2580. DOI: 10.1016/j.bbagen.2014.03.007.
18. Arpino V, Brock M, Gill SE. The role of TIMPs in regulation of extracellular matrix proteolysis. *Matrix Biol*. 2015; 44–46: 247–254. DOI: 10.1016/j.matbio.2015.03.005.
19. Robert S., Gicquel T., Victoni T., Valença S., Barreto E., Bailly-Maître B., Boichot E., Lagente V. Involvement of matrix metalloproteinases (MMPs) and inflammasome pathway in molecular mechanisms of fibrosis. *Biosci. Rep.* 2016; 36 (4): e00360. DOI: 10.1042/BSR20160107.
20. Laronha H., Caldeira J. Structure and function of human matrix metalloproteinases. *Cells*. 2020; 9 (5):1076. DOI: 10.3390/cells9051076.
21. Singh M., Tyagi S.C. Metalloproteinases as mediators of inflammation and the eyes: molecular genetic underpinnings governing ocular pathophysiology. *Int. J. Ophthalmol*. 2017; 10 (8):1308–1318. DOI: 10.18240/ijo.2017.08.20.
22. Roupakia E., Markopoulos G.S., Kolettas E. IL-12-mediated transcriptional regulation of matrix metalloproteinases. *Biosci. Rep*. 2018; 38 (3). DOI: BSR20171420. 10.1042/BSR20171420.
23. Singh S., Maniakis-Grivas G., Singh U.K., Asher R.M., Mauri F., Elkington P.T., Friedland J.S. Interleukin-17 regulates matrix metalloproteinase activity in human pulmonary tuberculosis. *J. Pathol.* 2018; 244 (3): 311–322. DOI: 10.1002/path.5013.
24. Zhang J.F., Wang G.L., Zhou Z.J., Fang X.Q., Chen S., Fan S.W. Expression of matrix metalloproteinases, tissue Inhibitors of metalloproteinases, and Interleukins in vertebral cartilage endplate. *Orthop. Surg.* 2018; 10 (4): 306–311. DOI: 10.1111/os.12409.
25. De Groef L, Van Hove I, Dekeyster E, Stalmans I, Moons L. MMPs in the trabecular meshwork: promising targets for future glaucoma therapies? *Invest. Ophthalmol. Vis. Sci.* 2013; 54 (12): 7756–7763. DOI: 10.1167/iovs.13-13088.
26. De Groef L., Van Hove I., Dekeyster E., Stalmans I., Moons L. MMPs in the neuroretina and optic nerve: modulators of glaucoma pathogenesis and repair? *Invest. Ophthalmol. Vis. Sci*. 2014; 55 (3): 1953–1964. DOI: 10.1167/iovs.13-13630.
27. Nga A.D., Yap S.L., Samsudin A., Abdul-Rahman P.S. Matrix metalloproteinases and tissue inhibitors of metalloproteinases in the aqueous humour of patients with primary angle closure glaucoma – a quantitative study. *BMC Ophthalmol*. 2014; 14: 33. DOI: 10.1186/1471-2415-14-33.
28. Sahay P., Rao A., Padhy D., Sarangi S., Das G., Reddy M.M., Modak R. Functional activity of matrix metalloproteinases 2 and 9 in tears of patients with glaucoma. *Invest. Ophthalmol. Vis. Sci.* 2017; 58 (6): 106–113. DOI: 10.1167/iovs.17-21723.
29. Markiewicz L., Pytel D., Mucha B., Szymanek K., Szaflik J., Szaflik J.P., Majsterek I. Altered expression levels of MMP1, MMP9, MMP12, TIMP1, and IL-1β as a risk factor for the elevated IOP and optic nerve head damage in the primary open-angle glaucoma patients. *Biomed. Res. Int.* 2015; 2015: 812503. DOI: 10.1155/2015/812503.
30. Zaleska-Żmijewska A., Strzemecka E., Wawrzyniak Z.M., Szaflik JP. Extracellular MMP-9-based assessment of ocular surface inflammation in patients with primary open-angle glaucoma. *J. Ophthalmol*. 2019; 2019: 1240537. DOI: 10.1155/2019/1240537.
31. Ashworth Briggs E.L., Toh T., Eri R., Hewitt A.W., Cook A.L. TIMP1, TIMP2, and TIMP4 are increased in aqueous humor from primary open angle glaucoma patients. *Mol. Vis*. 2015; 21: 1162–1172.

32. Fountoulakis N., Labiris G., Aristeidou A., Katsanos A., Tentes I., Kortsaris A., Kozobolis V.P. Tissue inhibitor of metalloproteinase 4 in aqueous humor of patients with primary open angle glaucoma, pseudoexfoliation syndrome and pseudoexfoliative glaucoma and its role in proteolysis imbalance. *BMC Ophthalmol.* 2013 13: 69. DOI: 10.1186/1471-2415-13-69.

Authors contribution

Chernykh V.V., Konenkov V.I. – conception and design, critical revision of the manuscript for important intellectual content, drafting of the article. Ermakova O.V. – selection and ophthalmic examination of patients, surgical treatment and sampling of the biomaterial for the study, analysis and interpretation of the data. Orlov N.B. – determination of biologically active molecules in the aqueous humor, statistical processing of the data. Trunov A.N. – substantiation of the manuscript, critical revision of the manuscript for important intellectual content, drafting of the manuscript.

Authors information

Chernykh Valery V., Dr. Sci. (Med.), Professor, Director of Novosibirsk Branch of S. Fyodorov Eye Microsurgery Federal State Institution, Novosibirsk, Russian Federation. ORCID 0000-0002-7623-3359.

Konenkov Vladimir I., Dr. Sci. (Med.), Professor, Academician of RAS, Head of the Laboratory of Clinical Immunogenetics, Research Institute of Clinical and Experimental Lymphology – a branch of the Federal Research Center "Institute of Cytology and Genetics of the Siberian Branch of the Russian Academy of Sciences", Novosibirsk, Russian Federation. ORCID 0000-0001-7385-6270.

Ermakova Olga V., Cand. Sci. (Med.), Ophthalmologist, Novosibirsk Branch of S. Fyodorov Eye Microsurgery Federal State Institution, Novosibirsk, Russian Federation. ORCID 0000-0003-0427-1564.

Orlov Nikolay B., Cand. Sci. (Med.), Senior Researcher, Laboratory of immunogenetics, Research Institute of Clinical and Experimental Lymphology – a branch of the Federal Research Center "Institute of Cytology and Genetics of the Siberian Branch of the Russian Academy of Sciences", Novosibirsk, Russian Federation. ORCID 0000-0002-3437-7151.

Trunov Alexander N., Dr. Sci. (Med.), Professor, Deputy Director for Research, Novosibirsk Branch of S. Fyodorov Eye Microsurgery Federal State Institution; Principal Researcher, Laboratory of Immunology, Federal Research Center for Fundamental and Translational Medicine SB RAS, Novosibirsk, Russian Federation. ORCID 0000-0002-7592-8984.

(✉) **Trunov Aleksander N.,** e-mail: trunov1963@yandex.ru

Received 10.11.2020
Accepted 28.12.2020

УДК 616.24-002-097.1-07:612.017.3
https://doi.org/10.20538/1682-0363-2021-4-93-102

Clinical, radiologic, and morphological diagnosis of hypersensitivity pneumonitis

Cherniaev A.L.[1, 2], Kusraeva E.V.[3], Samsonova M.V.[2, 4], Avdeev S.N.[5], Trushenko N.V.[5], Tumanova E.L.[3]

[1] *Pulmonology Scietific Research Institute*
28, Orekhovyi Blv., Moscow, 115682, Russian Federation

[2] *A.P. Avtsyn Research Institute of Human Morphology*
3, Tsyurupy Str., Moscow, 117418, Russian Federation

[3] *Pirogov Russian National Research Medical University*
1, Ostrovitianova Str., Moscow, 117997, Russian Federation

[4] *Loginov Moscow Clinical Scientific Center*
86, Entuziastov Highway, Moscow, 111123, Russian Federation

[5] *I.M. Sechenov First Moscow State Medical University (Sechenov University)*
8/2, Trubetskaya Str., Moscow, 119991, Russian Federation

ABSTRACT

Aim. To study the relationship between clinical, radiologic, and morphological features in nonfibrotic and fibrotic hypersensitivity pneumonitis.

Materials and methods. Clinical symptoms, data of high-resolution computed tomography, parameters of external respiration, and histological changes in the lung tissue obtained via open and transbronchial biopsies were studied retrospectively in 175 patients with hypersensitivity pneumonitis (HP). Statistical analysis was performed using the Statistica software.

Results. We found that the clinical error rate in the diagnosis of HP was 84.5%, among pathologists – 92%. Among all the variants of HP, the most common was fibrotic HP. It was shown that non-necrotizing granulomas and giant cells in the cavities of the alveoli, microcells, and interalveolar septa were more typical of nonfibrotic HP.

In fibrotic HP, peribronchial fibrosis, smooth muscle metaplasia in fibrotic areas, and the presence of fibroblastic foci in the walls of terminal bronchioles are signs of differential diagnosis with usual interstitial pneumonia. The classical triad of histological signs was observed in 19.2% of patients with nonfibrotic HP and in 5.6% of patients with fibrotic HP.

Conclusion. Diagnosis of HP is complex and should be based on a multidisciplinary approach involving clinicians (pulmonologists), radiologists, functional diagnostics specialists, and pathologists. In this case, it is imperative to take into account and identify factors causing development of the disease, as well as the age of patients.

Key words: nonfibrotic and fibrotic hypersensitivity pneumonitis, multidisciplinary approach, histological features.

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: Cherniaev A.L., Kusraeva E.V., Samsonova M.V., Avdeev S.N., Trushenko N.V., Tumanova E.L. Clinical, radiologic, and morphological diagnosis of hypersensitivity pneumonitis. *Bulletin of Siberian Medicine.* 2021; 20 (4): 93–102. https://doi.org/10.20538/1682-0363-2021-4-93-102.

✉ *Kusraeva Elina V.,* e-mail: elina.kusraeva@yandex.ru

Клинико-рентгено-морфологическая диагностика гиперчувствительного пневмонита

Черняев А.Л.[1, 2], Кусраева Э.В.[3], Самсонова М.В.[2, 4], Авдеев С.Н.[5], Трушенко Н.В.[5], Туманова Е.Л.[3]

[1] Научно-исследовательский институт (НИИ) пульмонологии
Россия, 115682, г. Москва, Ореховый бульвар, 28

[2] Научно-исследовательский институт морфологии человека (НИИМЧ) имени академика А.П. Авцына
Россия, 117418, г. Москва, ул. Цюрупы, 3

[3] Российский национальный исследовательский медицинский университет (РНИМУ) имени Н.И. Пирогова
Россия, 117997, г. Москва, ул. Островитянова, 1

[4] Московский клинический научно-практический центр имени А.С. Логинова Департамента здравоохранения города Москвы
Россия, 111123, г. Москва, шоссе Энтузиастов, 86

[5] Первый Московский государственный медицинский университет имени И.М. Сеченова (Сеченовский университет)
Россия, 119991, г. Москва, ул. Трубецкая, 8/2

РЕЗЮМЕ

Цель. Изучить взаимосвязь клинико-рентгено-морфологических признаков при нефиброзном и фиброзном вариантах гиперчувствительного пневмонита.

Материалы и методы. Ретроспективно у 175 пациентов с гиперчувствительным пневмонитом (ГП) были изучены клинические симптомы, данные компьютерной томографии высокого разрешения, показатели функции внешнего дыхания, гистологические изменения ткани легких, полученных при открытых и трансбронхиальных биопсиях. Статистический анализ осуществляли при помощи программы Statistica.

Результаты. Выявлено, что уровень ошибок в клинической практике при диагностике ГП составил 84,5%, среди патологоанатомов – 92%. Среди всех вариантов ГП наиболее часто встретился фиброзный. Показано, что ненекротические гранулемы, гигантские клетки в полостях альвеол, микросот и в межальвеолярных перегородках более характерны для нефиброзного ГП. При фиброзном ГП мозаичный перибронхиолярный фиброз, гладкомышечная метаплазия в зонах фиброза, наличие фибробластических фокусов в стенках терминальных бронхиол являются признаками дифференциальной диагностики с обычной интерстициальной пневмонией. Классическую триаду гистологических признаков наблюдали в 19,2% при нефиброзном ГП, при фиброзном – в 5,6%.

Заключение. Диагностика ГП сложна и должна быть основана на мультидисциплинарном подходе с участием клиницистов (пульмонологов), рентгенологов, специалистов по функциональной диагностике и патологоанатомов. При этом следует обязательно учитывать и выявлять факторы, вызывающие развитие заболевания и возраст пациентов.

Ключевые слова: нефиброзный и фиброзный гиперчувствительный пневмонит, мультидисциплинарная диагностика, гистологические признаки.

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: Черняев А.Л., Кусраева Э.В., Самсонова М.В., Авдеев С.Н., Трушенко Н.В., Туманова Е.Л. Клинико-рентгено-морфологическая диагностика гиперчувствительного пневмонита. *Бюллетень сибирской медицины.* 2021; 20 (4): 93–102. https://doi.org/10.20538/1682-0363-2021-4-93-102.

INTRODUCTION

Hypersensitivity pneumonitis (HP) (extrinsic allergic alveolitis) refers to a group of immune-mediated diseases of the lung tissue and terminal and respiratory bronchioles that develop in response to antigen inhalation [1].

The most well-known types of HP are bird fancier's lung [2], farmer's lung, air-conditioner lung caused by cold air in air-conditioned rooms, chemical worker's lung, and drug-induced bagassosis [3]. According to E. Fernández Pérez et al. [4], the prevalence of HP ranges from 0.3 to 0.9 per 100,000 population. According to F. Morell et al. [5], the incidence of HP amounts to 6.2 per 100,000 population sleeping on feather pillows and 54.6 per 100,000 among poultry breeders. S. Dhooria et al. [6] demonstrated that from 2015 to 2017, 10.7% out of 803 patients with interstitial lung disease (ILD) were diagnosed with HP.

The major pathogenetic mechanisms of the disease remain unclear; however, the development of types III and IV hypersensitivities has been shown. Antigen sensitization and manifestation of clinical symptoms after repeated exposure to the antigen play a key role [7].

Historically, three HP types were distinguished: acute, subacute, and chronic. Later, acute (inflammatory, cellular) and chronic HP were distinguished, which reflected the clinical course of the disease and differed in outcomes, survival, and treatment strategy [8, 9]. In 2020, the first international guidelines on HP were created, which suggest distinguishing nonfibrotic and fibrotic HP phenotypes [1].

Symptoms of nonfibrotic (inflammatory) HP are shortness of breath, cough, chills, and fever that occur within 4–8 hours (in farmer's lung, within 12–18 hours) after exposure to the antigen and can accelerate within several hours or days [9, 10]. In fibrotic HP, patients experience shortness of breath, slightly increasing with time, dry cough, malaise, fatigue, and loss of appetite [9, 10]. In high-resolution computed tomography (HRCT) of the lungs, nonfibrotic HP is characterized by multifocal, diffuse, and centrilobular ground-glass opacities, areas of mosaic attenuation, and "air traps" during the exhalation phase [11, 12]. Among all ILDs, areas of mosaic attenuation are more common in nonfibrotic HP, which makes this sign diagnostically significant and can lead to a correct diagnosis [13]. Major signs of fibrotic HP include alteration of lung architecture, reticular changes, areas of mosaic attenuation, the head cheese sign (juxtaposition of areas with ground-glass opacities, mosaic attenuation and normal lung tissue), traction bronchiectasis, and honeycomb lung [12, 14].

The gold standard for collecting a sample is a surgical lung biopsy. A transbronchial lung biopsy provides little information due to the small amount of lung tissue. However, a transbronchial cryobiopsy is believed to be promising in diagnosing HP [15, 16].

In nonfibrotic HP, histological examination reveals bronchiolocentric interstitial pneumonia (IP), chronic cellular bronchiolitis, granulomatous inflammation, with granulomas being usually small and loose masses with indistinct margins, consisting of epithelioid and multinucleated giant cells (MGCs) commonly located in peribronchiolar regions. Additionally, scattered MGCs are observed, containing asteroid bodies, needle-shaped cholesterol crystals, and calcifications (Schaumann bodies) in the cytoplasm.

In fibrotic HP, pulmonary arterial hypertension, fibrosis, honeycombing, obliterative bronchiolitis, and MGCs in the alveolar lumina, honeycombs, and interalveolar septa prevail.

The aim of the study was to perform a retrospective analysis of the relationship between clinical, radiologic, and morphological features in nonfibrotic and fibrotic HP.

MATERIALS AND METHODS

The research included 175 patients. We studied clinical symptoms from medical histories, performed HRCT of the lungs, and obtained open (via videothoracoscopy, through a small thoracotomy incision) and transbronchial biopsy specimens. Clinically, the following signs were studied: shortness of breath on the Modified Medical Research Council (mMRC) scale, cough, sputum production, and the presence or absence of generalized weakness.

When analyzing HRCT findings, attention was paid to the localization of changes in the lung tissue, the presence of ground-glass opacities, "air traps", reticular changes, the head cheese sign, traction bronchiectasis, and disseminated focal lung disease.

When studying the respiratory function, the following was taken into account: forced vital capacity (FVC), forced expiratory volume in 1 second (FEV_1), the forced expiratory volume in 1 second to forced vital capacity ratio (FEV1/FVC), total lung capacity (TLC), residual lung volume (RV), and diffusing lung capacity for carbon monoxide (DLCO).

Histological changes in the lungs were studied and then compared with the clinical referral diagnoses and pathology reports.

We conducted a histological examination of sections stained with hematoxylin and eosin and Van Gieson's stain to detect collagen and elastic fibers. The following changes were revealed: obliterative bronchiolitis; peribronchiolar fibrosis with lymphocytic infiltrates; organizing pneumonia; moderate fibrosis; smooth muscle metaplasia in fibrosis and interalveolar septa; nonspecific interstitial pneumonia (NSIP); loose non-necrotizing granulomas; honeycombing; MGCs in the alveolar lumina, interalveolar septa, and honeycombs; fibroblastic foci and their localization; bronchiolectasis; Schaumann bodies; and histological signs of secondary pulmonary arterial hypertension (SPAH).

The statistical analysis was carried out using STATISTICA 10.0 for Windows 10. The Shapiro – Wilk *W*-test was used to determine the nature of the sample and the Mann–Whitney U test was applied to determine the reliability of differences in the samples with non-normal distribution, which were considered statistically significant at $p < 0.05$. The correlations were assessed using the Spearman's rank order correlation coefficient, whereas the strength of the correlation coefficients was evaluated with the Chaddock scale.

RESULTS

HP was diagnosed only in 15.5% of all clinical referral diagnoses, i.e. clinicians misdiagnosed the disease in 84.5% of cases. Fig. 1 demonstrates the range of diagnoses.

Fig. 2 represents the range of histopathologic diagnoses. HP was diagnosed only in 8% of cases. Most often, patients were diagnosed with fibrosing lung disease (idiopathic pulmonary fibrosis). In other words, the error rate in the histological examination reached 92%. It should be noted that in 49.5% of cases, a histology report was not provided.

Fig. 3 demonstrates the frequency of the above-listed clinical symptoms in fibrotic and nonfibrotic HP. Shortness of breath, cough, and sputum production prevailed in fibrotic HP. However, the parameter of shortness of breath on the mMRC scale was not reliable.

Fig. 4 demonstrates the parameters of the pulmonary function tests. Parameters of bronchial obstruction prevailed in nonfibrotic HP; at the same time, the differences between the DLCO parameters were not significantly different in two HP types.

Fig. 5 presents data on the HRCT findings. We observed significantly more reticular changes in fibrotic HP and traction bronchiectasis was more common. Honeycombing was observed only in fibrotic HP. The differences between the remaining parameters in fibrotic and nonfibrotic HP were not statistically significant. Two HP types demonstrated diffuse changes in 42% of cases; lesions of the lower lobes prevailed in both lungs (47%). At the same time, the upper lobe lesions were detected in 11% of cases, which practically does not occur in usual interstitial pneumonia (UIP).

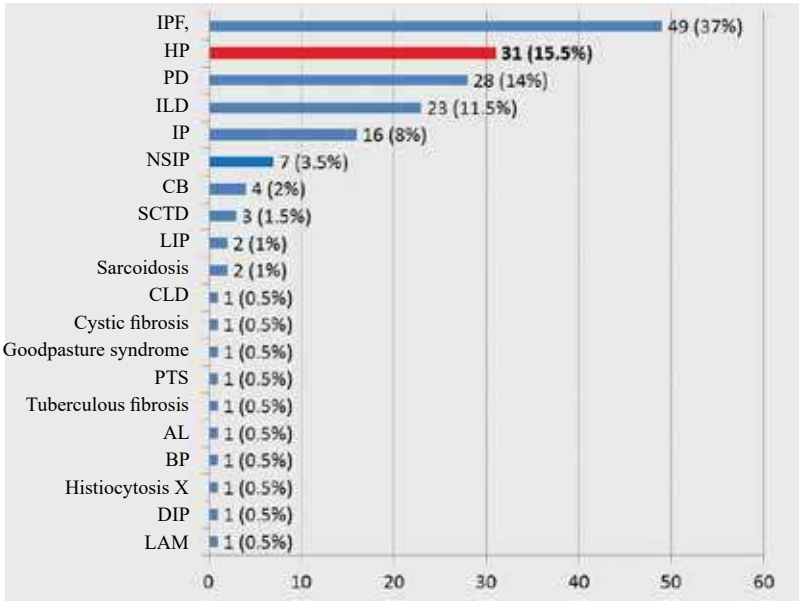


Fig. 1. Clinical referral diagnoses: IPF – idiopathic pulmonary fibrosis, HP – hypersensitivity pneumonitis, PD – pulmonary dissemination, ILD – interstitial lung disease, IP – interstitial pneumonia, NSIP – nonspecific interstitial pneumonia, CB – chronic bronchitis, SCTD – systemic connective tissue diseases, LIP – lymphoid interstitial pneumonia, CLD – cystic lung disease, PTS – post-thrombotic syndrome, AL – amiodarone lung, BP – bilateral pneumonia, DIP – desquamative interstitial pneumonia, LAM – lymphangioleiomyomatosis

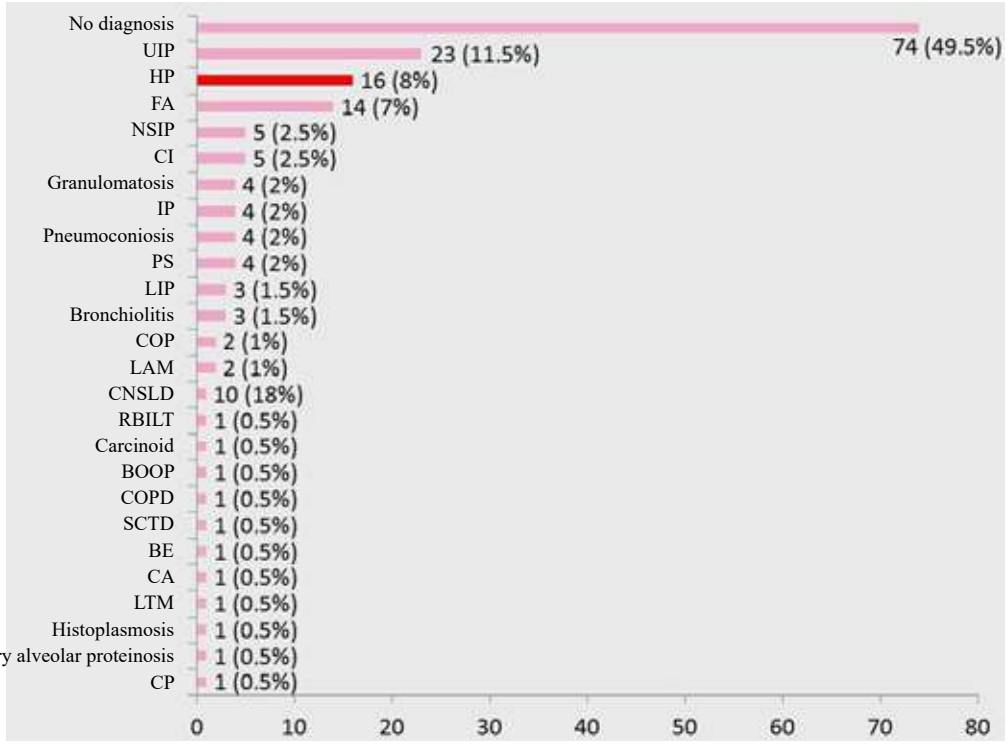


Fig. 2. Morphological referral diagnoses in HP: no diagnosis – no morphological report on the specimen from another institution was included in the medical history, FA – fibrosing alveolitis, CI – chronic inflammation, PS – pneumosclerosis, COP – cryptogenic organizing pneumonia, CNSLDs – chronic non-specific lung diseases, RBILT – respiratory bronchiolitis with another interstitial lung disease, BOOP – bronchiolitis obliterans organizing pneumonia, CA – capillary adenoma, LTM – lung tissue malformation, CP – chronic pneumonia

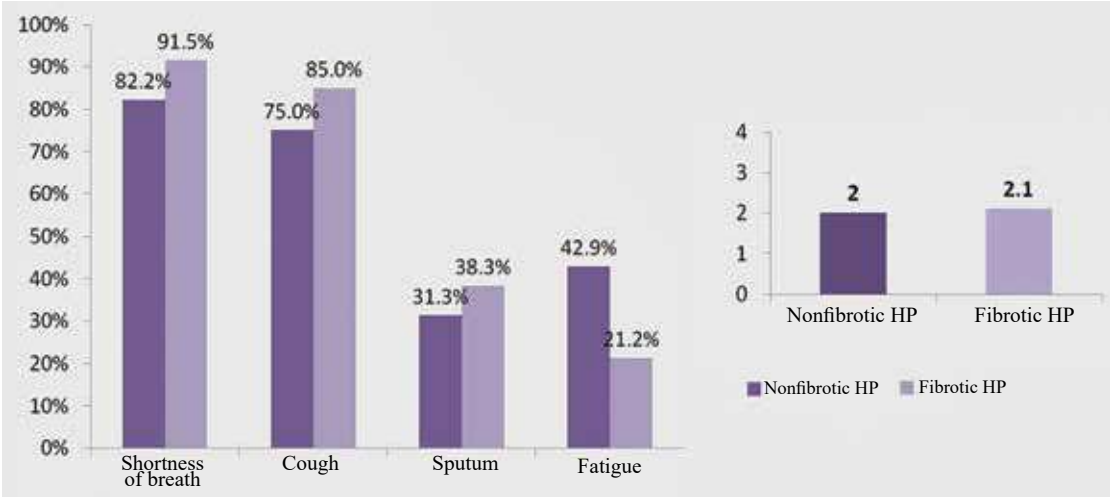


Fig. 3. The frequency of clinical symptoms in nonfibrotic and fibrotic HP

Parameter	FVC, %	FEV 1, %	FEV1/FVC	TLC, %	RV, %	DLCO, %
Nonfibrotic HP	78.37	**84.79**	**91**	76.9	103.5	53.32
Fibrotic HP	60.5	**64.22***	**88.19****	70.1	99.22	47.66

Fig. 4. Pulmonary function test parameters

Fig. 6 shows histological changes in the lung tissue in nonfibrotic HP. Obliterative bronchiolitis was the most common, granulomas and MGCs were observed to a lesser extent. NSIP, organizing pneumonia, and obliterative bronchiolitis with organizing pneumonia were detected in 96.1% of cases.

Fig. 7 demonstrates the frequency of histological signs in fibrotic HP. A microscopic examination revealed that in both fibrotic and nonfibrotic HP, obliterative bronchiolitis was mostly "string-like" (Fig. 8 *a*, *b*), and sometimes it was with fibroblastic foci in the walls of the terminal bronchioles (Fig. 8,*b*). In nonfibrotic HP, we observed MGCs in the alveolar lumina, cavities, and the interstitium (Fig. 10,*a*), non-necrotizing loose granulomas (Fig. 10,*b*), and NSIP (Fig. 11). Fibrotic HP can be also characterized by MGCs, moderately pronounced peribronchiolar interstitial fibrosis with smooth muscle metaplasia (Fig. 9), areas of bridging fibrosis (Fig. 12), peribronchiolar fibrosis with lymphocytic infiltrates, organizing pneumonia, honeycombing, Schaumann bodies, bronchiolectasis, and histological signs of SPAH (Fig. 13).

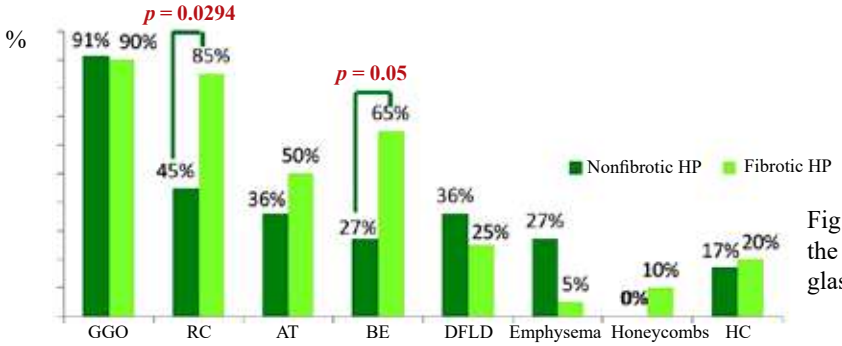


Fig. 5. Changes in the lung tissue on HRCT: HC – the head cheese sign, AT – air traps, GGO – ground-glass opacities, RC – reticular changes, DFLD disseminated focal lung disease

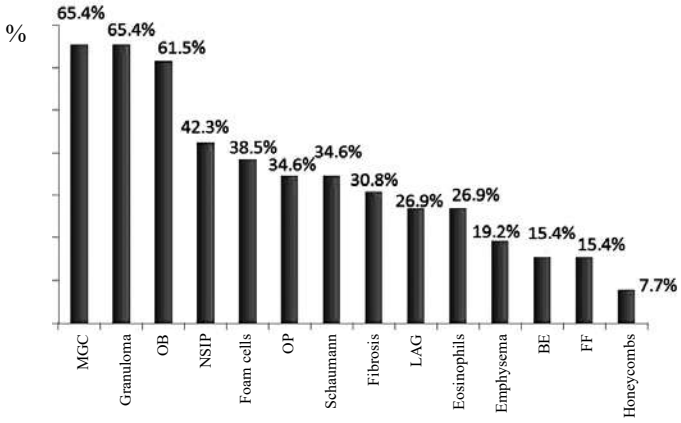


Fig. 6. The frequency of histological features in nonfibrotic HP: BE – bronchiectasis

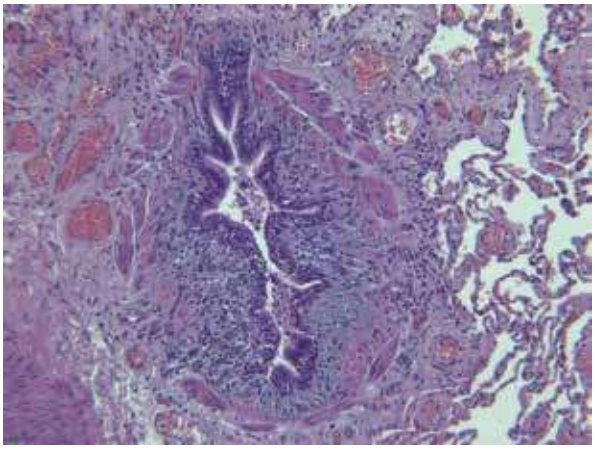
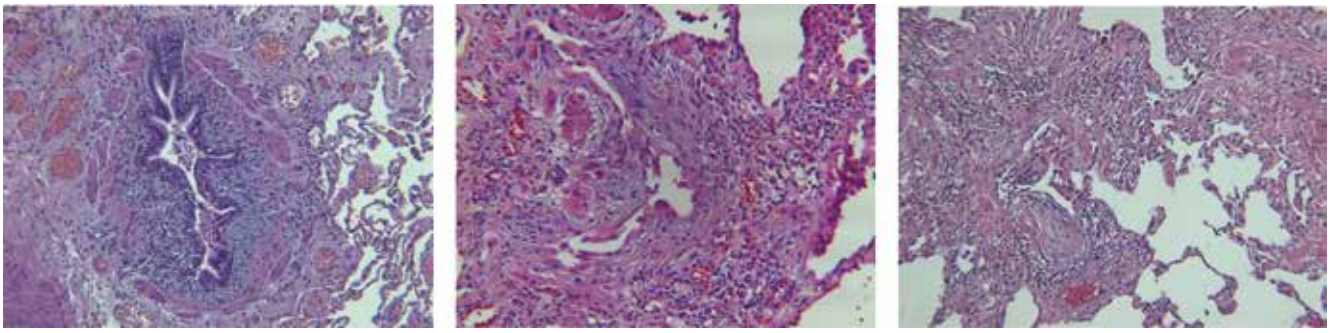
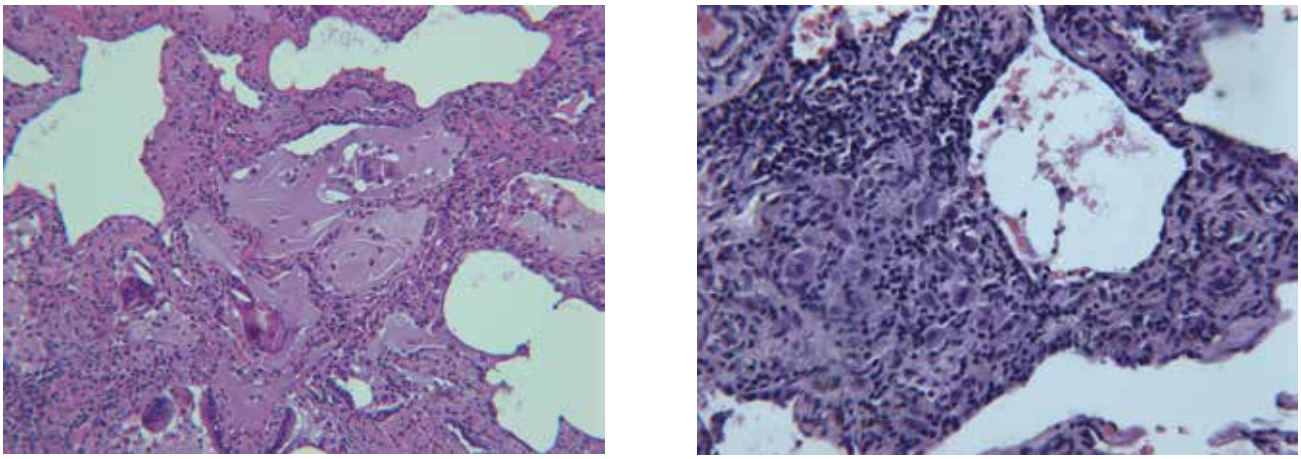


Fig. 7. The frequency of histological features in fibrotic HP



a *b* *c*

Fig. 8. Obliterative bronchiolitis in nonfibrotic and fibrotic HP: *a* – obliterative bronchiolitis, *b* – "string-like" bronchiolitis, *c* – fibroblastic foci in the wall of the terminal bronchiole with narrowing of the lumen; hematoxylin and eosin stain, ×100



a *b*

Fig. 9. Nonfibrotic HP. Giant cells and granuloma in nonfibrotic HP: *a* – MGCs in the alveolar lumina, *b* – peribronchiolar granuloma; hematoxylin and eosin stain, ×100

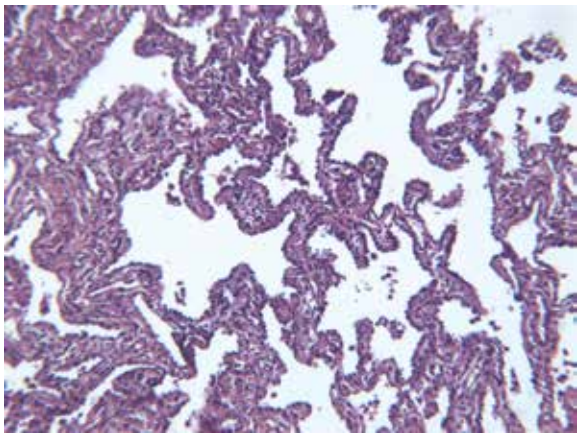


Fig. 10. Cellular NSIP in nonfibrotic HP; hematoxylin and eosin stain, ×100

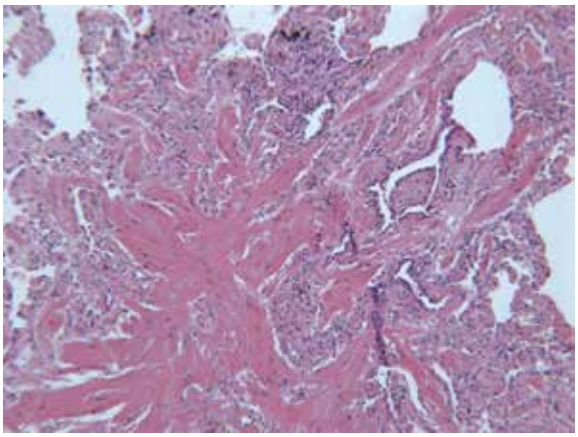


Fig. 11. Fibrotic HP. Smooth muscle metaplasia in the peribronchiolar fibrosis area; hematoxylin and eosin stain, ×100

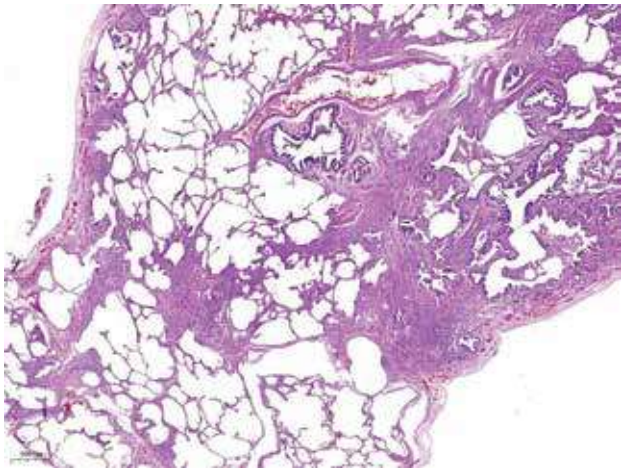


Fig. 12. Fibrotic HP. Bridging fibrosis; hematoxylin and eosin stain, ×200

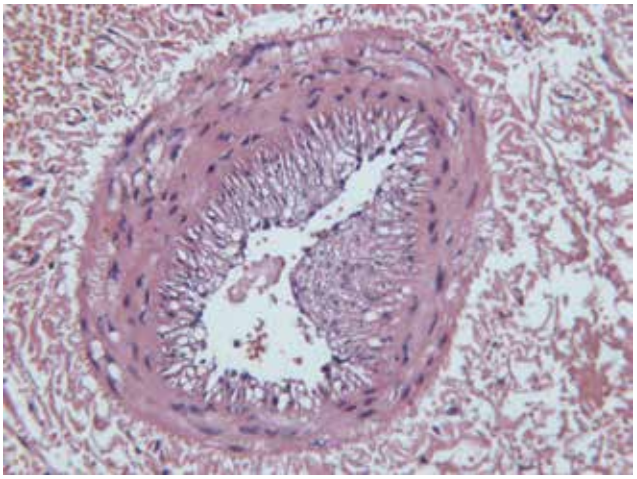


Fig. 13. SPAH in fibrotic HP. Proliferation of the intima with narrowing of the pulmonary artery branch lumen; hematoxylin and eosin stain, ×200

We compared the results of the histological analysis and lung CT findings and established that nonfibrotic HP was detected in 16% of cases, fibrotic HP – in 62% of cases, and possible HP – in 22% of cases. Pulmonary emphysema was detected only in 16 patients (9%), with one case having a combination of fibrotic HP and pulmonary alveolar proteinosis (PAP). The three key morphological HP signs that were described earlier (i.e. granulomas and/or MGCs, obliterative bronchiolitis, and NSIP) were found in 19.2% of cases in nonfibrotic HP and in 5.6% of cases in fibrotic HP.

The correlation analysis revealed a significant moderate relatioship between (1) NSIP frequency in the microscopic evaluation and the presence of honecombing in HRCT ($r = –0.34$); (2) between NSIP and the head cheese sign in HRCT ($r = 0.40$); (3) between the presence of the granuloma and reticular changes ($r = –0.34$); (4) between the presence of the granuloma and traction bronchiectasis ($r = –0.31$), (5) between honecombing and traction bronchiectasis ($r = 0.42$), and (6) between honeycombing and focal dissemination ($r = –0.32$).

DISCUSSION

Clinical HP manifestations are non-specific. The presence of shortness of breath in both HP types on the mMRC scale was not statistically significant. As in the studies by G. Raghu et al., M. Vasakova et al., M. Selman et al. [1, 9, 10], shortness of breath, cough, and sputum were 9.3, 10, and 7%, respectively, more common in fibrotic HP, but they were not statistically significant. At the same time, fatigue was observed twice as often in nonfibrotic HP. HRCT showed that, unlike UIP and lung changes in systemic connective tissue disease (SCTD), changes in the upper, middle, and lower parts of the lungs were observed in two HP types.

Consistent with the data by G. Raghu et al., B. Chong et al., and S. Kligerman et al. [1, 11, 13], pulmonary dissemination and emphysema were more often observed in nonfibrotic HP, but these changes were not statistically significant. The presence of reticular changes, “air traps”, and bronchiectasis was significantly more often observed in fibrotic HP, which did not differ from the data obtained by G. Raghu et al., L. Wang et al., and O. Dias et al. [1, 12, 14].

A microscopic evaluation of the lungs for nonfibrotic HP revealed bronchiolocentric IP, chronic cellular bronchiolitis, and granulomatous inflammation, with granulomas being usually small and loose, in the form of poorly defined clusters of epithelioid cells and MGCs, which were usually located in the peribronchiolar region. Moreover, scattered MGCs were observed in the alveolar lumina and honeycombs, terminal and respiratory bronchioles, and the interstitium. These cells often contained non-specific cytoplasmic inclusions, such as asteroid bodies and/or cholesterol crystals, and Schaumann bodies. Our data are consistent with those obtained by G. Raghu et al. and M. Kitaichi et al. [1, 17] on the fact that the described above histological signs were observed in possible nonfibrotic HP in the absence of granulomas.

Fibrotic HP is characterized by altered lung architecture due to centriacinar emphysema and bridging fibrosis; fibrous IP; the appearance of fibroblastic foci (usually in the walls of the terminal bronchioles), peribronchiolar metaplasia; and less often – by the presence of granulomas. Our data are also consistent with those of G. Raghu et al., M. Kitaichi et al., and S. Chiba et al. [1, 17, 18] in the fact that fibrosis covers both subpleural and centroacinar regions. However, as fibrosis progresses in HP, it is extremely difficult to distinguish its changes in the lungs from UIP. Moreover, the obtained data are consistent with the data of G. Raghu et al. [1] in the fact that the same histological signs are observed in possible fibrotic HP as in verified fibrotic HP, but without honeycombing and granulomas, with less pronounced peribronchiolar metaplasia and single MGCs.

In our opinion, the list of histological signs presented in the guidelines of 2020 should be supplemented with such signs as loose mosaic peribronchiolar fibrosis in fibrotic HP, smooth muscle metaplasia in the areas of fibrosis, and the presence of fibroblastic foci in the walls of terminal bronchioles, as opposed to the same foci in the walls of cells in UIP. The correlation analysis revealed significant moderate correlations between the HRCT parameters and histological changes in the lungs; however, we believe this does not allow to diagnose HP with certainty. HP is mainly diagnosed based on identifying the impact of an external factor, a CT scan of the lungs, and histopathological signs. The major problem is that no single HP sign alone is sufficient and its presence is not obligatory. This leads to possible multiple combinations of signs that contribute to correct HP diagnosis, presented in the guidelines of 2020 [1].

The age of patients with ILDs should be taken into account. Patients with different HP types are usually under 60 years of age, whereas patients with UIP are over 60.

CONCLUSIONS

1) Among clinicians, the error rate in HP diagnosis accounts for 84.5%, whereas among pathologists, it reaches 92%.

2) Among all HP types, fibrotic HP is the most common.

3) Non-necrotizing granulomas and giant cells in the alveolar lumina, honeycombs, and the interalveolar septa are more typical of nonfibrotic HP.

4) The following signs distinguish fibrotic HP from UIP: mosaic peribronchiolar fibrosis, smooth muscle metaplasia in the areas of fibrosis, and the presence of fibroblastic foci in the walls of terminal bronchioles.

4) The three key morphological HP signs were observed only in 19.2% of nonfibrotic HP cases and in 5.6% of fibrotic ones.

5) The diagnosis of HP is complex and should be based on a multidisciplinary approach involving clinicians (pulmonologists), radiologists, functional diagnostics specialists (pulmonary function technologists), and pathologists. At the same time, it is necessary to take into account and identify the factors that cause the development of the disease and the age of patients.

REFERENCES

1. Raghu G., Remy-Jardin M., Ryerson C.J., Myers J.L., Kreuter M., Vasakova M. et al. Diagnosis of hypersensitivity pneumonitis in adults. An official ATS/JRS/ALAT Clinical Practice Guideline. *Am. J. Respir. Crit. Care Med*. 2020; 202 (3): e36–e69. DOI: 10.1164/rccm.202005-2032ST.
2. Mikumo H., Yanagihara T., Hamada N., Hashisako M., Ijichi K., Suzuki K. et al. An autopsy case of bird-related chronic hypersensitivity pneumonitis presenting with repeated acute exacerbation. *Respir. Med. Case Rep*. 2018; 24: 92–94. DOI: 10.1016/j.rmcr.2018.04.016.
3. Roden A.C., Camus P. Iatrogenic pulmonary lesions. *Semin. Diagn. Pathol*. 2018; 35 (4): 260–271. DOI: 10.1053/j.semdp.2018.03.002.
4. Fernández Pérez E.R., Kong A.M., Raimundo K., Koelsch T.L., Kulkarni R., Cole A.L. Epidemiology of Hypersensitivity Pneumonitis among an Insured Population in the United States: A Claims-based Cohort Analysis. *Ann. Am. Thorac. Soc*. 2018; 15 (4): 460–469. DOI: 10.1513/AnnalsATS.201704-288OC.
5. Morell F., Villar A., Ojanguren I., Muñoz X., Cruz M.J., Sansano I. et al. Hypersensitivity pneumonitis and (idiopathic) pulmonary fibrosis due to feather duvets and pillows. *Arch. Bronconeumol.* 2021; 57 (2): 87–93. DOI: 10.1016/j.arbres.2019.12.003.
6. Dhooria S., Agarwal R., Sehgal I.S., Prasad K.T., Garg M., Bal A. et al. Spectrum of interstitial lung diseases at a tertiary center in a developing country: A study of 803 subjects. *PLoS One.* 2018; 13 (2): e0191938. DOI: 10.1371/journal.pone.0191938.
7. Johansson E., Boivin G.P., Yadav J.S. Early immunopathological events in acute model of mycobacterial hypersensitivity pneumonitis in mice. *J. Immunotoxicol.* 2017; 14 (1): 77–88. DOI: 10.1080/1547691X.2016.1273284.
8. Salisbury M.L., Myers J.L., Belloli E.A., Kazerooni E.A., Martinez F.J., Flaherty K.R. Diagnosis and treatment of fibrotic hypersensitivity pneumonia. Where we stand and where we need to go. *Am. J. Respir. Crit. Care Med*. 2017; 196 (6): 690–699. DOI: 10.1164/rccm.201608-1675PP.
9. Vasakova M., Morell F., Walsh S., Leslie K., Raghu G. Hypersensitivity pneumonitis: perspectives in diagnosis and management. *Am. J. Respir. Crit. Care Med*. 2017; 196 (6): 680–689. DOI: 10.1164/rccm.201611-2201PP.
10. Selman M., Pardo A., King T.E. Jr. Hypersensitivity pneumonitis: insights in diagnosis and pathobiology. *Am. J. Respir. Crit. Care Med*. 2012; 186 (4): 314–324. DOI: 10.1164/rccm.201203-0513CI.
11. Chong B.J., Kanne J.P., Chung J.H. Headcheese sign. *J. Thorac. Imaging*. 2014; 29 (1): W13. DOI: 10.1097/RTI.0000000000000067.
12. Wang L.J., Cai H.R., Xiao Y.L., Wang Y., Cao M.S. Clinical characteristics and outcomes of hypersensitivity pneumonitis: a population-based study in China. *Chin. Med. J. (Engl.).* 2019; 132 (11): 1283–1292. DOI: 10.1097/CM9.0000000000000256.
13. Kligerman S.J., Henry T., Lin C.T., Franks T.J., Galvin J.R. Mosaic attenuation: etiology, methods of differentiation, and pitfalls. *Radiographics.* 2015; 35 (5): 1360–1380. DOI: 10.1148/rg.2015140308.
14. Dias O.M., Baldi B.G., Pennati F., Aliverti A., Chate R.C., Sawamura M.V.Y. et al. Computed tomography in hypersensitivity pneumonitis: main findings, differential diagnosis and pitfalls. *Expert. Rev. Respir. Med.* 2018; 12 (1): 5–13. DOI: 10.1080/17476348.2018.1395282.
15. Myers JL. Hypersensitivity pneumonia: the role of lung biopsy in diagnosis and management. *Mod. Pathol*. 2012; 25 (1): S58–67. DOI: 10.1038/modpathol.2011.152.
16. Mooney J.J., Koth L.L. Surgical lung biopsy over bronchoalveolar lavage in chronic hypersensitivity pneumonitis. *Am. J. Respir. Crit. Care Med*. 2014; 189 (3): 371–372. DOI: 10.1164/rccm.201309-1736LE.
17. Kitaichi M., Shimizu S., Tamaya M., Takaki M., Inoue Y. Pathology of hypersensitivity pneumonitis. In: Sharma OP, ed. Clinical focus series, hypersensitivity pneumonitis. New Delhi, India: Jaypee Brothers Medical Publishers, 2013: 22–32. DOI: 10.5005/jp/books/12031_3.
18. Chiba S., Tsuchiya K., Akashi T, Ishizuka M., Okamoto T., Furusawa H. et alChronic hypersensitivity pneumonitis with a usual interstitial pneumonia-like pattern: correlation between histopathologic and clinical findings. *Chest.* 2016; 149 (6): 1473–1481. DOI: 10.1016/j.chest.2015.12.030.

Authors contribution

Cherniaev A.L., Samsonova M.V., Avdeev S.N., Trushenko N.V. – conception and design, analysis and interpretation of data, critical revision of the manuscript for important intellectual content, final approval of the manuscript for publication. Kusraeva E.V. – analysis and interpretation of data, substantiation of the manuscript. Tumanova E.L. – critical revision of the manuscript for important intellectual content.

Authors information

Cherniaev Andrey L., Dr. Sci. (Med.), Professor, Head of the Division of Fundamental Pulmonology, Pulmonology Scientific Research Institute; Leading Researcher, A.P. Avtsyn Research Institute of Human Morphology, Moscow, Russian Federation. ORCID 0000-0002-0158-7056.

Kusraeva Elina V., Resident, Pathology and Clinical Pathology Department, Pirogov Russian National Research Medical University, Moscow, Russian Federation. ORCID 0000-0002-1179-6070.

Samsonova Maria V., Dr. Sci. (Med.), Head of the Pathology Laboratory, Pulmonology Scientific Research Institute; Senior Researcher, Loginov Moscow Clinical Scientific Center, Moscow, Russian Federation. ORCID 0000-0001-8170-1260.

Avdeev Servey N., Dr. Sci. (Med.), Professor, Corresponding Member of the Russian Academy of Sciences, Head of the Clinical Department, Pulmonology Scientific Research Institute, Moscow, Russian Federation. ORCID 0000-0002-5999-2150.

Trushenko Natalya V., Cand. Sci. (Med.), Assistant, Pulmonology Department, I.M. Sechenov First Moscow State Medical University (Sechenov University); Researcher, Pulmonology Scientific Research Institute, Moscow, Russian Federation. ORCID 0000-0002-0685-4133.

Tumanova Elena L., Dr. Sci. (Med.), Professor, Head of the Pathology and Clinical Pathology Department, Pediatric Faculty, Pirogov Russian National Research Medical University, Moscow, Russian Federation. ORCID 0000-0003-1149-4061.

(✉) **Kusraeva Elina V.,** e-mail: elina.kusraeva@yandex.ru

Received 20.04.2021
Accepted 10.09.2021

УДК 616-056.257:577.121:577.112.387.4
https://doi.org/10.20538/1682-0363-2021-4-103-111

“Kynurenine switch” and obesity

Shestopalov A.V.[1, 2], Shatova O.P.[2], Karbyshev M.S.[2], Gaponov A.M.[1], Moskaleva N.E.[3], Appolonova S.A.[3], Tutelyan A.V.[4], Makarov V.V.[5], Yudin S.M.[5], Roumiantsev S.A.[2]

[1] Dmitry Rogachev National Medical Research Center of Pediatric Hematology, Oncology, and Immunology
1, Samory Mashela Str., Moscow, 117997, Russian Federation

[2] Pirogov Russian National Research Medical University
1, Ostrovityanova Str., Moscow, 117997, Russian Federation

[3] I.M. Sechenov First Moscow State Medical University
2/4 Bolshaya Pirogovskaya Str., Moscow, 119991, Russian Federation

[4] Central Research Institute of Epidemiology of the Federal Service for Surveillance on Consumer Rights Protection and Human Well-being
3A, Novogireevskaya Str., Moscow, 111123, Russian Federation

[5] Center for Strategic Planning and Management of Biomedical Health Risks
10/1, Pogodinskaya Str., Moscow, 119121, Russian Federation

ABSTRACT

Aim. To assess the concentrations of bacterial and eukaryotic metabolites mainly involved in indole, kynurenine, and serotonin pathways of tryptophan metabolism in a cohort of patients with obesity.

Materials and methods. Using high-performance liquid chromatography with mass spectrometric detection, the concentrations of several serum metabolites, such as kynurenine, kynurenic acid, anthranilic acid, xanthurenic acid, quinolinic acid, 5-hydroxyindole-3-acetate, tryptamine, serotonin, indole-3-lactate, indole-3-acetate, indole-3-butyrate, indole-3-carboxaldehyde, indole-3-acrylate, and indole-3-propionate, were analyzed in a cohort of obese patients compared with healthy volunteers.

Results. It was found that serum levels of tryptophan metabolites of microbial and eukaryotic origin were significantly increased in obese patients. Therefore, the concentration of kynurenine in the blood serum in obese patients was 2,413 ± 855 nmol / l, while in healthy volunteers of the same age group, the level of kynurenine in the blood serum was 2,122 ± 863 nmol / l. In obese patients, two acids formed due to kynurenine metabolism; the concentrations of kynurenic and quinolinic acids were increased in the blood serum. The concentration of kynurenic acid in the blood serum in obese patients was 21.1 ± 9.26 nmol / l, and in healthy patients, it was 16.8 ± 8.37 nmol / l. At the same time, the level of quinolinic acid in the blood serum in obese patients was 73.1 ± 54.4 nmol / l and in healthy volunteers – 56.8 ± 34.1 nmol / l. Normally, the level of quinolinic acid is 3.4 times higher than the concentration of kynurenic acid, and in case of obesity, there is a comparable increase in these acids in the blood serum.

From indole derivatives, mainly of microbial origin, the concentrations of indole-3-lactate, indole-3-butyrate, and indole-3-acetate were significantly increased in the blood serum of obese patients. In obese patients, the serum concentration of 5-hydroxyindole-3-acetate was elevated to 74.6 ± 75.8 nmol / l (in healthy volunteers – 59.4 ± 36.6 nmol / l); indole-3-lactate – to 523 ± 251 nmol / l (in healthy volunteers – 433 ± 208 nmol / l); indole-3-acetate – to 1,633 ± 1,166 nmol / l (in healthy volunteers – 1,186 ± 826 nmol / l); and indole-3-butyrate – to 4.61 ± 3.31 nmol / l (in healthy volunteers – 3.85 ± 2.51 nmol / l).

Conclusion. In case of obesity, the utilization of tryptophan was intensified by both the microbiota population and the macroorganism. It was found that obese patients had higher concentrations of kynurenine, quinolinic and kynurenic acids, indole-3-acetate, indole-3-lactate, indole-3-butyrate, and 5-hydroxyindole-3-acetate. Apparently, against the background of increased production of proinflammatory cytokines by adipocytes in obese patients,

✉ *Shatova Olga P.,* e-mail: shatova.op@gmail.com

the "kynurenine switch" was activated which contributed to subsequent overproduction of tryptophan metabolites involved in the immune function of the macroorganism.

Key words: microbiota, tryptophan, obesity, kynurenines, indoles, metabolic syndrome.

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 under the agreement No.037310012211900041 within the project "Creation of a bank of blood serum and fecal samples from healthy donors and patients with obesity, metabolic syndrome, type II diabetes mellitus, and breach in the gastrointestinal mucosal barrier to identify candidate species-nonspecific mediators of the human microbiota quorum sensing systems that modulate the endocrine and metabolic function of adipose tissue".

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 Pirogov Russian National Research Medical University (Protocol No. 186 of 26.06.2019).

For citation: Shestopalov A.V., Shatova O.P., Karbyshev M.S., Gaponov A.M., Moskaleva N.E., Appolonova S.A., Tutelyan A.V., Makarov V.V., Yudin S.M., Roumiantsev S.A. "Kynurenine switch" and obesity. *Bulletin of Siberian Medicine.* 2021; 20 (4): 103–111. https://doi.org/10.20538/1682-0363-2021-4-103-111.

«Кинурениновый переключатель» и ожирение

Шестопалов А.В.[1, 2], Шатова О.П.[2], Карбышев М.С.[2], Гапонов А.М.[1], Москалева Н.Е.[3], Апполонова С.А.[3], Тутельян А.В.[4], Макаров В.В.[5], Юдин С.М.[5], Румянцев С.А.[2]

[1] *Национальный медицинский исследовательский центр* (*НМИЦ*) *Детской гематологии, онкологии и иммунологии имени Дмитрия Рогачева*
Россия, 117997, г. Москва, ул. Саморы Машела, 1

[2] *Российский национальный исследовательский медицинский университет (РНИМУ) им. Н.И. Пирогова*
Россия, 117997, г. Москва, ул. Островитянова, 1

[3] *Первый Московский государственный медицинский университет им. И.М. Сеченова (Сеченовский университет)*
Россия, 119991, г. Москва, ул. Большая Пироговская, 2/4

[4] *Центральный научно-исследовательский институт (НИИ) эпидемиологии Роспотребнадзора Российской Федерации (РФ)*
Россия, 111123, г. Москва, ул. Новогиреевская, 3а

[5] *Центр стратегического планирования и управления медико-биологическими рисками здоровью*
Россия, 119121, г. Москва, ул. Погодинская, 10/1

РЕЗЮМЕ

Цель. Изучить содержание метаболитов бактериального и эукариотического происхождения индольного, кинуренинового и серотонинового путей обмена триптофана у пациентов с ожирением.

Материалы и методы. Методом высокоэффективной жидкостной хроматографии с масс-спектрометрическим детектированием изучили концентрации сывороточных метаболитов: кинуренина, кинуреновой кислоты, антраниловой кислоты, ксантуреновой кислоты, хинолиновой кислоты, 5-гидросииндол-3-ацетата, триптамина, серотонина, индол-3-лактата, индол-3-ацетата, индол-3-бутирата, индол-3-карбоксальдегида, индол-3-акрилата, индол-3-пропионата у пациентов с ожирением в сравнении с группой здоровых добровольцев.

Результаты. Установлено, что у пациентов с ожирением в сыворотке крови статистически значимо повышен уровень метаболитов триптофанового обмена микробиотического и эукариотического происхождения. Концентрация кинуренина в сыворотке крови у больных с ожирением составляла 2 413 ± 855 нмоль/л, тогда как у здоровых добровольцев такой же возрастной группы – 2 122 ± 863 нмоль/л. Также у пациентов с ожирением в сыворотке крови были повышены две кислоты, которые образуются в результате метабо-

лизма кинуренина – кинуреновая и хинолиновая. Концентрация кинуреновой кислоты в сыворотке крови у пациентов с ожирением составляла 21,1 ± 9,26 нмоль/л, а у здоровых 16,8 ± 8,37 нмоль/л соответственно. Тогда как концентрация хинолиновой кислоты в сыворотке крови при ожирении – 73,1 ± 54,4 нмоль/л, а у здоровых добровольцев – 56,8 ± 34,1 нмоль/л. В норме концентрация хинолиновой кислоты в 3,4 раза выше, чем концентрация кинуреновой кислоты, а при ожирении происходит сопоставимое их повышение.

Из производных индола, которые имеют преимущественно микробиотическое происхождение, в сыворотке крови пациентов с ожирением статистически значимо повышена концентрация индол-3-лактата, индол-3-бутирата и индол-3-ацетата. У пациентов с ожирением концентрация в сыворотке крови метаболита серотонина – 5-гидроксииндол-3-ацетата – была повышена и составляла 74,6 ± 75,8 нмоль/л (у здоровых добровольцев – 59,4 ± 36,6 нмоль/л); индол-3-лактата – 523 ± 251 нмоль/л (у здоровых добровольцев 433 ± 208 нмоль/л); индол-3-ацетата – 1 633 ± 1166 нмоль/л (у здоровых добровольцев 1 186 ± 826 нмоль/л); индол-3-бутирата – 4,61 ± 3,31 нмоль/л (у здоровых добровольцев 3,85 ± 2,51 нмоль/л).

Заключение. При ожирении происходит интенсификация утилизации триптофана как микробиотической популяцией кишечника, так и макроорганизмом. Установлено, что больные с ожирением имеют более высокие концентрации кинуренина, хинолиновой и кинуреновой кислот, индол-3-ацетата, индол-3-лактата, индол-3-бутирата и 5-гидроксииндол-3-ацетата. Видимо, на фоне гиперпродукции провоспалительных цитокинов адипоцитами у пациентов с ожирением срабатывает «кинурениновый переключатель», что и обеспечивает гиперпродукцию метаболитов триптофанового обмена, которые вовлечены в иммунологическую функцию макроорганизма.

Ключевые слова: микробиота, триптофан, ожирение, кинуренины, индолы, метаболический синдром.

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

Источник финансирования. Работа выполнена в рамках договора № 0373100122119000041 по проекту «Создание банка биообразцов сыворотки крови и фекалий от здоровых доноров и пациентов с ожирением, метаболическим синдромом, сахарным диабетом II типа, нарушением мукозального барьера желудочно-кишечного тракта с целью выявления кандидатных видонеспецифических медиаторов систем quorum sensing микробиоты человека, модулирующих эндокринную и метаболическую функцию жировой ткани».

Соответствие принципам этики. Все участники исследования подписали добровольное информированное согласие. Исследование одобрено локальным этическим комитетом РНИМУ им. Н.И. Пирогова (протокол № от 86 от 26.06.2019).

Для цитирования: Шестопалов А.В., Шатова О.П., Карбышев М.С., Гапонов А.М., Москалева Н.Е., Апполонова С.А., Тутельян А.В., Макаров В.В., Юдин С.М., Румянцев С.А. «Кинурениновый переключатель» и ожирение. *Бюллетень сибирской медицины.* 2021; 20 (4): 103–111. https://doi.org/10.20538/1682-0363-2021-4-103-111.

INTRODUCTION

Tryptophan is an essential amino acid for the human body [1]. However, the intestinal microbiota has an enzymatic system of the shikimate pathway of tryptophan synthesis and can provide the macroorganism with both amino acid and its derivatives. Among these metabolites are derivatives of the kynurenine, indole, and serotonin pathways of tryptophan metabolism [2]. Synthesis of aromatic amino acids is possible in the microbiome due to universal metabolic pathways – glycolysis and the pentose phosphate pathway (PPP). Therefore, glycolysis produces phosphoenolpyruvate (PEP), while PPP is a source of erythrose–4-phosphate for the shikimate pathway of tryptophan and anthranilic acid synthesis (Fig. 1,*a* and *b*).

The dominant mechanism of tryptophan utilization for eukaryotes is the kynurenine pathway. More than 90% of tryptophan not spent on biosynthesis is converted into metabolites of the kynurenine pathway [2]. They perform antioxidant and anti-inflammatory functions in the body, regulate immune responses, and act as toxins and signaling molecules in the molecular dialogue between the macroorganism and the microbiome [2]. For example, anthranilic acid (anthranilate) is an essential link in the metabolic coupling of the microbiome and the macroorganism: it has a mixed origin and is a precursor in the synthesis of quinolinic acid and, accordingly, NAD^+ and also participates in the formation of quinoline regulators of the quorum sensing (QS) in the microbiome [3]. So far, there is no information on the role

of microbiota enzymes in the synthesis of quinolinic acid and NAD^+ directly from tryptophan [4]. However, both quinolinic acid and NAD^+ are not species-specific metabolites, and their synthesis occurs in both eukaryotes and prokaryotes. It is important to note that quinolinic acid in eukaryotes is formed from tryptophan, whereas in prokaryotes, this acid is formed from aspartic acid [5].

Quinolinic acid is neuro- and gliotoxic and increases in animals with multiple sclerosis in brain tissues and blood serum [6]. Quinolinic acid is an ionotropic glutamate receptor antagonist that selectively binds N-methyl-D-aspartate, while kynurenic acid is an agonist of glutamatergic and cholinergic receptors and has antioxidant properties [7]. Therefore, kynurenic acid will have a neuroprotective effect.

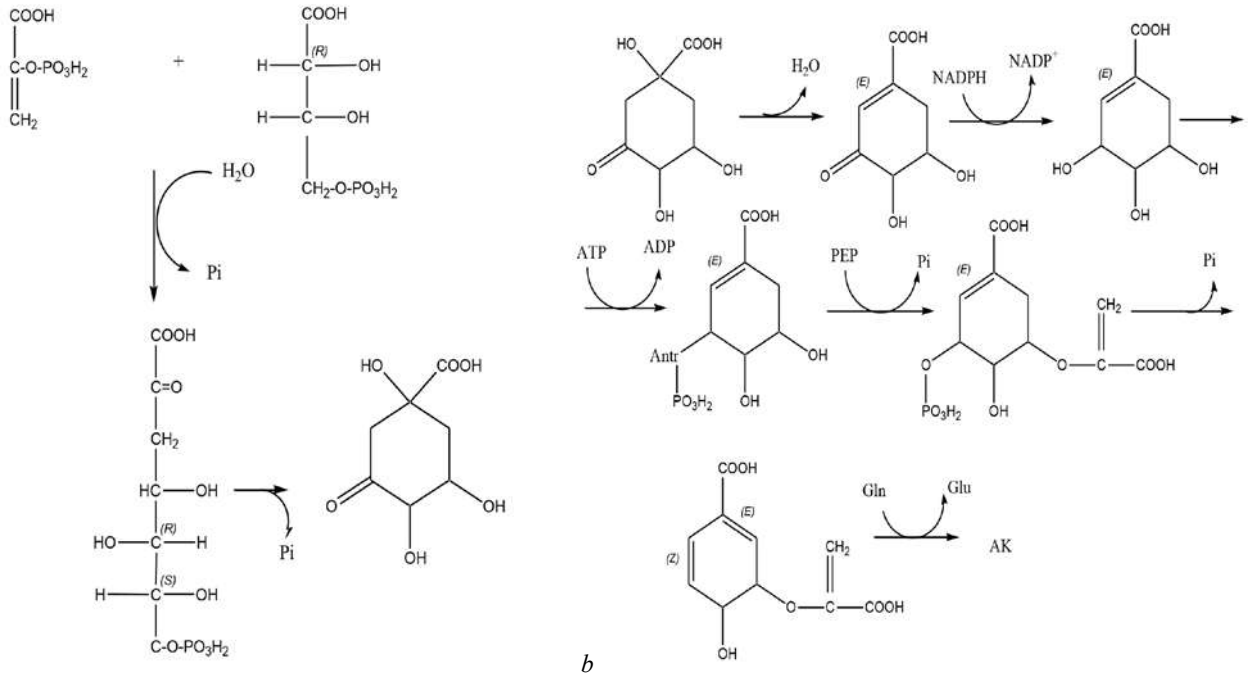


Fig. 1. The shikimate pathway: *a* – at the first stage, 3-dehydroquinate is synthesized: PEP and erythrose 4-phosphate are converted into deoxy-d-arabino-hept-2-ulosonate-7-phosphate (DAHP) with the participation of DAHP synthase; *b* – at the second stage, DAHP is converted into 3-dehydroquinate (3-DQ) with the participation of 3-DQ synthase, and then reduction to shikimate occurs, followed by a phosphorylation reaction to shikimate 3-phosphate. When interacting with PEP, shikimate 3-phosphate is converted into enolpyruvate shikimate-3-phosphate by a specific synthase, and then, after dephosphorylation, it becomes a chorismate, which, when interacting with glutamine (Gln), turns into anthranilic acid (AA)a

It is shown that after tryptophan loading or after immune stimulation, hepatocytes, which are constitutively responsible for NAD^+ synthesis, transiently accumulate quinolinic acid. At the same time, cells of the immune system, including macrophages, dendritic cells, Langerhans cells, Kupffer cells, etc., generate high, stable levels of intracellular quinolinic acid in response to various immune stimulators. This event regulates the mobility of immune cells, since it induces synthesis of cytoskeleton proteins in them [8]. It is important to note that in the liver, all tryptophan molecules not involved in protein synthesis are converted into NAD^+ or oxidized to CO_2 and H_2O. During immune stimulation, indolamine-2,3-dioxygenase (I-2,3-DO) is activated in the lung tissue, contributing to the "kynurenine switch" activation. Systemic kynurenine begins to actively engage immune cells for overproduction of NAD^+ [8].

Intracellular levels of quinolinic acid increase in response to immune stimulation by lipopolysaccharide in macrophages, microglia, dendritic cells, and other cells of the immune system [8]. The further fate of quinolinic acid depends on the activity of quinolinate phosphoribosyltransferase (QPRT), which catalyzes the formation of nicotinic acid mononucleotide from quinolinic acid and 5-phosphoribosyl-1-pyrophosphate (Fig. 2).

It is known that the inflammatory response requires higher levels of NAD^+ in immune cells. Thus, NAD^+ performs numerous functions (Fig. 2):

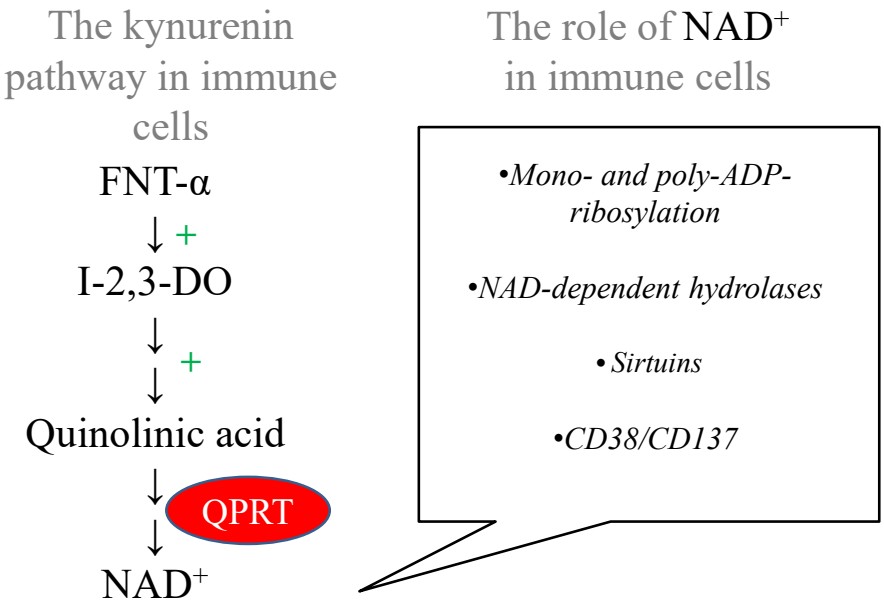


Fig. 2. The biological role of NAD+ in immune cells

participates in the respiratory burst and the polymerization reaction of poly(ADP-ribose) polymerase (which is involved in DNA repair mechanisms) and regulates the activity of NAD^+-dependent deacetylases (sirtuins) and NAD^+-dependent hydrolases, including CD38, CD157, and ADP-ribosyl cyclase/cyclic ADP-ribose hydrolase. The latter is expressed on many immune cells, including $CD4^+$, $CD8^+$, B-lymphocytes and natural killer cells, and CD157 – ADP-ribosyl cyclase/cyclic ADP-ribose hydrolase-2 is expressed by pre-B cells [8].

It was shown that obesity is a systemic inflammatory disease with overexpression of indoleamine 2,3-dioxygenase (I-2,3-DO) in both white adipose tissue and the liver, induced by adipocytes of proinflammatory cytokines [9]. In response to the activation of the regulatory enzyme of the kynurenine pathway, the entire kynurenine pathway of tryptophan metabolism is also intensified. Activation of I-2,3-DO in the intestine leads to an increase in the kynurenine concentration, which has an antimicrobial effect [2] and, thus, affects the gut microbiota.

It is possible that in obesity, the intensification of the shikimate pathway in the intestine is accompanied by excessive production of tryptophan, which can be metabolized into indole derivatives by various microbiota populations. It should be noted that indole-3-lactate regulates the kynurenine pathway of tryptophan conversion in cells of the macroorganism [10], and indole-3-acetate (and tryptamine) is an agonist of aryl hydrocarbon receptors [11].

Therefore, the aim of the study was to investigate the concentrations of metabolites of the kynurenine and indole pathways of tryptophan metabolism in the blood serum of patients with obesity.

MATERIALS AND METHODS

A cohort of 266 participants with an average age of 39.9 ± 4.2 years was examined. Two clinical groups were formed. Group 1 (n = 138) – a control group of healthy volunteers without obesity and/or metabolic syndrome with an average body mass index (BMI) of 22.7 kg / m^2 and waist circumference (WC) of 79.8 cm. Group 2 (n = 128) was an experimental group including patients with obesity and/or metabolic syndrome with an average BMI of 32.96 kg / m^2 and WC of 108.98 cm.

Samples of the whole blood and blood serum were obtained from all study participants according to the study protocol. Transportation and storage of the samples were carried out in compliance with the cold chain at a temperature not higher than –40 °C.

A quantitative analysis of tryptophan metabolites in the blood serum was performed by high-performance liquid chromatography with mass spectrometry (HPLC-MS/MS). The analysis was performed using an Agilent 1200 liquid chromatography (Agilent Inc., USA) with an automatic sample input system, a

column thermostat, and a degasser. Chromatographic separation was performed using the Discovery PFP HS F5 analytical column (2.1 × 150 mm; 3 microns). The composition of the mobile phase A – 0.1% formic acid solution in deionized water; phase B – 100% acetonitrile for chromatography. The mobile phase gradient ranged from 1 to 10 % within 4 minutes, then went up to 90% by the 9th minute of the analysis. The flow rate of the mobile phase was 0.40 ml / min.

A mass spectrometry detector based on the Agilent 6460 triple quadrupole (Agilent Inc., USA), MRM, and electrospray ionization were used. The characteristic parent and daughter ions for each compound for the MRM mode and ionization and dissociation parameters were optimized using the standards of the studied metabolites. The received signal was processed using MassHunter software (Agilent Inc., USA).

Metabolite concentrations were calculated by the internal standard method (2-hydroxynicotinic acid). The internal standards of the determined compounds were prepared using an artificial matrix containing bovine serum albumin and sodium chloride. The studied metabolites were added to the matrix and prepared according to the analysis method.

Serum sample preparation was conducted as follows: an internal standard (2-hydroxynicotinic acid) was added to 100 μl of serum, and proteins were precipitated with acetonitrile; the supernatant was evaporated and re-dissolved in 10% methanol in water with the addition of ascorbic acid to prevent oxidation of analytes. To prepare a stool sample, it was lyophilized to a dry residue, and then a sample of about 5 mg was extracted with 50% methanol in water with addition of an internal standard and ascorbic acid. After centrifugation, the sample was analyzed by HPLC-MS/MS.

The method was validated in terms of selectivity, linearity, accuracy, reproducibility, matrix effect, and analyte stability. The validation was conducted following the FDA guidelines for the validation of bioanalytical methods.

Statistical analysis of the obtained results was performed using Statistica 12.0 software package (StatSoft Inc, USA). The data are presented as the mean and standard deviation $M \pm \sigma$. After checking the data distribution for normality, the statistical significance of the differences in the mean values of independent samples was assessed using parametric analysis. The normality of the distribution of variables in the groups was assessed using the Shapiro – Wilk test. The Mann – Whitney test was used for comparative analysis of independent samples, with the Wilcoxon test used for comparative analysis of dependent samples. The differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

When analyzing the level of metabolites of the kynurenine and indole pathways in the blood serum in the control group, we found that concentrations of quinolinic acid, kynurenic acid, kynurenine, 5-hydroxyindole-3-acetate, indole-3-lactate, indole-3-acetate, and indole-3-butyrate significantly increased in obesity (Table).

Table

Serum content of tryptophan metabolites, nmol/l, $M \pm \sigma$

Parameter	Control group, $n = 138$	Experimental group, $n = 128$
Quinolinic acid	56.8 ± 34.1	73.1 ± 54.4*
Kynurenine	2,122 ± 863	2,413 ± 855**
5-hydrosyindole-3-acetate	59.4 ± 36.6	74.6 ± 75.8*
Kynurenic acid	16.8 ± 8.37	21.1 ± 9.26*
Indole-3-lactate	433 ± 208	523 ± 251**
Indole-3-acetate	1,186 ± 826	1,633 ± 1,166*
Indole-3-butyrate	3.85 ± 2.51	4.61 ± 3.31*
Serotonin	809 ± 356	782 ± 434
Anthranilic acid	33.3 ± 20.7	37.5 ± 20.9
Xanthurenic acid	4.31 ± 3.11	4.18 ± 2.91
Tryptamine	0.818 ± 0.541	0.731 ± 0.314
Indole-3-carboxaldehyde	40.4 ± 19.3	44.3 ± 25.7
Indole-3-acrylate	5.01 ± 14.6	4.29 ± 15.7
Indole-3-propionate	650 ± 845	753 ± 736

* differences are statistically significant relative to the control group at $p < 0.01$; ** differences are statistically significant relative to the control group at $p < 0.05$.

We did not find statistically significant differences in serum concentrations of such tryptophan metabolites as anthranilic acid, xanthurenic acid, tryptamine, indole-3-carboxaldehyde, indole-3-acrylate, and indole-3-propionate between healthy volunteers and obese patients (Table).

Many research groups demonstrated an increase in kynurenine concentration in the blood serum in obesity [4]. However, in this study, the statistically significant increase in serum kynurenine was not so significant in percentage terms, whereas we found a tremendous increase in the concentrations of quinolinic acid and kynurenic acid in obese patients.

We also showed that among all the kynurenine pathway metabolites in the blood serum in both groups, the concentration of kynurenine was significantly higher in comparison with other metabolites of the kynurenine pathway. In our opinion, this observation is natural, since kynurenine is the main catab-

olite of tryptophan for the macroorganism. However, kynurenine has a mixed origin, and it remains unclear what percentage of serum kynurenine is absorbed from the intestine and what percentage is formed in other organs of the macroorganism in normal conditions and obesity.

The depletion of taxa in the gut microbiota in obese patients is known [12]. Overproduction of various proinflammatory cytokines by adipocytes is also observed [13], accompanied by an increase in the kynurenine concentration in the blood serum with a subsequent rise in quinolinic acid. The increased concentration of quinolinic acid in the blood serum of obese patients reflects what is happening in the cells of the macroorganism. The significance of the "kynurenine switch" and the protective role of NAD^+ were discussed above, but there is a rate-limiting reaction in the synthesis of NAD^+ – CPRT [8]. Apparently, overproduction of quinolinic acid in obese patients is associated with low activity of this enzyme, also in immune cells.

Utilization of the excessive amount of kynurenine in obesity also follows the path of its conversion into kynurenic acid, as evidenced by data obtained on a statistically significant increase in the level of kynurenic acid in obese patients. This metabolite performs an antioxidant role and is seemingly a functional antagonist of quinolinic acid, not only in the nervous tissue. Probably that is why the increase in the serum concentration of quinolinic acid is comparable to the increase in the serum level of kynurenic acid in obese patients.

A pronounced statistically significant increase in the level of indole-3-acetate in the blood serum was found in the patients of the experimental group. This tryptophan metabolite is mainly of bacterial origin. It should be noted that the indole molecule itself suppresses the formation of *Pseudomonas* quinolone signal (PQS) in the intestine [14], the precursor of which is anthranilic acid.

It was shown that indole-3-acetate is a metabolite conjugating the indole and kynurenine tryptophan metabolism pathways; it can also be formed in eukaryotic cells from anthranilic acid. This metabolite is a ligand of aryl hydrocarbon receptors and performs a regulatory function in the human body, participating in the immune function [15]. Indole-3-lactate is also an agonist of aryl hydrocarbon receptors [16]. It is known that indole-3-lactate decreases the response to interleukin-8 after stimulation with interleukin-1 [17]. In obese patients, an increase in indole-3-lactate is microbiota metabolic compensation to reduce the production of proinflammatory cytokines. In further studies, it is necessary to analyze the relationship between the concentration of proinflammatory cytokines and indole-3-lactate in obese patients.

Indole-3-butyrate, like indole-3-lactate, is a metabolite of bacterial origin. So far, there are no studies that would show the eukaryotic origin of these metabolites of the indole pathway in tryptophan metabolism. The involvement of indole-3-butyrate in the pathogenesis of obesity has to be studied, while there are no studies that would determine the role of this tryptophan metabolite. We found that in obese patients, the concentration of indole-3-butyrate in the blood serum was higher than in healthy volunteers.

When serotonin is metabolized, 5-hydroxyindole-3-acetate is formed, which is significantly increased in the blood serum of obese patients, while the serum concentration of serotonin itself does not change significantly. It should be noted that the primary level of serum serotonin is determined by its overproduction in enterocytes. It can be assumed that an excessive amount of tryptophan in the intestine is metabolized to serotonin, indole-3-acetate, indole-3-butyrate, indole-3-lactate, and kynurenine.

CONCLUSION

It should be noted that all pathways of tryptophan metabolism in the body are intensified in obese patients. Utilization of the excessive amount of tryptophan occurs by both gut microbiota and cells of the macroorganism. An increase in the serum level of quinolinic acid has an adverse effect on the macroorganism and is a necessary consequence of immune stimulation. An increase in the concentration of kynurenic acid in the blood serum of patients with low-grade inflammation in obesity is an indispensable condition for simultaneous formation of a functional antagonist of quinolinic acid.

In obese patients, we have found an increase in the rate of tryptophan metabolism by the gut microbiota, and it has not been studied yet whether this tryptophan is of exogenous origin or a product of the shikimate pathway. The increased levels of kynurenine, quinolinic acid, and kynurenic acid in the blood serum may be a manifestation of the "kynurenine switch" of NAD^+ overproduction in immune cells in obese patients. A drastic increase in indole-3-acetate in the blood serum of obese patients performs a compensatory and adaptive function, also through suppression of the activity of some enzymes of the kynurenine pathway in different tissues of the macroorganism. The

increase in the concentrations of indole-3-lactate and indole-3-butyrate in the blood serum of obese patients reflects precisely the microbiotic activation of the utilization of excess tryptophan, possibly of shikimate origin. In obese patients, the utilization of serotonin is intensified, and the concentration of 5-hydroxyindole-3-acetate in the blood serum increases statistically significantly.

REFERENCES

1. Comai S., Bertazzo A., Brughera M., Crotti S. Tryptophan in health and disease. *Adv. Clin. Chem*. 2020; 95: 165–218. DOI: 10.1016/bs.acc.2019.08.005.
2. Dehhaghi M., Kazemi Shariat P.H., Guillemin G.J. Microorganisms, tryptophan metabolism, and kynurenine pathway: a complex interconnected loop influencing human health status. *Int. J. Tryptophan Res*. 2019; 12: 1178646919852996. DOI: 10.1177/1178646919852996.
3. Sas K., Szabo E., Vecsei L. Mitochondria, oxidative stress and the kynurenine system, with a focus on ageing and neuroprotection. *Molecules*. 2018; 23 (1): 191. DOI: 10.3390/molecules23010191.
4. Agus A., Planchais J., Sokol H. Gut microbiota regulation of tryptophan metabolism in health and disease. *Cell Host. Microbe*. 2018; 23 (6): 716–724. DOI: 10.1016/j.chom.2018.05.003.
5. Katoh A., Uenohara K., Akita M., Hashimoto T. Early steps in the biosynthesis of NAD in Arabidopsis start with aspartate and occur in the plastid. *Plant Physiol*. 2006; 141 (3): 851–857. DOI: 10.1104/pp.106.081091.
6. Sundaram G., Lim C.K., Brew B.J., Guillemin G.J. Kynurenine pathway modulation reverses the experimental autoimmune encephalomyelitis mouse disease progression. *J. Neuroinflammation*. 2020; 17 (1): 176. DOI: 10.1186/s12974-020-01844-y.
7. Ferreira F.S., Schmitz F., Marques E.P., Siebert C., Wyse A.T. Intrastriatal quinolinic acid administration impairs redox homeostasis and induces inflammatory changes: prevention by kynurenic acid. *Neurotox. Res*. 2020; 38(1): 50–58. DOI: 10.1007/s12640-020-00192-2.
8. Moffett J.R., Arun P., Puthillathu N., Vengilote R., Ives J.A., Badawy A.A., Namboodiri A.M. Quinolinate as a marker for kynurenine metabolite formation and the unresolved question of NAD(+) synthesis during inflammation and infection. *Front. Immunol*. 2020; 11: 31. DOI: 10.3389/fimmu.2020.00031.
9. Wolowczuk I., Hennart B., Leloire A., Bessede A., Soichot M., Taront S., Caiazzo R., Raverdy V., Pigeyre M., Guillemin G.J., Allorge D., Pattou F., Froguel P., Poulain-Godefroy O. Tryptophan metabolism activation by indoleamine 2,3-dioxygenase in adipose tissue of obese women: an *attempt* to maintain immune homeostasis and vascular tone. *Am. J. Physiol. Regul. Integr. Comp. Physiol*. 2012; 303 (2): R135–R143. DOI: 10.1152/ajpregu.00373.2011.
10. Han Q., Robinson H., Cai T., Tagle D.A., Li J. Structural insight into the inhibition of human kynurenine aminotransferase I/glutamine transaminase. *K. J. Med. Chem*. 2009; 52 (9): 2786–2793. DOI: 10.1021/jm9000874.
11. Jin U.H., Lee S.O., Sridharan G., Lee K., Davidson L.A., Jayaraman A., Chapkin R.S., Alaniz R., Safe S. Microbiome-derived tryptophan metabolites and their aryl hydrocarbon receptor-dependent agonist and antagonist activities. *Mol. Pharmacol*. 2014; 85 (5): 777–788. DOI: 10.1124/mol.113.091165.
12. Sitkin S., Pokrotnieks J. Gut microbiota as a host defender and a foe: the 2 faces of commensal Bacteroides thetaiotaomicron in inflammatory bowel disease. *Inflamm. Bowel Dis*. 2019; 25 (6): e71. DOI: 10.1093/ibd/izy377.
13. Brandacher G., Hoeller E., Fuchs D., Weiss H.G. Chronic immune activation underlies morbid obesity: is IDO a key player? *Curr. Drug Metab*. 2007; 8 (3): 289–295. DOI: 10.2174/138920007780362590.
14. Tashiro Y., Toyofuku M., Nakajima-Kambe T., Uchiyama H., Nomura N. Bicyclic compounds repress membrane vesicle production and Pseudomonas quinolone signal synthesis in Pseudomonas aeruginosa. *FEMS Microbiol. Lett*. 2010; 304 (2): 123–130. DOI: 10.1111/j.1574-6968.2010.01897.x.
15. Pernomian L., Duarte-Silva M., de Barros Cardoso C.R. The aryl hydrocarbon receptor (AHR) as a potential target for the control of intestinal inflammation: insights from an immune and bacteria sensor receptor. *Clin. Rev. Allergy Immunol.* 2020, 59 (3): 382–390. DOI: 10.1007/s12016-020-08789-3.
16. Li X., Lu C., Fan D., Lu X., Xia Y., Zhao H., Xu H., Zhu Y., Li J., Liu H., Xiao C. Human umbilical mesenchymal stem cells display therapeutic potential in rheumatoid arthritis by regulating interactions between immunity and gut microbiota via the aryl hydrocarbon receptor. *Front. Cell Dev. Biol*. 2020; 8: 131. DOI: 10.3389/fcell.2020.00131.
17. Meng D., Sommella E., Salviati E., Campiglia P., Ganguli K., Djebali K., Zhu W., Walker W.A. Indole-3-lactic acid, a metabolite of tryptophan, secreted by Bifidobacterium longum subspecies infantis is anti-inflammatory in the immature intestine. *Pediatr. Res.* 2020; 88 (2): 209–217. DOI: 10.1038/s41390-019-0740-x.

Authors contribution

Shestopalov A.V. – design of the study, compilation of a database, revision of the article, writing of all the sections, final approval of the manuscript for publication. Shatova O.P. – selection of the patient sample, review of publications of the topic of the study, statistical analysis of data and drafting of the article, compilation of tables and figures, final proofreading of the manuscript. Karbyshev M.S. – writing of the "Discussion" section, writing of the article in English. Gaponov A.M. – design of the study, writing of the "Materials and methods" section. Moskaleva N.E. – carrying out of the biochemical analyses, discussion of the obtained results. Appolonova S.A. – carrying out of the biochemical analyses, editing of the "Materials and methods" section. Tutelyan A.V. – critical revision of the manuscript for important

intellectual content. Makarov V.V. – discussion of the study design and the obtained data. Yudin S.M. – editing of the study concept, interpretation of the research results. Roumiantsev S.A. – design of the study, revision of the article, drafting of all the sections, final approval of the manuscript for publication.

Authors information

Shestopalov Aleksandr V., Dr. Sci. (Med.), Professor, Director of the Department of Postgraduate Education, Clinical Residency, and Doctoral Program, Dmitry Rogachev National Research Center of Pediatric Hematology, Oncology, and Immunology; Head of the Department of Biochemistry and Molecular Biology, Pirogov Russian National Research Medical University, Moscow, Russian Federation. ORCID 0000-0002-1428-7706.

Shatova Olga P., Cand. Sci. (Med.), Associate Professor, Department of Biochemistry and Molecular Biology, Pirogov Russian National Research Medical University, Moscow, Russian Federation. ORCID 0000-0003-4265-1293.

Karbyshev Mikhail S., Cand.Sci. (Biology), Associate Professor, Department of Biochemistry and Molecular Biology, Pirogov Russian National Research Medical University, Moscow, Russian Federation. ORCID 0000-0001-5588-3259.

Gaponov Andrei M., Cand. Sci. (Med.), Head of the Laboratory of Infectious Immunology, Dmitry Rogachev National Research Center of Pediatric Hematology, Oncology and Immunology, Moscow, Russian Federation. ORCID 0000-0002-3429-1294.

Moskaleva Natalya E., Cand. Sci. (Biology), Senior Researcher, Center for Biopharmaceutical Analysis and Metabolic Research, Institute of Translational Medicine and Biotechnology, I.M. Sechenov First Moscow State Medical University, Moscow, Russian Federation. ORCID 0000-0002-7309-8913.

Appolonova Svetlana A., Cand. Sci. (Chemistry), Head of the Center for Biopharmaceutical Analysis and Metabolic Research, Institute of Translational Medicine and Biotechnology, I.M. Sechenov First Moscow State Medical University, Moscow, Russian Federation. ORCID 0000-0002-9032-1558.

Tutelian Aleksei V., Dr. Sci. (Med.), Professor, Corresponding Member of the Russian Academy of Sciences, Head of Laboratory of Hospital Infections, Central Research Institute of Epidemiology of the Federal Service for Surveillance on Consumer Rights Protection and Human Well-being, Moscow, Russian Federation. ORCID 0000-0002-2706-6689.

Makarov Valentin V., Cand. Sci. (Biology), Analyst, Center for Strategic Planning and Management of Biomedical Health Risks, Moscow, Russian Federation. ORCID 0000-0001-9495-0266.

Yudin Sergei M., Dr. Sci. (Med.), Professor, Director General of Center for Strategic Planning and Management of Biomedical Health Risks, Moscow, Russian Federation. ORCID 0000-0002-7942-8004.

Rumyantsev Sergei A., Dr. Sci. (Med.), Professor, Corresponding Member of the Russian Academy of Sciences, Head of the Department of Oncology, Hematology and Radiation Therapy, Pirogov Russian National Research Medical University, Moscow, Russian Federation. ORCID 0000-0002-7418-0222.

(✉) **Shatova Olga P.,** e-mail: shatova.op@gmail.com

Received 29.09.2020
Accepted 28.12.2020

УДК 616.36-003.826-082.5:613.7/.8
https://doi.org/10.20538/1682-0363-2021-4-112-122

Adherence to lifestyle modification in patients with nonalcoholic fatty liver disease

Andreev K.A., Skirdenko Yu.P., Nikolaev N.A., Livzan M.A., Gorbenko A.V., Fedorin M.M., Krolevets T.S.

Omsk State Medical University
12, Lenina Str., Omsk, 644099, Russian Federation

ABSTRACT

Nonalcoholic fatty liver disease (NAFLD) makes a major impact on morbidity and mortality among the working-age population in developed countries. In the lack of effective pharmacological methods, the leading role in treatment of NAFLD belongs to lifestyle modification, consistent and gradual weight loss, and its maintenance. The qualitative and quantitative structure of the diet, intensity of physical activity, and most importantly, regularity and consistency of implementation of lifestyle modification activities are the key to successful management of patients with NAFLD.

To date, there are very few studies on adherence to lifestyle modification activities in this group of patients, which is mainly due to a deficiency of methodological tools. The questionnaire "QAA-25" recommended by the Russian Scientific Medical Society of Therapists for quantitative assessment of adherence to treatment allows to assess both adherence to therapy in general and adherence to its individual components (adherence to drug therapy, adherence to medical counseling, and adherence to lifestyle modification), which requires further study taking into account features of therapeutic strategies in treating NAFLD.

Key words: nonalcoholic fatty liver disease, lifestyle modification, adherence, insulin resistance, metabolic syndrome.

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 grant of the President of the Russian Federation for the state support for the leading scientific schools of the Russian Federation 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., Skirdenko Yu.P., Nikolaev N.A., Livzan M.A., Gorbenko A.V., Fedorin M.M., Krolevets T.S. Adherence to lifestyle modification in patients with nonalcoholic fatty liver disease. *Bulletin of Siberian Medicine.* 2021; 20 (4): 112–122. https://doi.org/10.20538/1682-0363-2021-4-112-122.

Приверженность модификации образа жизни при неалкогольной жировой болезни печени

Андреев К.А., Скирденко Ю.П., Николаев Н.А., Ливзан М.А., Горбенко А.В., Федорин М.М., Кролевец Т.С.

Омский государственный медицинский университет (ОмГМУ)
Россия, 644099, г. Омск, ул. Ленина, 12

РЕЗЮМЕ

Неалкогольная жировая болезнь печени (НАЖБП) вносит серьезный вклад в заболеваемость и смертность трудоспособного населения развитых стран. При отсутствии эффективных фармакологических подходов

✉ *Andreev Kirill A.,* e-mail: kivi2104@gmail.com

ведущую роль в лечении НАЖБП играет модификация образа жизни, в первую очередь последовательная и постепенная потеря массы тела, а также ее поддержание. Качественный и количественный состав диеты, уровень физической активности, а главное, регулярность выполнения мероприятий по модификации образа жизни являются залогом успешного ведения пациентов с НАЖБП.

На сегодняшний день исследований, посвященных изучению вопросов приверженности мероприятиям по изменению образа жизни у данной группы больных, крайне мало, что во многом связано со скудностью методологического инструментария. Рекомендованный российским научным медицинским обществом терапевтов опросник количественной оценки приверженности лечению «КОП-25» позволяет оценить как приверженность терапии в целом, так и по отдельным ключевым компонентам (приверженность лекарственной терапии, приверженность медицинскому сопровождению и приверженность модификации образа жизни), что требует изучения, учитывая особенности терапевтических стратегий при НАЖБП.

Ключевые слова: неалкогольная жировая болезнь печени, приверженность, модификация образа жизни, инсулинорезистентность, метаболический синдром.

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

Источник финансирования. Публикация подготовлена за счет финансирования по гранту Президента России для государственной поддержки ведущих научных школ 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., Скирденко Ю.П., Николаев Н.А., Ливзан М.А., Горбенко А.В., Федорин М.М., Кролевец Т.С. Приверженность модификации образа жизни при неалкогольной жировой болезни печени. *Бюллетень сибирской медицины.* 2021; 20 (4): 112–122. https://doi.org/10.20538/1682-0363-2021-4-112-122.

RELEVANCE

Nonalcoholic fatty liver disease (NAFLD) includes a range of liver changes which are morphologically characterized by excessive accumulation of fat in hepatocytes (associated with insulin resistance) in patients who do not consume significant amounts of alcohol and/or steatogenic drugs and do not have other possible causes of secondary steatosis [1]. The established risk factors for the development and progression of this disease include obesity, type 2 diabetes mellitus, and dyslipidemia [2, 3].

Currently, NAFLD is one of the most common liver diseases worldwide, affecting 20–40% of the population in developed countries [4, 5]. This trend is expected to worsen as obesity rates increase [6, 7]. NAFLD is considered as a hepatic manifestation of the metabolic syndrome, whose pathogenetic mechanisms of formation determine the interdisciplinary relationship between the progression of the disease (steatohepatitis, fibrosis, cirrhosis) and cardiovascular events, renal pathology, etc. This, in turn, becomes the reason for high mortality among these patients.

Despite the growing interest in the study of NAFLD in the global and Russian research community, the issue of effective specific treatment of NAFLD remains unresolved. The main directions of drug therapy for NAFLD are increasing tissue sensitivity to insulin and reducing the stage of liver damage [8], which are used only with lifestyle modification [9–12]. Therefore, lifestyle modification is the cornerstone of any NAFLD treatment regimen and implies namely changes in the quantity and quality of nutrition, weight loss, and increased physical activity. Changing habits and lifestyle as established functional systems in human behavior requires significant efforts. Long-term effectiveness of non-pharmacological treatment depends on patient motivation and adherence [13–15].

In few studies available on adherence to treatment in patients with NAFLD, there is evidence of significant effectiveness of non-pharmacological treatment, but information on adherence to these treatment methods is extremely limited. This may be determined by difficulties of studying the adherence to lifestyle modification, as well as by imperfection of the methodology.

EATING BEHAVIOR MODIFICATION IN THE TREATMENT OF NAFLD

The 2016 guidelines of the European Association for the Study of the Liver, European Association for the Study of Diabetes, and European Association for the Study of Obesity describe the main provisions of diet therapy: weight loss by 7–10% from the initial weight, which should be achieved by reducing the daily calorie intake by 500–1,000 kcal; a change in the ratio of macronutrients in favor of an increase in the amount of complex carbohydrates, plant-based proteins, and fiber and a decrease in the proportion of fat; exclusion of food containing fructose from the diet; consumption of alcoholic beverages not exceeding the daily safe dose (30 ml for men, 20 ml for women in terms of ethanol) [16].

The most effective therapeutic intervention in the treatment of NAFLD is diet modification, which promotes weight loss in patients [16]. Despite this, some diets that involve excessive and/or rapid weight loss (for example, diets very low in carbohydrates and high in fat) contribute to the development of insulin resistance. This, in turn, worsens the course of the disease [17]. The use of elimination diets and the so-called healthy fasting for the treatment of patients with NAFLD is absolutely unacceptable, since fatty liver disease is aggravated, and a relatively favorable stage of steatosis can advance to steatohepatitis and fibrosis [18].

Given a strong association between NAFLD and obesity, it is not surprising that significant histological and biochemical improvements are seen in patients after weight loss. A study by M. Palmer and S. Schaffner demonstrated that weight loss of more than 10% in obese patients with NAFLD is associated with a significant decrease in serum aminotransferase levels [19]. In another study, S. Zelber-Sagi et al. found that a 9% decrease in body weight leads to significant morphological changes [20].

Diet therapy to achieve the target body weight should have both quantitative and qualitative changes. Most studies demonstrate that energy restriction alone is not enough to treat NAFLD, modulation of both macro- and micronutrients in the diet is critical, so a balanced diet and moderate weight loss may now be considered the best therapeutic approach [21]. The balance and qualitative composition of the diet are even more important in a situation when we face the so-called metabolically inactive NAFLD in patients with initially normal body weight. The main goal in diet therapy is to maintain normal body weight and realize the antifibrinogen effects of the corresponding diets [22, 23].

Diet is the main moderator of triglyceride accumulation in the liver parenchyma and can be crucial for potentiating antioxidant activity [2, 24–26]. To date, there are no solid data on the effect of certain products on the development and course of NAFLD, but such studies are being conducted globally [22, 24, 27, 28]. There have been reports that diets high in carbohydrates and low in fat, but with the same amount of protein and total calories are associated with greater severity of NAFLD [24], or that patients with NAFLD consume more saturated fatty acids compared with healthy individuals [23].

A population-based study in Germany showed that it is typical of people with NAFLD to consume less tea, confectionery, fat, bread, cereals, and cheese and have higher consumption of soups, beer, wine, juice, poultry, and eggs [29]. Among 999 Chinese adults included in the study by C.Q. Yang et al., patients who had a vegetarian diet had the lowest risks of developing NAFLD [30].

A number of European studies also suggested that a plant-based diet is beneficial for patients with liver diseases [31–33]. In a crossover study of the Chinese population, a vegetarian diet was associated with a lower risk of developing fatty liver disease [34]. However, there are studies that do not confirm this effect [27, 35]. For example, in another crossover study involving 615 Buddhists, a vegetarian diet was not shown to be effective in preventing NAFLD [35]. According to the Rotterdam study of 3,882 patients with NAFLD, it was found that a diet rich in animal proteins was associated with the development of NAFLD, regardless of the traditional risk factors. According to that study, sugar consumption did not increase the risk of the development and progression of NAFLD [36].

One of the most preferred diets in patients with NAFLD is the Mediterranean diet [37]. This diet is a traditional approach to eating among the population in the states surrounding the Mediterranean Sea. Although due to cultural, religious, and agricultural characteristics, each region is characterized by its own variant of the diet, the general features of

the diet are as follows: eating a large amount of unrefined grains, vegetables, fresh fruits, olive oil and nuts; fish, white meat and legumes in moderation; limiting the intake of red meat, meat products, and sweets. The main characteristics of the Mediterranean diet are a healthy fatty acid profile, consisting in low intake of saturated fat and cholesterol and, conversely, high intake of monounsaturated fatty acids with a balanced ratio of omega-6 to omega-3, along with a high content of complex carbohydrates and dietary fiber [38].

The MEDINA study in a large cohort of patients with NAFLD demonstrated that a Mediterranean diet, regardless of weight loss, can lead to significant improvements in biochemical liver markers, and these changes were maintained for at least 12 months [39]. It was suggested that the positive effect of the Mediterranean diet is partly due to the high content of substances with high antioxidant and anti-inflammatory activity [40–42].

Cognitive behavioral therapy (CBT) can be an important aspect of lifestyle modification therapy. S. Moscatiello et al. compared the effectiveness of psychotherapy support in weight loss in patients with NAFLD [43]. After 2 years, in the group of patients receiving psychotherapy, there was a statistically significant decrease in body weight, normalization of liver parameters and a greater likelihood of maintaining it within the required therapeutic interval, compared with the group receiving diet therapy alone. Evidence on long-term treatment efficacy and adherence to lifestyle modifications and diet emphasizes the role of psychotherapy support in the treatment of NAFLD [44].

PHYSICAL ACTIVITY IN THE TREATMENT OF PATIENTS WITH NAFLD

In the aforementioned consensus statement of the European Association for the Study of the Liver, European Association for the Study of Diabetes, and European Association for the Study of Obesity, increasing physical activity to 120–150 minutes per week (in 3–5 workouts) is one of the main recommendations for non-drug treatment of NAFLD. Preference should be given to aerobic activities (brisk walking, exercise bike, swimming) [16].

In a retrospective analysis of 813 adults with biopsy-confirmed NAFLD, physical activity was assessed using clinical questionnaires [45]. Similarly, the level of physical activity was assessed in the crossover study by S. Zelber-Sagi, which involved 375 patients with NAFLD [46]. Patients with NAFLD were significantly less physically active than those without NAFLD. An important limitation of these studies is the use of survey and questionnaire data rather than objective measures of physical activity.

The relationship between low physical activity and NAFLD was also studied among adolescents [28, 47–49]. L.N. Hattar et al. in a prospective cohort study compared the physical activity of pediatric patients (aged 8–16 years) with NAFLD in patients without liver diseases [50]. The results showed that the group of obese children with NAFLD had the lowest physical activity scores, and more than 50% of them did not exercise actively at all.

All this led to a new concept that a simple decrease in the level of "inactivity", even in the absence of proper physical education sessions, can be useful [51–53]. The lack of adequate physical activity in patients with NAFLD prompted the American Association for the Study of Liver Diseases, the American College of Gastroenterology, and the American Gastroenterological Association to revise the guidelines on the role of physical exercise in 2012 [54]. The Association Joint Clinical Guidelines mention physical exercise as an effective method for reducing hepatic steatosis, however, it should be noted that only a limited number of studies use physical exercises as the main treatment for NAFLD, and the significance of improvements in the course of the disease, provided no weight loss, is yet to be studied.

Several studies focused on finding the optimal frequency and intensity of physical exercise that would lead to clinically significant reduction of hepatic steatosis [55–58]. The study by K.D. Kistler et al. showed that patients who performed high-intensity exercise, such as exercising on a treadmill or using a step machine, were significantly less likely to develop NAFLD [45]. The average time spent on performing intense physical exercise by the studied patients was 3 hours per week. A decrease in the risk of developing progressive fibrosis was also noted, when the recommended duration of high-intensity exercise was doubled. In contrast, patients who did moderate-intensity exercise, such as brisk walking, did not show a difference in the risk of

developing NAFLD or fibrosis compared with those who did not exercise.

These results are consistent with previously published studies showing great cardiovascular health benefits of intense exercise [46]. Based on the available data, it can be concluded that high-intensity exercise appears to be of the greatest benefit to patients with NAFLD and should be considered the preferred option when discussing the treatment strategy [59, 60].

Strength training deserves special attention, due to its wider application. Often, patients with NAFLD have underlying medical conditions that can complicate aerobic exercises. To date, there are few studies examining the effectiveness of strength training in the treatment of patients with NAFLD, but the available data suggest this area is promising [61, 62].

The study by K. Hallsworth et al. evaluated the effect of resistance training on the biochemical parameters of patients with NAFLD [63]. The training consisted of eight strength exercises with a total duration of 45–60 minutes, done 3 times a week. At the end of the 8-week test period, a decrease in steatosis and lipid oxidation and an increase in muscle tissue sensitivity to insulin were recorded. These effects were recorded regardless of body weight loss. Since the data on the effect of resistance training on the course and outcome of NAFLD are insufficient, attention should be paid to studies evaluating the effectiveness of such exercises in patients who meet the criteria for metabolic syndrome, given high probability of their having NAFLD.

A meta-analysis of 13 randomized controlled trials using resistance training to improve metabolic syndrome showed that this approach can significantly influence such parameters as obesity, glycated hemoglobin levels, and systolic blood pressure [64]. There was also a decrease in the amount of visceral fat and the level of proinflammatory cytokines [65].

COMBINING DIET AND EXERCISE IN THE TREATMENT OF NAFLD

Sustained weight loss is most effectively achieved by combining diet changes with a specific level of exercise. Thus, in the prospective study by S.S. Baba et al. 44 patients with histologically confirmed NAFLD underwent aerobic physical activity for at least 45 minutes a day 5 times a week in combination with a diet aimed at reducing body weight (daily deficit in the range of 500–1000 kcal). After 3 months, these patients showed a significant decrease in aspartate aminotransferase (from 70.5 to 41.4 U/l, $p < 0.0001$) and alanine aminotransferase (from 104 to 63.2 U/l, $p < 0.0001$). In approximately 45% of patients adhering to the recommended regimen for 3 months, the level of aminotransferases became fully normal [66].

A similar study of 96 patients with NAFLD compared the effectiveness of intensive lifestyle interventions (exercise and dietary changes) to achieve a minimum weight loss of 7% and exceptional nutritional education. According to magnetic resonance spectroscopy, a significant decrease in the degree of steatosis was noted in the intensive intervention group (50.8 and 22.8%, respectively; $p = 0.04$) [67].

Despite the fact that statistically significant results were achieved in all of the above-mentioned studies, a number of limitations may prevent the use of these approaches in real clinical practice. Thus, in the study by C.S. Baba et al., 25% of the participants were unable to complete their prescribed exercise program due to difficulty and physical fatigue. In addition, significant specialized assistance was required to achieve the results obtained.

In the aforementioned study by K. Promrat et al., all patients were closely monitored by a nutritionist and a professionally trained physical education instructor for a 48-week trial period [68]. This study included extensive psychological support throughout the period. These financial, human, and physical obligations significantly increase the burden of managing the NAFLD patient.

ADHERENCE IN NAFLD PATIENTS

Even without significant weight loss, lifestyle changes improve the course of NAFLD, especially when adherence is high. Meanwhile, it is the adherence to lifestyle modification that is the main problem of all such interventions [69]. To date, there are very few studies examining the issues of adherence to lifestyle change in this group of patients. This is largely due to a deficiency of methodological tools, since the widely used questionnaires of patient adherence to MMAS-4 and MMAS-8 therapy do not adequately assess adherence to lifestyle modification [70–72].

The Russian Scientific Medical Society of Therapists recommends to use the Russian questionnaire for quantitative assessment of adherence to treatment "QAA-25" to determine adherence to therapy [73]. It allows to estimate (in %) patient's adherence to therapy by its main components: adherence to drug therapy, medical counseling, and lifestyle modification, which makes it extremely promising [74].

A number of studies which were carried out in 2001–2006 in Greece and dedicated to the influence of the Mediterranean diet on the development of cardiovascular diseases in the Greek population are of interest. To assess the adherence and effectiveness of the Mediterranean diet, the Mediterranean diet score system was used, which implies a score assigned to respondents depending on what foods and with what frequency they consume. Typically, the score ranges from 0 (minimum adherence to the Mediterranean diet) to 9 (maximum adherence) [75, 76].

Another tool for assessing adherence to a particular type of nutrition is the international diet quality indicator (DQI-I), which examines four main aspects: its composition and variety, adequacy, moderation, and balance (the sources of energy that a person receives from food are analyzed). The score is estimated from 0 to 100; the higher the score, the higher the quality of the diet [77, 78].

Bearing in mind that pharmacological and surgical methods of NAFLD treatment are a backup therapy strategy, the prevalence of their use can indirectly indicate the degree of ineffectiveness of non-drug methods, due to low adherence of patients to the doctor's recommendations in the long term. In this regard, it is worth noting that there are data that in the vast majority of patients after bariatric surgeries, no manifestations of fibrosis were registered; however, after a short period of time, they relapsed and progressed at a higher rate [8].

The use of metabolic surgery techniques in the treatment of patients with NAFLD remains a matter of debate. To date, there is no evidence for the safety and efficacy of bariatric surgery in the treatment of patients with nonalcoholic steatohepatitis. In addition, there is evidence of both progression of fibrosis in patients with NAFLD after surgical treatment of obesity and cases of NAFLD manifestation in patients undergoing similar operations [79, 80].

CONCLUSION

Diseases associated with metabolic syndrome, in particular, NAFLD, make a significant contribution to the morbidity and mortality of the working population in developed countries. In the absence of effective pharmacological approaches, lifestyle modification, primarily consistent and gradual weight loss, as well as its maintenance are crucial in the treatment of NAFLD. Diet and exercise should be the main elements of any NAFLD treatment plan. The macro- and microelement composition of the diet has a significant impact on the progression of liver damage. Diets that are low in carbohydrates and saturated fats should be preferred.

Exercise is clearly essential in the management of NAFLD and should be recommended for all patients with hepatic steatosis. Even regardless of weight loss, physical activity improves disease progression rates. Increasing the intensity of exercise leads to a gradual increase in the beneficial effect in the treatment of NAFLD. The effect of strength training is less studied, but preliminary results show that it is highly effective in patients with NAFLD, even in the absence of significant weight loss.

As with many therapies, there is no single approach that would apply to every patient. At this stage of understanding the problem, there is a need for strategies focused on patient's individual characteristics. Regularity and consistency are the most important characteristics of any NAFLD management program.

Previous studies have investigated adherence to in patients with NAFLD treatment in general, while it is fundamentally important to study the nature of patients' adherence to lifestyle modifications in the short and long term, since eating behavior and physical activity are currently the only effective treatments for this pathology. Until recently, there have been no tools to assess adherence to lifestyle modification. However, at present, the Russian questionnaire for quantitative assessment of adherence to treatment "QAA-25" makes it possible to determine both adherence to therapy in general and adherence by individual key components: adherence to drug therapy, medical counseling, and lifestyle modification, which undoubtedly takes into account treatment strategies in patients with NAFLD.

REFERENCES

1. Byrne C.D., Targher G. NAFLD: A multisystem disease. *Journal of Hepatology.* 2015; 61 (1 Suppl.): 47–64. DOI: 10.1016/j.jhep.2014.12.012.
2. Livzan M.A., Lapteva I.V., Miller T.S. The role of leptin and leptin resistance in the formation of non-alcoholic fatty liver disease in obese and overweight individuals. *Experimental and Clinical Gastroenterology.*2014; 108 (8): 27–33 (in Russ.).
3. Rodriguez B., Torres D.M., Harrison S.A. Physical activity: An essential component of lifestyle modification in NAFLD. *Nature Reviews Gastroenterology and Hepatology*. 2012; 9 (12): 726–731. DOI: 10.1038/nrgastro.2012.200.
4. Bedogni G., Nobili V., Tiribelli C. Epidemiology of fatty liver: An update. *World Journal of Gastroenterology.* 2014; 20 (27): 9050–9054. DOI: 10.3748/wjg.v20.i27.9050.
5. Pappachan J.M., Babu S., Krishnan B., Ravindran N.C. Non-alcoholic fatty liver disease: a clinical update. *J. Clin. Transl. Hepatol.* 2017; 5 (4): 384–393. DOI: 10.14218/jcth.2017.00013.
6. Keating S.E., Hackett D.A., George J., Johnson N.A. Exercise and non-alcoholic fatty liver disease: A systematic review and meta-analysis. *J. Hepatol.* 2012; 57 (1): 157–166. DOI: 10.1016/j.jhep.2012.02.023.
7. LabrecqueD.R.,AbbasZ.,AnaniaF.,FerenciP.,KhanA.G., Goh K.L., Hamid S.S., Isakov V., Lizarzabal M., Peñaranda M.M., Ramos J.F.R., Sarin S., Stimac D., Thomson A.B.R., Umar M., Krabshuis J., Lemair A. World gastroenterology organisation global guidelines: Nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. *J. Clin. Gastroenterol.* 2014; 48 (6): 467–473. DOI: 10.1097/MCG.0000000000000116.
8. Lee Y., Doumouras A.G., Yu J., Brar K., Banfield L., Gmora S., Anvari M., Hong D. Complete Resolution of Nonalcoholic Fatty Liver Disease After Bariatric Surgery: A Systematic Review and Meta-analysis. *Clin. Gastroenterol. Hepatol.* 2019; 17 (6): 1040–1060. DOI: 10.1016/j.cgh.2018.10.017.
9. Li Y., Liu L., Wang B., Wang J., Chen D. Metformin in non-alcoholic fatty liver disease: A systematic review and meta-analysis. *Biomed. Reports.* 2013; 1 (1): 57–64. DOI: 10.3892/br.2012.18.
10. Armstrong M.J., Gaunt P., Aithal G.P., Barton D., Hull D., Parker R., Hazlehurst J.M., Guo K., Abouda G., Aldersley M.A., Stocken D., Gough S.C., Tomlinson J.W., Brown R.M., Hübscher S.G., Newsome P.N. Liraglutide safety and efficacy in patients with non-alcoholic steatohepatitis (LEAN): A multicentre, double-blind, randomised, placebo-controlled phase 2 study. *Lancet.* 2016; 387 (10019): 679–690. DOI: 10.1016/S0140-6736(15)00803-X.
11. Rakoski M.O., Singal A.G., Rogers M.A.M., Conjeevaram H. Meta-analysis: Insulin sensitizers for the treatment of non-alcoholic steatohepatitis. *Aliment Pharmacol. Ther.* 2010; 32 (10): 1211–1221. DOI: 10.1111/j.1365-2036.2010.04467.x.
12. Boettcher E., Csako G., Pucino F., Wesley R., Loomba R. Meta-analysis: Pioglitazone improves liver histology and fibrosis in patients with non-alcoholic steatohepatitis. *Aliment Pharmacol. Ther.* 2012; 35 (1): 66–75. DOI: 10.1111/j.1365-2036.2011.04912.x.
13. Mazidi M., Kengne A.P. Higher adherence to plant-based diets are associated with lower likelihood of fatty liver. *Clin. Nutr.* 2019; 38 (4): 1672–1677. DOI: 10.1016/j.clnu.2018.08.010.
14. Khalatbari-Soltani S.. Imamura F., Brage S., De Lucia Rolfe E., Griffin S.J., Wareham N.J., Marques-Vidal P., Forouhi N.G. The association between adherence to the Mediterranean diet and hepatic steatosis: Cross-sectional analysis of two independent studies, the UK Fenland Study and the Swiss CoLaus Study. *BMC Med.* 2019; 17 (1): 19. DOI: 10.1186/s12916-019-1251-7.
15. Drapkina O.M., Livzan M.A., Martynov A.I., Moiseev S.V., Nikolaev N.A., Skirdenko Yu.P. on behalf of the Working Group and the Committee of co-authors: Bunova S.S., Viktorova I.A., Drapkina O.M., Zadionchenko V.S., Lazebnik L.B., Livzan M.A., Malyavin A.G., Martynov A.I., Moiseev S.V., Nelidova A.V., Nechaeva G.I., Nikolaev N.A., Pushkareva L.A., Rebrov A.P., Saifutdinov R.I., Skirdenko Yu.P., Sovalkin V.I., Terentiev V.P., Tyurin V.P., Urazov V.P., Fedoseev G.B., Yagoda A.V., Yakushin S.S., Yasnitsky L.N. The first Russian expert consensus on the quantitative evaluation of the treatment adherence: pivotal issues, algorithms and recommendations. *Medical News of the North Caucasus.* 2018; 13 (1.2): 259–271 (in Russ.). DOI: 10.14300/mnnc.2018.13039.
16. Marchesini G., Day C.P., Dufour J.F., Canbay A., Nobili V., Ratziu V., Tilg H., Roden M., Gastaldelli A., Yki-Jarvinen H., Schick F., Vettor R., Fruhbeck G., Mathus-Vliegen L. EASL-EASD-easo clinical practice guidelines for the management of non-alcoholic fatty liver disease. *J. Hepatol.* 2016; 64 (6): 1388–1402. DOI: 10.1016/j.jhep.2015.11.004.
17. Asrih M., Jornayvaz F.R. Diets and nonalcoholic fatty liver disease: The good and the bad. *Clinical Nutrition.* 2014; 33 (2): 186–190. DOI: 10.1016/j.clnu.2013.11.003.
18. Drapkina O.M., Korneeva O.N., Ivashkin V.T. Impact on the parameters of abdominal obesity in patients with metabolic syndrome: focus on adherence to dietary recommendations. *Lechaschi Vrach Journal.* 2010; 7: 29–34 (in Russ.).
19. Palmer M., Schaffner F. Effect of weight reduction on hepatic abnormalities in overweight patients. *Gastroenterology.* 1990; 99 (5): 1408–1413. DOI: 10.1016/0016-5085(90)91169-7.
20. Zelber-Sagi S., Kessler A., Brazowsky E., Webb M., Lurie Y., Santo M., Leshno M., Blendis L., Halpern Z., Oren R. A Double-Blind Randomized Place-

bo-Controlled Trial of Orlistat for the Treatment of Nonalcoholic Fatty Liver Disease. C*lin. Gastroenterol. Hepatol.* 2006; 4 (5): 639–644. DOI: 10.1016/j.cgh.2006.02.004.
21. Thoma C., Day C.P., Trenell M.I. Lifestyle interventions for the treatment of non-alcoholic fatty liver disease in adults: A systematic review. *Journal of Hepatology*. 2012; 56 (1): 255–266. DOI: 10.1016/j.jhep.2011.06.010.
22. Rodriguez-Ramiro I., Vauzour D., Minihane A.M. Polyphenols and non-alcoholic fatty liver disease: Impact and mechanisms. *Proceedings of the Nutrition Society.* 2016; 75 (1): 47–60. DOI: 10.1017/S0029665115004218.
23. Musso G., Gambino R., De Michieli F., Cassader M., Rizzetto M., Durazzo M., Fagà E., Silli B., Pagano G. Dietary habits and their relations to insulin resistance and postprandial lipemia in nonalcoholic steatohepatitis. *Hepatology.* 2003; 37 (4): 909–916. DOI: 10.1053/jhep.2003.50132.
24. Kang H., Greenson J.K., Omo J.T., Chao C., Peterman D., Anderson L., Foess-Wood L., Sherbondy M.A., Conjeevaram H.S. Metabolic syndrome is associated with greater histologic severity, higher carbohydrate, and lower fat diet in patients with NAFLD. *Am. J. Gastroenterol.* 2006; 101 (10): 2247–2253. DOI: 10.1111/j.1572-0241.2006.00719.x.
25. Krolevets T.S., Livzan M.A., Kolbina M.V. Matrix metalloproteinases and their tissue inhibitors in patients with nonalcoholic fatty liver disease. J. *Pharm. Pharmacol.* 2016; 4 (12). DOI: 10.17265/2328-2150/2016.12.008.
26. Efremenko E.S., Titov D.S., Nikonov D.A., Sidorov G.G., Andreev K.A. Glutathione-dependent antioxidant defense mechanisms in alcoholism. *International Journal of Applied and Fundamental Research.* 2019; 4: 105–108 (in Russ.).
27. Eilat-Adar S., Sinai T., Yosefy C., Henkin Y. Nutritional recommendations for cardiovascular disease prevention. Nut*rients.* 2013; 5 (9): 3646–3683. DOI: 10.3390/nu5093646.
28. 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.
29. Koch M., Borggrefe J., Barbaresko J., Groth G., Jacobs G., Siegert S., Lieb W., Müller M.J., Bosy-Westphal A., Heller M., N̈othlings U. Dietary patterns associated with magnetic resonance imaging-determined liver fat content in a general population study 1–3. *Am. J. Clin. Nutr.* 2014; 99 (2): 369–377. DOI: 10.3945/ajcn.113.070219.
30. Yang C.Q., Shu L., Wang S., Wang J.J., Zhou Y., Xuan Y.J., Wang S.F. Dietary patterns modulate the risk of non-alcoholic fatty liver disease in Chinese adults. Nut*rients.* 2015; 7 (6): 4778–4791. DOI: 10.3390/nu7064778.
31. Cicero A.F.G., Colletti A., Bajraktari G., Descamps O., Djuric D.M., Ezhov M., Fras Z., Katsiki N., Langlois M., Latkovskis G., Panagiotakos D.B., Paragh G., Mikhailidis D.P., Mitchenko O., Paulweber B., Pella D., Pitsavos C., Reiner Ž., Ray K.K., Rizzo M., Sahebkar A., Serban M.C., Sperling L.S., Toth P.P., Vinereanu D., Vrablík M., Wong N.D., Banach M. Lipid-lowering nutraceuticals in clinical practice: position paper from an international lipid expert panel. *Nutr. Rev.* 2017; 75 (9): 731–767. DOI: 10.1093/nutrit/nux047.
32. Martínez-González M.A., Sánchez-Tainta A., Corella D., Salas-SalvadóJ.,RosE.,ArósF.,Gómez-GraciaE.,FiolM., Lamuela-Raventós R.M., Schröder H., Lapetra J., Serra-Majem L., Pinto X., Ruiz-Gutierrez V., Estruch R. A provegetarian food pattern and reduction in total mortality in the Prevención con Dieta Mediterránea (PREDIMED) study. *American Journal of Clinical Nutrition.* 2014; 100 (Suppl. 1): 320–328. DOI: 10.3945/ajcn.113.071431.
33. Fraser G.E. Vegetarian diets: What do we know of their effects on common chronic diseases? A*merican Journal of Clinical Nutrition.* 2009; 89 (5): 1607–1612. DOI: 10.3945/ajcn.2009.26736K.
34. Chiu T.H., Lin M.N., Pan W.H., Chen Y.C., Lin C.L. Vegetarian diet, food substitution, and nonalcoholic fatty liver. Tzu. *Chi. Med. J.* 2018; 30 (2): 102–109. DOI: 10.4103/tcmj.tcmj_109_17.
35. Choi S.H., Oh D.J., Kwon K.H., Lee J.K., Koh M.S., Lee J.H., Kang H.W. A vegetarian diet does not protect against nonalcoholic fatty liver disease (NAFLD): A cross-sectional study between Buddhist priests and the general population. *Turkish J. Gastroenterol.* 2015; 26 (4): 336–343. DOI: 10.5152/tjg.2015.0046.
36. Alferink L.J.M., Kiefte-De Jong J.C., Erler N.S., Veldt B.J., Schoufour J.D., De Knegt R.J., Ikram M.A., Metselaar H.J., Janssen H.L.A., Franco O.H., Darwish M.S. Association of dietary macronutrient composition and non-alcoholic fatty liver disease in an ageing population: The Rotterdam Study. *Gut.* 2019; 68 (6): 1088–1098. DOI: 10.1136/gutjnl-2017-315940.
37. Anania C., Massimo Perla F., Olivero F., Pacifico L., Chiesa C. Mediterranean diet and nonalcoholic fatty liver disease. *World Journal of Gastroenterology*. 2018; 24 (19): 2083–2094. DOI: 10.3748/wjg.v24.i19.2083.
38. Serra-Majem L., Ortiz-Andrellucchi A., Sánchez-Villegas A. Mediterranean diet. Encyclopedia of Food Security and Sustainability. 2018. DOI: 10.1016/B978-0-08-100596-5.22054-4.
39. Papamiltiadous E.S., Roberts S.K., Nicoll A.J., Ryan M.C., Itsiopoulos C., Salim A., Tierney A.C. A randomised controlled trial of a Mediterranean Dietary Intervention for Adults with Non Alcoholic Fatty Liver Disease (MEDINA): Study protocol. *BMC Gastroenterol.* 2016; 16: 14. DOI: 10.1186/s12876-016-0426-3.

40. Teixeira J.S., Campos A.B.F., Cordeiro A., Pereira S.E., Saboya C.J., Ramalho A. Vitamin D nutritional status and its relationship with metabolic changes in adolescents and adults with severe obesity. *Nutr. Hosp.* 2018; 35 (4): 847–853. DOI: 10.20960/nh.1657.
41. Cordeiro A., Pereira S., Saboya C.J., Ramalho A. Relationship between nonalcoholic fatty liver disease and vitamin D nutritional status in extreme obesity. *Can. J. Gastroenterol. Hepatol.* 2017; 2017: 9456897. DOI: 10.1155/2017/9456897.
42. Villaça Chaves G., Pereira S.E., Saboya C.J., Ramalho A. Non-alcoholic fatty liver disease and its relationship with the nutritional status of vitamin A in individuals with class III obesity. *Obes. Surg.* 2008; 18 (4): 78–85. DOI: 10.1007/s11695-007-9361-2.
43. Moscatiello S., Di Luzio R., Bugianesi E., Suppini A., Hickman I.J., Di Domizio S., Dalle Grave R., Marchesini G. Cognitive-behavioral treatment of nonalcoholic fatty liver disease: A propensity score-adjusted observational study. *Obesity.* 2011; 19 (4): 763–770. DOI: 10.1038/oby.2010.254.
44. Greaves C.J., Sheppard K.E., Abraham C., Hardeman W., Roden M., Evans P.H., Schwarz P. Systematic review of reviews of intervention components associated with increased effectiveness in dietary and physical activity interventions. B*MC Public Health.* 2011; 11: 119. DOI: 10.1186/1471-2458-11-119.
45. Kistler K.D., Brunt E.M., Clark J.M., Diehl A.M., Sallis J.F., Schwimmer J.B. Physical activity recommendations, exercise intensity, and histological severity of nonalcoholic fatty liver disease. *Am. J. Gastroenterol.* 2011; 106 (3): 460–468. DOI: 10.1038/ajg.2010.488.
46. Zelber-Sagi S., Nitzan-Kaluski D., Goldsmith R., Webb M., Zvibel I., Goldiner I., Blendis L., Halpern Z., Oren R. Role of leisure-time physical activity in nonalcoholic fatty liver disease: A population-based study. *Hepatology.* 2008; 48 (6): 1791–1798. DOI: 10.1002/hep.22525.
47. Anderson E.L., Howe L.D., Jones H.E., Higgins J.P.T., Lawlor D.A., Fraser A. The prevalence of non-alcoholic fatty liver disease in children and adolescents: A systematic review and meta-analysis. *PLoS One.* 2015; 10 (10): e0140908. DOI: 10.1371/journal.pone.0140908.
48. Lavine J.E., Schwimmer J.B., Van Natta M.L., Molleston J.P., Murray K.F., Rosenthal P., Abrams S.H., Scheimann A.O., Sanyal A.J., Chalasani N., Tonascia J., Ünalp A., Clark J.M., Brunt E.M., Kleiner D.E., Hoofnagle J.H., Robuck P.R. Effect of Vitamin E or metformin for treatment of nonalcoholic fatty liver disease in children and adolescents the tonic randomized controlled trial. JA*MA.* 2011; 305 (16): 1659–1668. DOI: 10.1001/jama.2011.520.
49. Younossi Z., Tacke F., Arrese M., Chander Sharma B., Mostafa I., Bugianesi E., Wai-Sun Wong V., Yilmaz Y., George J., Fan J., Vos M.B. Global perspectives on nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. *Hepatology.* 2019; 69 (6): 2672–2682. DOI: 10.1002/hep.30251.
50. Hattar L.N., Wilson T.A., Tabotabo L.A., Smith E.O.B., Abrams S.H. Physical activity and nutrition attitudes in obese Hispanic children with non-alcoholic steatohepatitis. *World J. Gastroenterol.* 2011; 17 (39): 4396–4403. DOI: 10.3748/wjg.v17.i39.4396.
51. Kwak M.S., Kim D., Chung G.E., Kim W., Kim Y.J., Yoon J.H. Role of physical activity in nonalcoholic fatty liver disease in terms of visceral obesity and insulin resistance. *Liver Int.* 2015; 35 (3): 944–952. DOI: 10.1111/liv.12552.
52. Golabi P., Otgonsuren M., Cable R., Felix S., Koenig A., Sayiner M., Younossi Z.M. Non-alcoholic fatty liver disease (NAFLD) is associated with impairment of Health Related Quality of Life (HRQOL). *Health Qual Life Outcomes*. 2016; 14: 18. DOI: 10.1186/s12955-016-0420-z.
53. Gaus O.V., Akhmedov V.A. Affect of metabolic syndrome on the state of the liver parenchyma and biliary system in patients with cholelithiasis. *Ural Medical Journal*. 2015; 1 (124): 132–137 (in Russ.).
54. Chalasani N., Younossi Z., Lavine J.E., Diehl A.M., Brunt E.M., Cusi K., Charlton M., Sanyal A.J. The diagnosis and management of non-alcoholic fatty liver disease: Practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. *Hepatology.* 2012; 55 (6): 2005–2023. DOI: 10.1002/hep.25762.
55. Katsagoni C.N., Georgoulis M., Papatheodoridis G.V., Panagiotakos D.B., Kontogianni M.D. Effects of lifestyle interventions on clinical characteristics of patients with non-alcoholic fatty liver disease: A meta-analysis. *Metabolism.* 2017; 68: 119–132. DOI: 10.1016/j.metabol.2016.12.006.
56. Medrano M., Cadenas-Sanchez C., Álvarez-Bueno C., Cavero-Redondo I., Ruiz J.R., Ortega F.B., Labayen I. Evidence-Based Exercise Recommendations to Reduce Hepatic Fat Content in Youth- a Systematic Review and Meta-Analysis. *Progress in Cardiovascular Diseases.* 2018; 61 (2): 222–231. DOI: 10.1016/j.pcad.2018.01.013.
57. Hashida R., Kawaguchi T., Bekki M., Omoto M., Matsuse H., Nago T., Takano Y., Ueno T., Koga H., George J., Shiba N., Torimura T. Aerobic vs. resistance exercise in non-alcoholic fatty liver disease: A systematic review. *J. Hepatol.* 2017; 66 (1): 142–152. DOI: 10.1016/j.jhep.2016.08.023.
58. Golabi P., Locklear C.T., Austin P., Afdhal S., Byrns M., Gerber L., Younossi Z.M. Effectiveness of exercise in hepatic fat mobilization in nonalcoholic fatty liver disease: Systematic review. *World Journal of Gastroenterology.* 2016; 22 (27): 6318–6327. DOI: 10.3748/wjg.v22.i27.6318.
59. Kantartzis K., Thamer C., Peter A., Machann J., Schick F., Schraml C., Königsrainer A., Königsrainer I., Kröber S.,

Niess A., Fritsche A., Häring H.U., Stefan N. High cardiorespiratory fitness is an independent predictor of the reduction in liver fat during a lifestyle intervention in non-alcoholic fatty liver disease. *Gut.* 2009; 58 (9): 1281–1288. DOI: 10.1136/gut.2008.151977.

60. Liu W.Y., Lu D.J., Du X.M., Sun J.Q., Ge J., Wang R.W., Wang R., Zou J., Xu C., Ren J., Wen X.F., Liu Y., Cheng S.M., Tan X., Pekkala S., Munukka E., Wiklund P., Chen Y.Q., Gu Q., Xia Z.C., Liu J.J., Liu W.B., Chen X.B., Zhang Y.M., Li R., Borra R.J.H., Yao J.X., Chen P.J., Cheng S. Effect of aerobic exercise and low carbohydrate diet on pre-diabetic non-alcoholic fatty liver disease in postmenopausal women and middle aged men – The role of gut microbiota composition: Study protocol for the AELC randomized controlled trial. *BMC Public Health*. 2014; 14: 48. DOI: 10.1186/1471-2458-14-48.
61. Kawaguchi T., Shiba N., Maeda T., Matsugaki T., Takano Y., Itou M., Sakata M., Taniguchi E., Nagata K., Sata M. Hybrid training of voluntary and electrical muscle contractions reduces steatosis, insulin resistance, and IL-6 levels in patients with NAFLD: A pilot study. *J. Gastroenterol.* 2011; 46 (6): 746–757. DOI: 10.1007/s00535-011-0378-x.
62. Oh S., Maruyama T., Eguchi K., Shida T., Arai E., Isobe T., Okamoto Y., Shoda J. Therapeutic effect of hybrid training of voluntary and electrical muscle contractions in middle-aged obese women with nonalcoholic fatty liver disease: A pilot trial. *Ther. Clin. Risk Manag.* 2015; 11: 371–380. DOI: 10.2147/TCRM.S75109.
63. Hallsworth K., Fattakhova G., Hollingsworth K.G., Thoma C., Moore S., Taylor R., Day C.P., Trenell M.I. Resistance exercise reduces liver fat and its mediators in non-alcoholic fatty liver disease independent of weight loss. *Gut.* 2011; 60 (9): 1278–1283. DOI: 10.1136/gut.2011.242073.
64. Strasser B., Siebert U., Schobersberger W. Resistance training in the treatment of the metabolic syndrome: A systematic review and meta-analysis of the effect of resistance training on metabolic clustering in patients with abnormal glucose metabolism. *Sports Medicine*. 2010; 40 (5): 397–415. DOI: 10.2165/11531380-000000000-00000.
65. Strasser B., Arvandi M., Siebert U. Resistance training, visceral obesity and inflammatory response: A review of the evidence. *Obesity Reviews.* 2012; 13 (7): 578–591. DOI: 10.1111/j.1467-789X.2012.00988.x.
66. Baba C.S., Alexander G., Kalyani B., Pandey R., Rastogi S., Pandey A., Choudhuri G. Effect of exercise and dietary modification on serum aminotransferase levels in patients with nonalcoholic steatohepatitis. *J. Gastroenterol. Hepatol*. 2006; 21 (1 Pt 1): 191–198. DOI: 10.1111/j.1440-1746.2005.04233.x.
67. Lazo M., Solga S.F., Horska A., Bonekamp S., Diehl A.M., Brancati F.L., Wagenknecht L.E., Pi-Sunyer F.X., Kahn S.E., Clark J.M. Effect of a 12-month intensive lifestyle intervention on hepatic steatosis in adults with type 2 diabetes. *Diabetes Care*. 2010; 33 (10): 2156–2163. DOI: 10.2337/dc10-0856.
68. Promrat K., Kleiner D.E., Niemeier H.M., Jackvony E., Kearns M., Wands J.R., Fava J.L., Wing R.R. Randomized controlled trial testing the effects of weight loss on nonalcoholic steatohepatitis. *Hepatology*. 2010; 51 (1): 121–129. DOI: 10.1002/hep.23276.
69. Chalasani N., Younossi Z., Lavine J.E., Charlton M., Cusi K., Rinella M., Harrison S.A., Brunt E.M., Sanyal A.J. The diagnosis and management of nonalcoholic fatty liver disease: Practice guidance from the American Association for the Study of Liver Diseases. *Hepatology.* 2018; 67 (1): 328–357. DOI: 10.1002/hep.2936.7.
70. Morisky D.E., Green L.W., Levine D.M. Concurrent and predictive validity of a self-reported measure of medication adherence. *Med. Care.* 1986; 24 (1): 67–74. DOI: 10.1097/00005650-198601000-00007.
71. Tan X., Patel I., Chang J. Review of the four item Morisky Medication Adherence Scale (MMAS-4) and eight item Morisky Medication Adherence Scale (MMAS-8). *Inov. Pharm.* 2014; 5 (3): 1–8. DOI: 10.24926/iip.v5i3.347.
72. Morisky D., Ang A., Krousel-Wood M., Ward H.J. Predictive validity of a medication adherence measure in an outpatient setting. *J. Clin. Hypertens.* 2008; 10 (5): 348–354. DOI: 10.1111/j.1751-7176.2008.07572.x.
73. Nikolaev N.A., Martynov A.I., Drapkina O.M., Livzan M.A., Moiseev S.V., Skirdenko Yu.P., Bunova S.S., Viktorova I.A., Zadionchenko V. S., Lazebnik L.B., Malyavin A.G., Nelidova A.V., Nechaeva G.I., Pushkareva L.A., Rebrov A.P., Saifutdinov R.I., Sovalkin V.I. ., Terent'ev V.P., Tyurin V.P., Urazov V.P., Fedoseev G.B., Yagoda A.V., Yakushin S.S., Yasnitsky L.N. The first Russian expert consensus on the quantitative evaluation of the treatment adherence (approved by the XII National Congress of Physicians. Moscow, November 22–24, 2017). *Therapy*. 2018; 5: 11–32 (in Russ.). DOI: 10.18565/therapy.2018.5.11-32.
74. Nikolaev N.A., Skirdenko Yu.P. Russian generic questionnaire for evaluation of compliance to drug therapy (QAA-25). *Clinical Pharmacology and Therapy.* 2018; 27 (1): 74–78 (in Russ.).
75. Trichopoulou A., Costacou T., Bamia C., Trichopoulos D. Adherence to a Mediterranean diet and survival in a Greek population. *N. Engl. J. Med.* 2003; 348: 2599–2608. DOI: 10.1056/NEJMoa025039.
76. Panagiotakos D.B., Pitsavos C., Stefanadis C. Dietary patterns: A Mediterranean diet score and its relation to clinical and biological markers of cardiovascular disease risk. *Nutr. Metab. Cardiovasc. Dis.* 2006; 16 (8): 559–568. DOI: 10.1016/j.numecd.2005.08.006.
77. Tur J.A., Romaguera D., Pons A. The Diet Quality Index-International (DQI-I): is it a useful tool to evaluate the quality of the Mediterranean diet? *Br. J. Nutr.* 2005; 93 (3): 369–376. DOI: 10.1079/bjn20041363.

78. Kim S., Haines P.S., Siega-Riz A.M., Popkin B.M. The Diet Quality Index-International (DQI-I) provides an effective tool for cross-national comparison of diet quality as illustrated by China and the United States. *J. Nutr.* 2003; 133 (11): 3476–3484. DOI: 10.1093/jn/133.11.3476.
79. Mathurin P., Hollebecque A., Arnalsteen L., Buob D., Leteurtre E., Caiazzo R., Pigeyre M., Verkindt H., Dharancy S., Louvet A., Romon M., Pattou F. Prospective Study of the Long-Term Effects of Bariatric Surgery on Liver Injury in Patients Without Advanced Disease. *Gastroenterology.* 2009; 137 (2): 532–540. DOI: 10.1053/j.gastro.2009.04.052.
80. Kral J.G., Thung S.N., Biron S., Hould F.S., Lebel S., Marceau S., Simard S., Marceau P. Effects of surgical treatment of the metabolic syndrome on liver fibrosis and cirrhosis. *Surgery.* 2004; 135 (1): 48–58. DOI: 10.1016/j.surg.2003.10.003.

Authors contribution

Skirdenko Yu.P., Nikolaev N.A. – conception and design. Andreev K.A., Gorbenko A.V., Fedorin M.M. – analysis and interpretation of data. Livzan M.A., Krolevets T.S. – substantiation of the manuscript or critical revision of the manuscript for important intellectual content. All authors – 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.

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.

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.

Fedorin Maksim M., Inspector, Department for the Development of Regional Healthcare and Medical Activity, Omsk State Medical University, Omsk, Russian Federation. ORCID 0000-0002-0238-4664.

Krovelets Tatyana S., Cand. Sci. (Med.), Assistant, Department of Internal Medicine and Gastroenterology, Omsk State Medical University, Omsk, Russian Federation. ORCID 0000-0002-7452-7230.

(✉) **Andreev Kirill A.,** e-mail: kivi2104@gmail.com

Received 17.08.2020
Accepted 28.12.2020

УДК 616-008.9-06:616.62-003.7]-055.1/2
https://doi.org/10.20538/1682-0363-2021-4-123-130

Gender aspects of urolithiasis development in patients with metabolic syndrome

Bespalova I.D.[1], Boshchenko V.S.[1], Koshchavtseva Yu.I.[1], Tsoy A.V.[2], Teteneva A.V.[1], Mesko P.E.[1], Karzilov A.I.[1], Porovskiy Ya.V.[1], Mishustina E.L.[1], Tetenev K.F.[1], Kalyuzhina E.V.[1], Kalyuzhin V.V.[1]

[1] Siberian State Medical University (SSMU)
2, Moscow Trakt, Tomsk, 634050, Russian Federation

[2] St. Luke's Clinical Hospital
46, Chugunnaya Str., St. Petersburg, 194044, Russian Federation

ABSTRACT

The review summarizes and analyzes the results of domestic and major foreign studies of recent years concerning gender characteristics of the epidemiology and development mechanisms of metabolic syndrome and urolithiasis as an associated disease. A deep understanding of gender aspects in the pathogenesis of these pathologies can form the basis for development of high-quality diagnostic algorithms and pathogenetically grounded approaches to treatment.

Key words: metabolic syndrome, urolithiasis, gender characteristics.

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: Bespalova I.D., Boshchenko V.S., Koshchavtseva Yu.I., Tsoy A.V., Teteneva A.V., Mesko P.E., Karzilov A.I., Porovskiy Ya.V., Mishustina E.L., Tetenev K.F., Kalyuzhina E.V., Kalyuzhin V.V. Gender aspects of urolithiasis development in patients with metabolic syndrome. *Bulletin of Siberian Medicine.* 2021; 20 (4): 123–130. https://doi.org/10.20538/1682-0363-2021-4-123-130.

Гендерные аспекты развития уролитиаза у пациентов с метаболическим синдромом

Беспалова И.Д.[1], Бощенко В.С.[1], Кощавцева Ю.И.[1], Цой А.В.[2], Тетенева А.В.[1], Месько П.Е.[1], Карзилов А.И.[1], Поровский Я.В.[1], Мишустина Е.Л.[1], Тетенев К.Ф.[1], Калюжина Е.В.[1], Калюжин В.В.[1]

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

[2] Клиническая больница Святителя Луки
Россия, 194044, г. Санкт-Петербург, ул. Чугунная, 46

✉ *Bespalova Inna D.,* e-mail: innadave@mail2000.ru

РЕЗЮМЕ

В обзоре обобщены и проанализированы результаты отечественных и крупных зарубежных исследований последних лет, касающиеся гендерных особенностей эпидемиологии и механизмов развития метаболического синдрома и мочекаменной болезни как ассоциированного с ним заболевания. Глубокое понимание гендерных аспектов в патогенезе данных патологических процессов может лечь в основу разработки качественных диагностических алгоритмов и патогенетически обоснованных подходов к лечению.

Ключевые слова: метаболический синдром, уролитиаз, гендерные особенности.

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: Беспалова И.Д., Бощенко В.С., Кощавцева Ю.И., Цой А.В., Тетенева А.В., Месько П.Е., Карзилов А.И., Поровский Я.В., Мишустина Е.Л., Тетенев К.Ф., Калюжина Е.В., Калюжин В.В. Гендерные аспекты развития уролитиаза у пациентов с метаболическим синдромом. *Бюллетень сибирской медицины.* 2021; 20 (4): 123–130. https://doi.org/10.20538/1682-0363-2021-4-123-130.

INTRODUCTION

For four decades, the interest of many clinicians and researchers in the problem of metabolic syndrome (MS) has continued to grow. First of all, this is determined by the growth of its social and medical importance associated with its high prevalence and negative consequences. The results of the latest epidemiological studies on all continents of the world make it possible to establish a global trend towards an increase in the spread of MS in the adult world population, a significant part of which are people of working age.

The concept of MS, which united the interests of therapists, cardiologists, and endocrinologists at the end of the last century, is to recognize the existence of a cluster of factors that have a common pathogenetic basis. This symptom complex, according to most researchers, is a combination of risk factors for the development, severe course, and complications of a large number of socially sensitive diseases, which currently determine the structure of morbidity, disability, and mortality among the population. MS and its components (abdominal obesity, arterial hypertension, impaired lipid, carbohydrate, and purine metabolism, and low-grade inflammation) are known to negatively affect the quality of life in patients, which further increases its social significance [1, 2]. The list of diseases and pathological conditions, the development of which is based on modifiable factors united under the concept of MS, continues to grow. Among the pathological conditions associated with MS, in addition to diseases of the cardiovascular system and type 2 diabetes mellitus (DM), urolithiasis should be also mentioned.

Urolithiasis is considered one of the most common chronic diseases. According to a number of recent epidemiological studies, its prevalence varies from 3.5 to 9.6% [3–6] and accounts for about 40% of all diseases of the urinary tract. At the same time, attention is paid to significant differences in this parameter not only in different countries, but also in different social groups. There is a persistent trend towards an increase in the incidence of urolithiasis among the population, which does not depend on age, gender, and race [5]. The social significance of this disease is determined not only by its big share in the structure of morbidity, but also by its propensity for a recurrent course and high frequency of emergency conditions and disability, while the existing methods for removing calculi do not save patients from recurrent urolithiasis [7, 8].

There is still no single point of view on the pathogenesis of urolithiasis. The development of this disease is explained by complex physicochemical processes occurring both in the body as a whole and directly in the urinary tract and kidneys. Urolithiasis is currently considered a multifactorial pathological process resulting from disorders of urodynamics and genetic, hormonal, and metabolic disorders [9]. In the etiology of urolithiasis, the so-called non-modifiable factors, such as gender, ethnic and genetic characteristics, as well as geographic location, play an essential role [6]. However, it is the increasing role of modifiable risk factors in the increase in the urolithiasis incidence that

currently explains the great interest of researchers in studying the influence of such factors as obesity, DM, and MS on the formation of kidney stones to improve the quality of diagnosis and develop effective methods of drug and non-drug correction [10].

For the first time, data on the relationship between urolithiasis and MS were published in 2008 by American researchers, who demonstrated a close correlation between the severity of MS symptoms and cases of urolithiasis. At the same time, the simultaneous presence of four or more components of MS, diagnosed according to the National Cholesterol Education Program (NCEP) criteria, almost doubled the risk of developing urolithiasis [11].

Later, the results of a large-scale study conducted in South Korea at the Asan Medical Center were presented. 34,895 people were examined: they underwent general screening tests, which included anthropometry, blood and urine tests, chest X-ray, respiratory function test, and ECG. The presence of MS was also determined according to the NCEP criteria. The presence of kidney stones was assessed using computed tomography or ultrasound.

Of all the examined individuals, kidney stones were detected in 839 (2.4%) people, MS was diagnosed in 4,779 (13.7%) people. The likelihood of calculi formation grew with an increase in the waist circumference quintile and the level of systolic / diastolic blood pressure ($p < 0.001$). Age, gender, arterial hypertension, and metabolic status were independent risk factors for urolithiasis. The presence of MS had a probability coefficient of 1.25 (95% confidence interval (CI), 1.03–1.50) for the formation of urolithiasis. In subjects with arterial hypertension, the probability coefficient for the presence of kidney stones was 1.47 (95% CI, 1.25–1.71) compared with that for people without arterial hypertension after adjustment for other variables [12].

In the Iranian population, the prevalence of urolithiasis was 14.53%, while patients with this disease were significantly younger and had higher body mass index and blood uric acid levels compared with the average values in the general population [13]. Chinese researchers found that in the presence of urolithiasis, MS was detected 1.74 times more often. A statistically significant relationship was found between these pathological processes, the strength of which increased with a rise in the number of MS components [14].

Currently, contribution of the following factors to the pathogenesis of urolithiasis in MS has been proven: insulin resistance, hyperuricemia, and high levels of free fatty acids. Hyperuricemia is quite common in the clinical practice of a doctor. An asymptomatic increase in the concentration of uric acid in the blood is observed in 5–8% of the population. A close relationship between the level of this parameter in the blood and MS components allowed to consider hyperuricemia one of the manifestations of this symptom complex [15, 16]. Much more often in patients with MS, calculi originating from uric acid salts are found. In individuals with type 2 diabetes, the incidence of uric acid nephrolithiasis is 6 times higher than in patients from the general population [17].

One of the key mechanisms of the uric acid stone formation, along with a decrease in urine output and hyperuricosuria, is the acidic reaction of urine, which is a consequence of insulin resistance. Insulin resistance reduces production and transport of ammonium, which leads to a change in urine pH towards oxidation. Free fatty acids (FFA) are released from adipose tissue in visceral obesity, which contributes to an increase in production of glucose, triglycerides, very low-density lipoproteins (VLDL) and low-density lipoproteins (LDL) and a decrease in production of high-density lipoproteins (HDL) in the liver. It underlies the development of insulin resistance and characterizes dyslipidemia.

Calcium oxalate urolithiasis is the most common type of urolithiasis [17], and its pathogenetic relationship with MS is very complex. The role of MS as a risk factor for calcium oxalate stone formation is often considered insignificant. The pathogenesis of calcium oxalate nephrolithiasis includes increased excretion of lithogenesis predictors, such as oxalates, calcium, uric acid, etc., against the background of a decrease in the excretion of citrates, which act as inhibitors of kidney stone formation. It is followed by a decrease in urine acidity, increased formation of Randall's plaques, and inflammation in the renal tubular epithelium due to oxidative stress associated with insulin resistance [18].

In addition, in the mechanisms of calcium oxalate urolithiasis in patients with MS, the presence of obesity and associated lipid metabolism disorders with chronic low-grade inflammation is of greater importance, in contrast to uric acid lithiasis, where insulin resistance is considered the main pathogenetic factor. Experimental studies and clinical observations indicate a negative effect of dyslipidemia on renal tubular function with the development of hypercalciuria, hyperoxaluria, and hyperphosphaturia with a decrease in the level of citrates in the urine, which characterizes

an increase in its lithogenic properties. Data were also obtained confirming the relationship between other MS components and oxalate urolithiasis. A positive correlation was described between body mass index (BMI) and calcium and oxalate excretion, and a negative correlation – between BMI and citrate excretion.

In the study of the epidemiology, clinical presentation, and pathogenesis of MS and urolithiasis as an associated disease, the attention of some researchers was drawn to gender characteristics in the development of metabolic disorders, understanding of which would make it possible to more effectively select pathogen-specific therapy for patients. Gender differences in the epidemiological parameters of MS are characterized by earlier (from the age of 30 years) appearance of abdominal obesity in men and, as a consequence, a risk of atherosclerosis of different localization and other pathological conditions. For women, the problem of MS and the emergence of related diseases become more relevant during menopause. On the one hand, these features are associated with the influence of sex hormones: the stimulating effect of estrogens and progesterone and the suppressive effect of testosterone. On the other hand, they are related to the peculiarities of adipose tissue distribution, since the morphofunctional features of the adipose tissue in the subcutaneous fat are well known. According to the lipokine theory, white adipose tissue is considered an endocrine organ that synthesizes a large amount of biologically active substances – adipokines, which realize their systemic action by participating in the regulation of various body functions [19–25].

It is currently believed that in the pathogenesis of MS and associated pathological conditions, adipokine imbalance is of great importance, the main manifestations of which are hypoadiponectinemia, hyperleptinemia, and leptin resistance. At the same time, adipokine imbalance has also gender characteristics [26]. There are literature data confirming the statistically significant predominance of the concentrations of adiponectin [27, 28], leptin [29, 30], and resistin [31] in women. Meanwhile, some researchers established gender characteristics of the relationship between some adipokines and both insulin resistance [32] and other clinical and metabolic parameters, including markers of the acute phase of inflammation [26].

Inflammation and oxidative stress, which significant role in the mechanisms associated with MS in pathological conditions has been proven, are also regulated by adipokines. The relationship of clinical and laboratory symptoms of MS (the severity of abdominal obesity, arterial hypertension, impaired carbohydrate metabolism), the intensity of inflammation, and activation of free radical oxidation with adipokine imbalance has gender characteristics. For men, hypoadiponectinemia is crucial in this relationship, and for women – hyperleptinemia [26]. Gender characteristics of urolithiasis development are multifaceted.

Researchers studied gender differences in urine concentration, which may affect the prevalence of urolithiasis in men and women. It was found that both in healthy individuals and in patients with diabetes and / or chronic kidney disease, urine osmolality, calculated urine osmolality, and relative urine concentration index (urine creatinine / plasma creatinine) were higher in men than in women [33]. Urine osmolality increases after protein loading [34]. Urine osmolality in men is already higher before puberty, which is not found in women. Therefore, it is unlikely that sex hormones directly affect urine concentration. A comparative analysis of the concentration of some electrolytes in urine showed that excretion of calcium and oxalates was significantly higher in men, and the level of citrate – in women ($p < 0.05$). These data suggest that lower calcium and oxalate concentrations and higher citrate excretion may reduce the risk of kidney stone formation in women [35].

Seasonal fluctuations of risk factors for the formation of urolithiasis were found. In summer, both men and women had moderate sodium depletion with a corresponding decrease in urinary calcium, while men had a significant decrease in the volume of urine with an increase in calcium oxalate concentration. In women, the level of calcium oxalate was at its highest at the beginning of winter, both due to a decrease in the urine volume and an increase in urinary calcium excretion. A decrease in urine pH in both gender groups was observed in summer, but the pH level in men was significantly lower, mainly due to a high concentration of uric acid [36]. Thus, the risk of kidney stone formation is seasonal. In men, an increase in the concentration of calcium oxalate and uric acid was noted in summer, while in women, a high level of calcium oxalate was noted at the beginning of winter.

Gender differences in the level of vasopressin were determined. They were characterized by higher values for this parameter in the blood plasma and urine of men, as well as by higher threshold of sensitivity to osmotic stimuli in this group [33]. On a biological animal model, the concept of gender differences in antidiuretic reactivity to endogenous vasopressin was confirmed [37, 38]. Higher values of urine con-

centration may be a risk factor for the development of urolithiasis and / or chronic kidney disease and arterial hypertension in men, which requires in-depth study.

Two large studies from France and Germany demonstrate a clear gender correlation of urolithiasis formation in relation to age [39, 40]. In the first decade of life, urolithiasis is more common in boys, while in the second decade, girls, according to these data, suffer from it more often [41]. The largest number of people with urolithiasis was observed in the age groups of 40–49 years and 30–39 years, regardless of gender [39]. Italian researchers found that the process of kidney stone formation can be the result of environmental factors, such as dietary habits and lifestyle. In particular, they noted the influence of increased consumption of animal protein [42].

In Germany, a large-scale study of more than 200,000 urinary stone compositions was carried out for almost 30 years (from 1977 to 2006). The overall ratio of men and women with urolithiasis was 2.4 : 1, and throughout the study, it changed: in 1977 – 1.86 : 1, and in 2006 – 2.7 : 1. At the same time, the peak of kidney stone formation in women was in the age group of 60–69 years, while in men, a plateau at the age of 30–69 years was observed [40]. It was also found that calcium stones were widespread regardless of gender (84% of men, 81% of women). However, in the age group of 60–69 years, the proportion of men among patients with urolithiasis with calcium stone formation was 3 times higher than among women.

Calcium phosphate in the form of carbonate apatite was twice as common in women than in men; it was the third most common calculi after calcium oxalate monohydrate and calcium oxalate dihydrate. Hyperuricemia was more common in men with a 4 : 1 ratio [40]. The incidence of uric acid lithiasis remained steady, with an overall rate of 11.7% in men and 7.0% in women and a peak at an older age in both groups [40]. It was found that 0–9.6% of all analyzed kidney stones were cystine. The level of cystine stone formation remained low; this type of urolithiasis occurred in 0.4% of men and 0.7% of women. The peak incidence in women was between 20 and 29 years of age, while in men, the peak incidence occurred 10 years later, between 30 and 29 years, respectively [40].

In order to study the influence of the MS components on kidney stone formation in relation to gender aspects, a study was carried out in the child population. The study included 94 children (the ratio of boys and girls was 1 : 1.8) who did not take any medications and did not follow a diet prior to treatment for urolithiasis [43]. Overweight children were found to have hypocitraturia, hypercalciuria, and hyperoxaluria compared with children with normal weight.

An assessment of risk factors for kidney stone formation in both groups confirms that being overweight can cause stone formation in both sexes. Likewise, A.L. Negri et al. [44] found that with an increase in BMI in both sexes, there was a significant increase in the excretion of uric acid and oxalate, but a significant decrease in urine pH was noted only in men. In another study of more than 500 people with calcium oxalate stones, a positive relationship was found between BMI and urinary oxalate excretion in women and urinary calcium excretion in men [45].

To determine a possible relationship between urolithiasis and obesity, a subanalysis was carried out. It showed that obesity was a risk factor for the development of urolithiasis in all age groups and in both gender groups, in persons with hypertension and diabetes mellitus. In this subanalysis, obesity in women showed the greatest effect: women with obesity were significantly more susceptible to stone formation than nonobese women (odds ratio (OR) 1.35, 95% CI 1.33–1.37). This effect was less pronounced in men (OR 1.04, 95% CI 1.02–1.06). Being overweight in women increased the risk of urolithiasis by 35% compared with any other parameter [46].

A. Trinchieri et al. conducted a study aimed at assessing the impact of overweightness and obesity on the risk of kidney stone formation in the population following a Mediterranean diet, as well as elucidating the mechanisms underlying the increased risk of urolithiasis observed in obese individuals [47]. A retrospective analysis of data from 1,698 patients with urolithiasis (average age of 45.9 ± 14.6 years; 984 men and 714 women) attending outpatient clinics in Milan and Florence from January 1986 to June 2014 was carried out.

Italian scientists reviewed the records and collected data regarding age, sex, body weight, height, calculus composition, association with type 2 diabetes or gout, and daily urine metabolic profile. In the studied population, overweightness and obesity occurred in 40.7% and 8% of men, respectively, and in 19.9% and 8.7% of women, respectively [47]. The average BMI in patients with urolithiasis was 24.5 ± 7.5 kg / m^2 [47]. BMI values positively correlated with age (p = 0.000), and the average BMI was higher in men than in women (25.5 ± 8.9 versus 23.2 ± 4.4 kg / m^2) [47]. In men with urolithiasis, the rates of overweightness and obesity were higher than in the general population

of Italy in 2004 for the age group of 25–44 years alone [47].

The rates of overweightness and obesity varied significantly in patients with different chemical composition of calculi. In particular, patients with uric acid stones had higher rates of overweightness and obesity than patients with calcium or other types of stones [47]. Additionally, the rates characterizing type 2 diabetes and gout were significantly higher in overweight and obese patients. Besides, in this category of patients, urinary excretion of risk factors for stone formation (calcium, oxalates, and urates) and inhibitory substances (citrate) was significantly higher than in patients with normal weight or who were underweight. The prevalence of overweightness and obesity in patients with urolithiasis in a country following a Mediterranean diet is no higher than in the general population [47].

There is a growing body of evidence suggesting a relationship between insulin resistance or type 2 diabetes and urolithiasis. To assess this correlation, a study was conducted including three large cohorts of over 200,000 participants: the Nursing Health Study (I) (older women), the Nursing Health Study (II) (young women), and the Health Professionals Follow-Up Study (men) [48]. The relationship between diabetes and nephrolithiasis was studied for over 44 years of follow-up. Analysis of the results in the groups listed above showed a relative risk of urolithiasis prevalence: 1.67 in young women with diabetes, 1.38 in older women with diabetes, and 1.31 in men with diabetes.

The relative risk of urolithiasis in participants with diabetes compared with participants without diabetes was 1.60 in young women, 1.29 in older women, and 0.81 in men [48]. It was found that not only type 2 diabetes is associated with an increased risk of urolithiasis, but also a history of urolithiasis increases the likelihood of type 2 diabetes in the future. The authors consider it relevant to diagnose diabetes mellitus in new patients with urolithiasis [48]. This opinion is confirmed by a study which found that the proportion of uric acid stones is 2.2 times higher in patients with diabetes mellitus than in patients with urolithiasis without diabetes, with statistically significant predominance in women compared with men (3.8 versus 1.7) ($p = 0.003$) [49].

CONCLUSION

This literature review showed that gender aspects are of significant importance in the mechanisms of development of MS and urolithiasis as an associated disease. Their further study and understanding are necessary for the development of high-quality diagnostic algorithms and pathogenetically grounded approaches to treatment.

REFERENCES

1. Bespalova I.D., Bychkov V.A., Kalyuzhin V.V., Ryazantseva N.V., Medyantsev Yu.A., Osikhov I.A., Murashev B.Yu. Quality of life in hypertensive patients with metabolic syndrome: interrelation with markers of systemic inflammation. *Bulletin of Siberian Medicine.* 2013; 12 (6): 5–15 (in Russ.). DOI: 10.20538/1682-0363-2013-6-5-11.
2. Bespalova I.D., Kalyuzhin V.V., Medyantsev Y.A., Osikhov I.A., Murashev B.Y., Ryazantseva N.V. The effect of the hyperleptinemia on the quality of life of hypertensive patients with metabolic syndrome. *Arterial Hypertension.* 2013; 19 (5): 428–434 (in Russ.) DOI: 10.18705/1607-419X-2013-19-5-428-424.
3. Curhan G., Goldfarb D. Epidemiology of stone disease. *Urol. Clin. North Am.* 2007; 34 (3): 287–293. DOI: 10.1016/j.ucl.2007.04.003.
4. Lieske J.C., Pena de la Vega L.S., Slezak J.M., Bergstralh E.J., Leibson C.L., Ho K.-L., Gettman M.T. Renal stone epidemiology in Rochester, Minnesota: an update. *Kidney Int.* 2006; 69 (4): 760–764. DOI: 10.1038/sj.ki.5000150.
5. Romero V., Akpinar H., Assimos D.G. Kidney stones: a global picture of prevalence, incidence, and associated risk factors. *Rev. Urol.* 2010; 12 (2-3): e86–96.
6. Scales C.D., Smith A.C., Hanley J.M., Saigal C.S. Prevalence of kidney stones in the United States. *Eur. Urol.* 2012; 62 (1): 160–165. DOI: 10.1016/j.eururo.2012.03.052.
7. Gudkov A.V., Boshenko V.S., Afonin V.Ya., Losovsky M.S. Dependence of the efficacy and safety of the retrograde contact electroimpulse ureterolythotripsy on the stone location and size. *Experimental and Clinical Urology.* 2013; 3: 100–106 (in Russ.).
8. Shestaev A.U., Paronnikov M.V., Protoshak V.V., Babkin P.A., Gulko A.M., Kubin N.D. An efficiency of preventive treatments for recurrence of patients with urolithiasis and metabolic syndrome. *Herald of the North-Western State Medical University named after I.I. Mechnikov.* 2013; 5 (2): 85–89 (in Russ.).
9. Gadzhiev N.K., Malhasyan V.A., Mazurenko D.A., Guseynov M.A., Tagirov N.S. Urolithiasis and metabolic syndrome. The pathophysiology of stone formation. *Experimental and Clinical Urology.* 2018; 1: 66–75 (in Russ.). DOI: 10.29188/2222-8543-2018-9-1-66-75.
10. Golovanov S.A., Sivkov A.V., Anokhin N.V., Drozhzheva V.V. Body-mass index and chemical composition of urinary stones. *Experimental and Clinical Urology.* 2015; (4): 94–99 (in Russ.).
11. West B., Luke A., Durazo-Arvizu R.A., Cao G., Shoham D., Kramer H. Metabolic syndrome and self-reported history of kidney stones: the National Health and Nutrition Examination Survey (NHANES III) 1988–1994. *Am. J. Kidney Dis.* 2008; 51 (5): 741–747. DOI: 10.1053/j.ajkd.2007.12.030.

12. Jeong I.G., Kang T., Bang J.K., Park J., Kim W., Hwang S.S., Kim H.K., Park H.K. Association between metabolic syndrome and the presence of kidney stones in a screened population. *Am. J. Kidney Dis.* 2011; 58 (3): 383–388. DOI: 10.1053/j.ajkd.2011.03.021.
13. Moudi E., Hosseini S.R., Bijani A. Nephrolithiasis in elderly population; effect of demographic characteristics. *J. Nephropathol.* 2017; 6 (2): 63–68. DOI: 10.15171/jnp.2017.11.
14. Lee Y.C., Huang S.P., Juan Y.S., Huang T.Y., Liu C.C. Impact of metabolic syndrome and its components on kidney stone in aging Taiwanese males. *Aging Male.* 2016; 19 (3): 197–201. DOI: 10.1080/13685538.2016.1174987.
15. Bespalova I.D., Kalyuzhin V.V., Medyantsev Yu.A. Asymptomatic hyperuricemia as a component of metabolic syndrome. *Bulletin of Siberian Medicine.* 2012; 11 (3): 14–17 (in Russ.). DOI: 10.20538/1682-0363-2012-3-14-17.
16. Kobalava Zh.D., Troitskaya E.A. Asymptomatic Hyperuricemia and risk of cardiovascular and renal diseases. *Cardiology.* 2020; 60 (10): 113–121 (in Russ.). DOI: 10.18087/cardio.2020.10.n1153.
17. Sevostyanova O.A., Boschenko V.S., Osadchiy V.K., Parnachev V.P., Polienko A.K. The study of mineral composition and structure of uroliths in the residents of Tomsk district (Tomsk). *Urology.* 2017; 2: 52–57 (in Russ.). DOI: 10.18565/urol.2017.2.76-81.
18. Konstantinova O.V., Katibov M.I., Yanenko E.K., Kalinichenko D.N., Gecaev T.K. Obesity and urolithiasis. *Experimental and Clinical Urology.* 2013; 2: 65–67. (in Russ.).
19. Solntseva A.V. Endocrine effects of adipose tissue. Medical News. 2009; 3: 7–12 (in Russ.).
20. Schwarz V. Inflammation of adipose tissue. Part 1. Morphological and functional manifestations. *Endocrinology Problems.* 2009; 55 (4): 44–49 (in Russ.). DOI: 10.14341/PROBL200955444-49.
21. Maury E., Brichard S.M. Adipokine dysregulation, adipose tissue inflammation and metabolic syndrome. *Molecular and Cellular Endocrinology.* 2010; 314 (1): 1–16. DOI: 10.1016/j.mce.2009.07.031.
22. Vasyukova O.V. Adipokines for obesity in children and adolescents. *Endocrinology Problems.* 2012; 4 (2): 9–10 (in Russ.).
23. Dunmore S.J., Brown J. The role of adipokines in β-cell failure of type 2 diabetes. *J. Endocrinol.* 2013; 216 (1): 37–45. DOI: 10.1530/JOE-12-0278.
24. Bespalova I.D. Leptin as an inducer of inflammation and oxidative stress in metabolic syndrome. *Bulletin of Siberian Medicine.* 2014; 13 (1): 20–26 (in Russ.). DOI: 10.20538/1682-0363-2014-1-20-26.
25. Bespalova I.D., Ryazantseva N.V., Kalyuzhin V.V., Dzyuman A.N., Osikhov I.A., Medyantsev Y.A., Klinovitskiy I.Y., Murashev B.Y., Afanas'eva D.S., Bychkov V.A. Clinicomorphological parallels in abdominal obesity. *The Siberian Scientific Medical Journal.* 2014; 34 (4): 51–58 (in Russ.).
26. Bespalova I.D., Ryazantseva N.V., Kalyuzhin V.V., Osikhov I.A., Murashev B.Yu., Medyantsev Yu.A., Rudnitsky V.A. Gender features of interaction hormonal activity adipose tissue and proinflammatory status in hypertension with metabolic syndrome. *Bulletin of Siberian Medicine.* 2014; 13 (5): 12–19 (in Russ.). DOI: 10.20538/1682-0363-2014-5-12-19.
27. Laughlin G.A., Barret-Connor E., May S. Sex-specific determinants of serum adiponectin in older adults: the role endogenous sex hormones. *Int. J. Obes.* (Lond.). 2007; 31 (3): 457–465. DOI: 10.1038/sj.ijo.0803427.
28. Dontsov A., Vasilyeva L. Gender anthropometric and hormonal features in metabolic syndrome. *Doctor.* 2014; (7): 72–74 (in Russ.).
29. Janeckova R. The role of leptin in human physiology and pathophysiology. *Physiol. Res.* 2001; 50 (5): 443–459.
30. Minyaylova N.N., Sundukova E.L., Rovda Ju.I. Hyperleptinemia and its clinical and metabolic associations in insulin resistance syndrome in children and adolescents. *Pediatria. Journal named after G.N. Speransky.* 2009; 88 (6): 6–13 (in Russ.).
31. Baranova A.V. Adipokine genetics: unbalanced protein secretion by human adipose tissue as a cause of the metabolic syndrome. *Russian Journal of Genetics.* 2008; 44 (10): 1338–1355 (in Russ.).
32. Boyarinova M.A., Rotar O.P., Erina A.M. Gender peculiarities of adipokine association with insulin resistance in patients with risk factors. Materials of the Russian National Congress of Cardiology "Integration of Knowledge in Cardiology". 2012: 82. (in Russ.).
33. Perucca J., Bouby N., Valeix P., Bankir L. Sex difference in urine concentration across differing ages, sodium intake, and level of kidney disease. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 2007; 292 (2): 700–705. DOI: 10.1152/ajpregu.00500.2006.
34. Hadj-Aissa A., Bankir L., Fraysse M., Bichet D.G., Laville M., Zech P., Pozet N. Influence of the level of hydration on the renal response to a protein meal. *Kidney Int.* 1992; 42 (5): 1207–1216. DOI: 10.1038/ki.1992.406.
35. Sarada B., Satyanarayana U. Urinary composition in men and women and the risk of urolithiasis. *Clin. Biochem.* 1991; 24 (6): 487–490. DOI: 10.1016/s0009-9120(05)80007-4.
36. Parks J.H., Barsky R., Coe F.L. Gender differences in seasonal variation of urine stone risk factors. *J. Urol.* 2003; 170 (2 Pt 1): 384–388. DOI: 10.1097/01.ju.0000071721.91229.27.
37. Wang Y.X., Crofton J.T., Liu H., Sato K., Brooks D.P., Share L. Estradiol attenuates the antidiuretic action of vasopressin in ovariectomized rats. *Am. J. Physiol.* 1995; 268 (4 Pt 2): 951–957. DOI: 10.1152/ajpregu.1995.268.4.R951.
38. Wang Y.X., Crofton J.T., Miller J., Sigman C.J., Liu H., Huber J.M., Brooks D.P., Share L. Sex difference in urinary concentrating ability of rats with water deprivation. *Am. J. Physiol.* 1996; 270 (3 Pt 2): 550–555. DOI: 10.1152/ajpregu.1996.270.3.R550.
39. Daudon M., Doré J.-C., Jungers P., Lacour B. Changes in stone composition according to age and gender of patients: a multivariate epidemiological approach. *Urol. Res.* 2004; 32 (3): 241–247. DOI: 10.1007/s00240-004-0421-y.
40. Knoll T., Schubert A.B., Fahlenkamp D., Leusmann D.B., Wendt-Nordahl G., Schubert G. Urolithiasis through the ages: data on more than 200,000 urinary stone analyses. *J. Urol.* 2011; 185 (4): 1304–1311. DOI: 10.1016/j.juro.2010.11.073.

41. Novak T.E., Lakshmanan Y., Trock B.J., Gearhart J.P., Matlaga B.R. Sex prevalence of pediatric kidney stone disease in the United States: an epidemiologic investigation. *Urology.* 2009; 74 (1): 104–107. DOI: 10.1016/j.urology.2008.12.079.
42. Trinchieri A., Coppi F., Montanari E., Del Nero A., Zanetti G., Pisani E. Increase in the prevalence of symptomatic upper urinary tract stones during the last ten years. *Eur. Urol.* 2000; 37 (1): 23–25. DOI: 10.1159/000020094.
43. Sarica K., Eryildirim B., Yencilek F., Kuyumcuoglu U. Role of overweight status on stone-forming risk factors in children: a prospective study. *Urology.* 2009; 73 (5): 1003–1007. DOI: 10.1016/j.urology.2008.11.038.
44. Negri A.L., Spivacow F.R., Del Valle E.E., Forrester M., Rosende G., Pinduli I. Role of overweight and obesity on the urinary excretion of promoters and inhibitors of stone formation in stone formers. *Urol. Res.* 2008; 36 (6): 303–307. DOI: 10.1007/s00240-008-0161-5.
45. Taylor E.N., Curhan G.C. Body size and 24-hour urine composition. Am. J. Kidney Dis. 2006; 48 (6): 905–915. DOI: 10.1053/j.ajkd.2006.09.004.
46. Nowfar S., Palazzi-Churas K., Chang D.C., Sur R.L. The relationship of obesity and gender prevalence changes in United States inpatient nephrolithiasis. *Urology.* 2011; 78 (5): 1029–1033. DOI: 10.1016/j.urology.2011.04.011.
47. Trinchieri A., Croppi E., Montanari E. Obesity and urolithiasis: evidence of regional influences. *Urolithiasis.* 2017; 45 (3): 271–278. DOI: 10.1007/s00240-016-0908-3.
48. Taylor E.N., Stampfer M.J., Curhan G.C. Diabetes mellitus and the risk of nephrolithiasis. *Kidney Int.* 2005; 68 (3): 1230–1235. DOI: 10.1111/j.1523-1755.2005.00516.x.
49. Daudon M., Lacour B., Jungers P. High prevalence of uric acid calculi in diabetic stone formers. *Nephrol. Dial Transplant.* 2005; 20 (2): 468–469. DOI: 10.1093/ndt/gfh594.

Authors information

Bespalova Inna D., Dr. Sci. (Med.), Professor, Department of Advanced-Level Therapy, Acting Head of the Department of Propaedeutics of Internal Diseases with a Course of Therapy, Siberian State Medical University, Tomsk, Russian Federation. ORCID 0000-0002-4513-6329.

Boschenko Vyacheslav S., Dr. Sci. (Med.), Professor, Department of General and Pediatric Urology and Andrology, Siberian State Medical University, Tomsk, Russian Federation. ORCID 0000-0002-2448-9870.

Koshchavtseva Yulia I., Resident, Department of Propaedeutics of Internal Diseases with a Course of Therapy, Siberian State Medical University, Tomsk, Russian Federation.

Tsoy Alexey V., Urologist, St. Luke's Clinical Hospital, St. Petersburg, Russian Federation.

Teteneva Anna V., Dr. Sci. (Med.), Professor, Department of Propaedeutics of Internal Diseases with a Course of Therapy, Siberian State Medical University, Tomsk, Russian Federation. ORCID 0000-0002-4323-2798.

Mesko Pavel E., Cand. Sci. (Med.), Associate Professor, Department of Propaedeutics of Internal Diseases with a Course of Therapy, Siberian State Medical University, Tomsk, Russian Federation. ORCID 0000-0003-2183-4402.

Karzilov Alexander I., Dr. Sci. (Med.), Professor, Department of Propaedeutics of Internal Diseases with a Course of Therapy, Siberian State Medical University, Tomsk, Russian Federation. ORCID 0000-0002- 3919-720.

Porovskiy Yaroslav V., Dr. Sci. (Med.), Professor, Department of Propaedeutics of Internal Diseases with a Course of Therapy, Siberian State Medical University, Tomsk, Russian Federation. ORCID 0000-0003-3378-0608.

Mishustina Elena L., Cand. Sci. (Med.), Associate Professor, Department of Propaedeutics of Internal Diseases with a Course of Therapy, Siberian State Medical University, Tomsk, Russian Federation. ORCID 0000-0003-2498-801X.

Tetenev Konstantin F., Cand. Sci. (Med.), Associate Professor, Department of Propaedeutics of Internal Diseases with a Course of Therapy, Siberian State Medical University, Tomsk, Russian Federation. ORCID 0000-0001-5306-6589.

Kalyuzhina Elena V., Dr. Sci. (Med.), Professor, Department of Advanced-Level Therapy, Siberian State Medical University, Tomsk, Russian Federation. ORCID 0000-0002-7978-5327.

Kalyuzhin Vadim V., Dr. Sci. (Med.), Professor, Head of the Department of Advanced-Level Therapy, Siberian State Medical University, Tomsk, Russian Federation. ORCID 0000-0001-9640-2028.

(✉) **Bespalova Inna D.,** e-mail: innadave@mail2000.ru

Received 22.01.2021
Accepted 25.05.2021

УДК 616.831-006-073.916
https://doi.org/10.20538/1682-0363-2021-4-131-142

Modern methods for radionuclide diagnosis of tumors and non-tumor pathologies of the brain

Zelchan R.V.[1, 2], Medvedeva A.A.[1], Bragina O.D.[1, 2], Ribina A.N.[1], Ryabova A.I.[1], Chernov V.I.[1, 2], Choynzonov E.L.[1]

[1] Cancer Research Institute, Tomsk National Research Medical Center (NRMC), Russian Academy of Sciences
5, Kooperativny Str., Tomsk, 634009, Russian Federation

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

ABSTRACT

The review analyzes the global experience in the application of nuclear medicine techniques for diagnosis of tumors and non-tumor pathologies of the brain. The main groups of radiopharmaceuticals currently used for imaging of malignant brain tumors and diagnosis of cognitive impairments and neurotransmitter system disturbances by means of single-photon emission computed tomography and positron emission tomography are described.

Modern approaches to the application of methods for radionuclide diagnosis in neuro-oncology and neurology are compared, and the main trends in production of new, more specific radiopharmaceuticals for visualizing brain tumors of various degrees of malignancy and diagnosing non-tumor pathologies of the brain are described. The review discusses the advantages and disadvantages of currently used techniques and radiopharmaceuticals for imaging of central nervous system disorders, depending on the clinical situation and specific diagnostic tasks.

In addition, the review presents consolidated recommendations of the leading scientific schools in neuro-oncology on the use of nuclear medicine techniques in patients with brain tumors at the stages of treatment and follow-up. The presented article examines the experience of domestic scientific schools in the development of radiopharmaceuticals for neuro-oncology. The features of the development and use of new radiopharmaceuticals in patients with brain tumors and neurodegenerative diseases are highlighted. The review is based on the analysis of literature included in the Scopus, Web of Science, MedLine, The Cochrane Library, EMBASE, Global Health, and RSCI databases.

Key words: nuclear medicine, brain tumor, dementia, radionuclide diagnosis, radiopharmaceutical.

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 federal targeted program “Development of the pharmaceutical and medical industry in the Russian Federation up to 2020 and beyond” on the topic “Preclinical trials of the radiopharmaceutical on the basis of ^{99m}Tc-labeled glucose derivative for radionuclide diagnosis of cancers” (Code 2015-14-№ 08-0008. State contract 14. № 08.11.0033 of 19.05.2015).

For citation: Zelchan R.V., Medvedeva A.A., Bragina O.D., Ribina A.N., Ryabova A.I., Chernov V.I., Choynzonov E.L. Modern methods for radionuclide diagnosis of tumors and non-tumor pathologies of the brain. *Bulletin of Siberian Medicine.* 2021; 20 (4): 131–142. https://doi.org/10.20538/1682-0363-2021-4-131-142.

✉ *Zelchan Roman V.,* e-mail: r.zelchan@yandex.ru

Современные методы радионуклидной диагностики опухолей и неопухолевой патологии головного мозга

Зельчан Р.В.[1, 2], Медведева А.А.[1], Рыбина А.Н.[1], Брагина О.Д.[1, 2], Рябова А.И.[1], Чернов В.И.[1, 2], Чойнзонов Е.Л.[1]

[1] *Научно-исследовательский институт (НИИ) онкологии, Томский национальный исследовательский медицинский центр (НИМЦ) Российской академии наук*
Россия, 634009, г. Томск, пер. Кооперативный, 5

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

РЕЗЮМЕ

В обзоре обсуждается мировой опыт применения методов ядерной медицины в диагностике опухолей головного мозга и его неопухолевых изменений. Рассматриваются основные группы применяемых сегодня радиофармацевтических препаратов (РФП) для визуализации злокачественных опухолей головного мозга, диагностики когнитивных нарушений и нарушений системы нейротрансмиссии методом однофотонной эмиссионной компьютерной томографии и позитронно-эмиссионной томографии.

В сравнительном аспекте освещаются современные подходы к применению методов радионуклидной диагностики в нейроонкологии и неврологии, отражаются основные тенденции в производстве новых, более специфичных РФП для визуализации опухолей головного мозга различной степени злокачественности и диагностики неопухолевых заболеваний мозга. Обсуждаются преимущества и недостатки применяемых сегодня методик и РФП для визуализации заболеваний центральной нервной системы в зависимости от клинической ситуации и конкретных диагностических задач.

Представлены консолидированные рекомендации ведущих научных школ нейроонкологии по применению методов ядерной медицины у пациентов с опухолями головного мозга на этапах лечения и динамического наблюдения. Рассмотрен опыт отечественных научных школ в разработке РФП для нейроонкологии. Освещены особенности разработки и применения новых РФП у пациентов с опухолями головного мозга и нейродегенеративных заболеваний. Обзор выполнен на анализе литературы, входящей в базы данных Scopus, Web of Science, MedLine, The Cochrane Library, EMBASE, Global Health и РИНЦ.

Ключевые слова: ядерная медицина, опухоль головного мозга, деменция, радионуклидная диагностика, радиофармацевтичекий препарат.

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

Источник финансирования. Работа выполнена в рамках Федеральной целевой программы «Развитие фармацевтической и медицинской промышленности Российской Федерации на период до 2020 года и дальнейшую перспективу» по теме «Доклинические исследования радиофармацевтического препарата на основе меченной ^{99m}Tc производной глюкозы для радионуклидной диагностики онкологических заболеваний». Шифр «2015-14-№ 08-0008». Государственный контракт 14. № 08.11.0033 от 19.05.2015.

Для цитирования: Зельчан Р.В., Медведева А.А., Рыбина А.Н., Брагина О.Д., Рябова А.И., Чернов В.И., Чойнзонов Е.Л. Современные методы радионуклидной диагностики опухолей и неопухолевой патологии головного мозга. *Бюллетень сибирской медицины.* 2021; 20 (4): 131–142. https://doi.org/10.20538/1682-0363-2021-4-131-142.

INTRODUCTION

Currently, nuclear medicine technologies are quite firmly entrenched in modern medical science and clinical practice in the world in general and in the Russian Federation in particular. Oncology, neurology, and cardiology remain the main areas of application of nuclear medicine techniques. Radionuclide studies are successfully used for primary diagnosis of brain tumors, assessment of the effectiveness of combination treatment, and as an objective method of disease control at the follow-up stage, as well as for early di-

agnosis of neurotransmitter system disturbances of the central nervous system and various types of dementia [1–3].

These methods make it possible to study the activity of various enzymes, synthesis and metabolism of neurotransmitters, density of receptors, and expression of various genes [4–6]. Modern techniques of radionuclide imaging allow to conduct differential diagnosis of pathological changes in the brain and clarify their biological nature.

According to numerous studies, such radiopharmaceuticals (RP) as a ^{99m}Tc-MIBI, $^{Tl-201}$ chloride, and ^{123}I-labeled amino acids are most commonly used for the diagnosis of brain tumors by single-photon emission computed tomography (SPECT). As a rule, ^{99m}Tc-MIBI SPECT makes it possible to visualize primary malignant brain tumors and hypervascular benign neoplasms [7]. The major limiting factor of ^{99m}Tc-MIBI SPECT in imaging of brain tumors is the intensity of blood flow in the tumor. The degree of ^{99m}Tc-MIBI accumulation in malignant tumors varies widely and does not correlate with the degree of malignancy of the tumor and its histological type [8–10]. Despite this, some authors argue that hyperintense accumulation of ^{99m}Tc-MIBI is characteristic of glioblastoma multiforme, and high values of the drug accumulation index in this type of tumor make it possible to differentiate it from other malignant neoplasms of the brain [11, 12].

Due to the nonspecific accumulation of ^{99m}Tc-MIBI in the zones of post-radiation changes, its use is very limited in the follow-up of patients after radiation therapy of brain tumors [13]. Although some researchers testify to the opposite and admit the use of ^{99m}Tc-MIBI SPECT for assessing the effectiveness of chemoradiotherapy of malignant brain tumors and detecting relapses in this group of patients at early stages [14,15].

Various amino acids labeled with iodine-123 (^{123}I) are still of great interest in terms of diagnosing brain tumors by SPECT. Most of the studies are devoted to the study of the diagnostic capabilities of SPECT with the following amino acids labeled with iodine-123: α-methyl-L-tyrosine and L-phenylalanine [16–18]. It was shown that the level of accumulation of ^{123}I-α-methyl-L-tyrosine in the tumor is slightly higher than that of ^{123}I-L-phenylalanine, and does not depend on the density of tumor cells. SPECT with the indicated iodine-123-labeled amino acids can effectively visualize gliomas of various grades of malignancy (grade I–IV), while metastases to the brain, for example, in lung cancer and non-neoplastic brain lesions, do not accumulate these radioactive tracers.

It was noted that when imaging grade I–II gliomas by SPECT with ^{123}I-L-phenylalanine, false-negative results are more common. Thus, the sensitivity of SPECT with iodine-123-labeled amino acids according to various studies is 78–90%, and the specificity reaches 100% [19– 21].

At the end of the last century, the possibility of using thallium-201 (^{201}Tl) for the diagnosis of brain tumors by SPECT was actively studied. Numerous clinical trials demonstrated the effectiveness of ^{201}Tl SPECT for detecting intracerebral tumors with an average sensitivity and specificity of 95% and 87–93%, respectively. Some authors showed that the degree of ^{201}Tl accumulation in malignant gliomas is significantly higher than in low-grade gliomas and benign brain tumors, which allows for their differential diagnosis [22–27]. It should be noted that ^{201}Tl is currently not used, and large-scale studies on investigating the possibility of using SPECT with another thallium isotope, ^{199}Tl, which favorably differs in low radiation exposure for a patient, have not been carried out in the diagnosis of brain tumors.

In recent years, positron emission tomography (PET) with various radiopharmaceuticals has been the undisputed leader among nuclear medicine techniques for the diagnosis of brain tumors [28–31]. The main radiopharmaceutical for PET is ^{18}F-FDG. The specified RP has physicochemical characteristics that are convenient for the diagnostic process, including a relatively long half-life (110 minutes), which makes it possible to transport it to the nearest PET centers that are not equipped with cyclotrons.

Already in the first studies on the use of ^{18}F-FDG PET in imaging of brain tumors, it was demonstrated that an increase in glucose metabolism in a tumor correlates with the grade of its malignancy and aggressiveness of the course of the disease in general. Based on the results of various studies, a consolidated decision was made to consider the degree of glucose metabolism in the intact gray and white matter of the brain as a background level and rely on it both in visual assessment of the study results and in calculation of quantitative parameters of PET [32, 33].

Numerous studies demonstrated the differences in the levels of ^{18}F-FDG accumulation in grade I–IV brain tumors. Thus, the level of ^{18}F-FDG accumulation in low-grade tumors is more often the same as in the intact white matter or lower, and the level of accumulation in high-grade tumors is more often equal to

or higher than that in the gray matter of the brain [34]. This feature makes it difficult to visualize low-grade brain tumors and interpret the results of the study. It should also be noted that the specificity of ^{18}F-FDG PET remains low. For example, the level of SUV_{max} in primary cerebral lymphoma is significantly higher than that in glioblastoma [35, 36].

The results of ^{18}F-FDG PET remain ambiguous in the differential diagnosis of grade III–IV gliomas and brain metastases, because the SUV_{max} values in these formations are often the same. According to many authors, ^{18}F-FDG PET has significant limitations in the differentiation of gliomas and various non-neoplastic brain lesions, such as brain abscesses, tumor-like demyelination, inflammatory changes caused by fungal infections, and neurosarcoidosis. All of the above-mentioned pathological processes, as well as tumor damage, one way or another, lead to an increase in glucose metabolism, which complicates interpretation of the study results [37].

At a certain historical stage, ^{18}F-FDG PET of the brain was of great importance for choosing a site of tumor tissue for targeted stereotactic biopsy, because most glial tumors (82%) are characterized by heterogeneity. It should be noted that imaging of such tumor differentiation is absolutely impossible with traditional methods for diagnostic radiology – CT and MRI, even with the use of contrast-enhanced techniques and cutting-edge software algorithms [38–42].

Considering the complexity and multistage nature of the treatment process in patients with brain tumors, assessment of treatment effectiveness and timely detection of relapses remain some of the most important problems in modern neuro-oncology. Studies showed that the change in the level of ^{18}F-FDG accumulation in the brain tumor after radiation therapy or chemoradiation correlates with the tumor response to therapy, which means that a decrease in the level of ^{18}F-FDG metabolism indicates the effectiveness of treatment [43– 46].

It is known that, due to increased proliferation, tumor cells are characterized by increased metabolism, including enhanced protein synthesis, for which a sufficient supply of amino acids is required. It should be noted that amino acids labeled with various isotopes do not differ in their physicochemical properties from natural amino acids and are their complete biological analogs. In neuroimaging, labeled amino acids have a significant advantage over ^{18}F-FDG, which consists in an extremely low level of physiological accumulation in intact brain structures, including the cortex and basal nuclei. Carbon-11-labeled methionine (^{11}C-MET) was the first amino acid-based radiopharmaceutical. Since that time, ^{11}C-MET has become the most commonly used radiopharmaceutical in oncology after ^{18}F-FDG [47, 48].

In most cases, ^{11}C-MET PET makes it possible to visualize brain tumors of various degrees of malignancy (grade I–IV according to the classification of the World Health Organization (WHO)), with a fairly clear definition of the boundaries of the tumor lesion and normal brain tissues, as well as to delimit the area of edema and true tumor infiltration [49]. According to different authors, the averaged indices of the sensitivity and specificity of ^{11}C-MET PET in imaging of brain tumors of various grades of malignancy are 89–90% and 94–100%, respectively [50–52].

Some researchers argue that, when using purely visual assessment of ^{11}C-MET PET without resorting to quantification, the sensitivity and specificity of the method in imaging brain tumors are 94% and 56.5%, respectively. At the same time, the accuracy of the method with this approach is 84.4%, and the level of positive predictive value and negative predictive value is 86.3% and 76.5%, respectively. Determination of semi-quantitative parameters of ^{11}C-MET PET of the brain in most cases contributes to the differential diagnosis between a malignant lesion and benign changes and allows to determine the grade of malignancy of glial brain tumors according to the WHO [53–55].

When studying the possibility of using ^{11}C-MET PET in the differential diagnosis between low-grade (grade I–II) and high-grade (grade III–IV) gliomas according to the classification of the WHO, the researchers also focused on the level of ^{11}C-MET accumulation in the tumor. It turned out that the degree of ^{11}C-MET accumulation in grade III–IV gliomas is significantly higher than in low-grade tumors.

Another important aspect in the use of ^{11}C-MET PET in the diagnosis of brain tumors is the possibility of using the level of RP accumulation in the tumor as a prognostic factor for the course of the disease. It was found that a high level of ^{11}C-MET uptake in the primary brain tumor before treatment indicates a poor prognosis of the disease. It should also be noted that a decrease in the level of ^{11}C-MET uptake in the course of conservative therapy reliably reflects the effectiveness of treatment [56–58].

According to literature, ^{11}C-MET PET is effectively used in follow-up of patients with benign brain gliomas, and the index of RP accumulation in the tumor reflects the grade of its malignancy. It is believed that

when the threshold value of the ^{11}C-MET accumulation index in the tumor is reached, it indicates its transition to the group of malignant gliomas, which requires a change in patient management strategy from a passive to an active radical approach [59–62].

Timely detection of recurrent malignant gliomas is still an important aspect in the treatment of patients with brain tumors. Most authors claim that ^{11}C-MET PET is capable of detecting tumor recurrence even against the background of post-therapeutic changes with sensitivity of 88% and specificity of 85% [63].

Therefore, ^{11}C-MET PET is widely used today in the oncological practice at all stages of treatment and follow-up of patients with brain tumors. This method is now considered routine and quite effective, and its availability for the population is constantly growing.

18F-fluoroethyl-L-tyrosine (^{18}F-FET) is another RP based on labeled amino acids, which has proven to be effective in diagnosing brain tumors. The indicated RP, as well as ^{11}C-MET, is characterized by low-intensity background accumulation in unchanged structures and parts of the brain. In addition, ^{18}F-FET is less accumulated in macrophages and granulocytes than ^{11}C-MET, which, in turn, leads to an increase in the specificity of ^{18}F-FET PET in detecting brain tumors [64].

The main diagnostic difference in the use of ^{18}F-FET PET for imaging of brain masses is the ability to assess the dynamic characteristics of drug accumulation in the tumor in addition to the usual accumulation indices in the area of interest. The use of data from a dynamic scanning protocol in ^{18}F-FET PET allows for differential diagnosis between grade I–II and grade III–IV gliomas and indicates the presence of tumor relapse, reliably differentiating it from the zones of radiation necrosis [65, 66].

This is of particular importance in clinical situations when a patient with suspected grade II glioma does not show contrast agent accumulation on MRI. In about 40% of these patients, an anaplastic lesion is found on a ^{18}F-FET PET scan. Application of the kinetic characteristics of the method increases its sensitivity and specificity up to 95% [67]. According to different authors, the averaged indicators of the sensitivity and specificity for ^{18}F-FET PET in the diagnosis of brain tumors are 94% and 100%, respectively.

Many authors also suggest focusing on the index of RP accumulation in the tumor during ^{18}F-FET PET. A number of studies highlight the role of ^{18}F-FET PET in planning radiation therapy in patients with brain tumors. The authors argue that the use of ^{18}F-FET in planning radiation therapy reduces the error in determining tumor boundaries and, thereby, increases the effectiveness of radiation therapy [68–70].

In recent years, the possibility of using a synthetic analog of the amino acid, L-6- [^{18}F] fluoro-3,4-dioxyphenylalanine (^{18}F-DOPA), for the diagnosis of brain tumors has been studied [71]. Studies have shown that the sensitivity of ^{18}F-DOPA PET in detecting malignant and benign brain tumors is 96%, and the specificity is about 40%. At the same time, the specificity of the method allows to significantly increase the use of threshold values of the accumulation index – tumor / striatum. It has also been shown that ^{18}F-DOPA PET can be used to differentiate the relapse of malignant gliomas and radiation necrosis [72].

In addition to amino acids labeled with various isotopes that have proven their effectiveness, other synthetic analogs of biological molecules are also used for imaging of brain tumors. Thus, to assess the proliferative activity of tumor cells, an RP based on thymidine, 3-deoxy-3- [^{18}F] -fluorothymidine (^{18}F-FLT), was proposed. Studies have shown that the degree of ^{18}F-FLT accumulation in the tumor correlates with the level of Ki-67 expression [73]. ^{18}F-FLT PET can be used to assess the malignancy grade of gliomas, predict the course of the disease, and plan radiation therapy. ^{18}F-FLT PET is of particular importance in determining the prognosis in patients with malignant brain gliomas [74]. The main disadvantage of ^{18}F-FLT is the dependence of its accumulation on the degree of damage to the blood – brain barrier and the intensity of blood flow in the tumor.

In addition, there are currently RPs for imaging brain tumors, the use of which is based on the assessment of tumor cell hypoxia, for example, [^{18}F] -fluoromisonidazole (^{18} F-FMISO). In studies on a group of patients with malignant gliomas before surgical and radiation treatment, the effectiveness of this RP was shown in determining the exact boundaries of the tumor, as well as in assessing the severity of hypoxia [75]. The disadvantages of ^{18}F-FMISO include high background accumulation of the RP, due to which there is a need for a delayed study, 2–4 hours after intravenous administration.

Currently, several RPs have been synthesized to assess the expression level of VEGF receptors. First of all, these are [^{64}Cu]-DOTA-VEGF (DEE), [^{89}Zr] -ranibizumab, as well as [^{11}C]-gefitinib ([^{11}C]-iressa) [76, 77]. Preliminary studies showed the effectiveness of these RPs in the diagnosis of tumors of various localization, including the brain, as well as in

assessing the effectiveness of their treatment. Along with the above-mentioned radioactive tracers, RPs are currently being developed to assess the state of adhesion receptors of the αVβ3integrin class. RPs with high tropism for αVβ3integrins include, in particular, [^{18}F]-galacto-arginylglycylaspartic acid, ^{64}Cu-DOTAE {E [c (RGDfK)] 2} 2, as well as a number of arginylglycylaspartic acid derivatives [78, 79]. Currently, these RPs are at different stages of development and study, so they have not yet found widespread use in the clinical practice.

Analyzing the above-presented information, we can say that PET with various RPs is firmly entrenched in the algorithms for diagnosing brain tumors at all stages of treatment and follow-up of patients with such lesions. The undisputed leaders among all RPs used in neuro-oncology today are drugs based on labeled amino acids. This is confirmed by the recommendations of the European Association of Neuro-Oncology (EANO) on the clinical use of PET in brain gliomas at various stages of patient management (Figure).

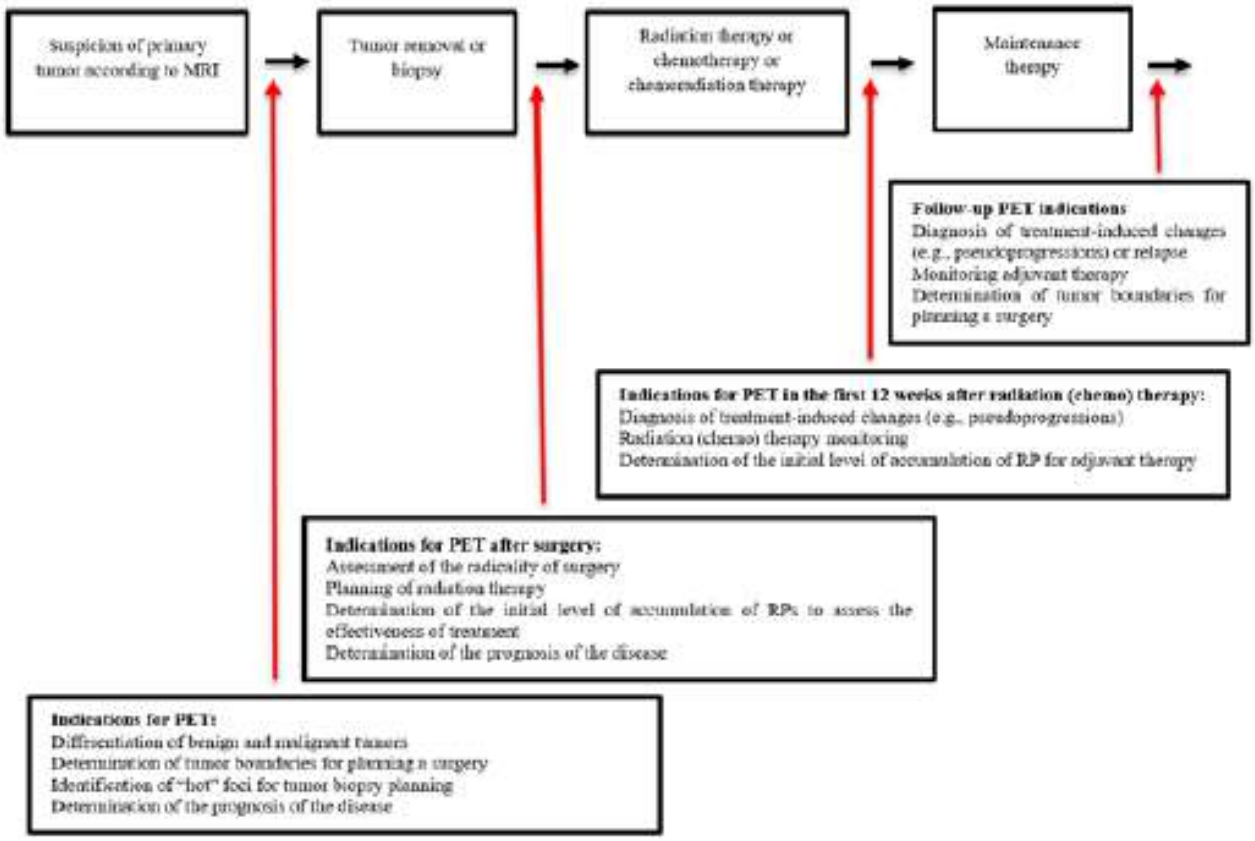


Figure. EANO General Guidelines for the Clinical Use of PET with Labeled Amino Acids in Brain Gliomas, 2016

Despite high diagnostic effectiveness of PET with various RPs, the widespread use of this method in our country is limited due to the high cost of the diagnostic procedure and the complexity of the cycle of manufacturing RPs in cyclotron facilities. From this point of view, it is of interest to develop new RPs based on technetium-99m, which is widely used in numerous SPECT centers. For example, the use of glucose-based RPs labeled with technetium-99m will make it possible to study the biochemical processes occurring in the body at the molecular level due to inclusion of glucose derivatives in normal and pathological metabolic processes, as well as to obtain information in terms of uniqueness and reliability that is not inferior to PET studies.

A number of studies found that the most promising glucose derivatives for being labeled with the ^{99m}Tc radioactive isotope, which retain the biochemical properties of glucose itself, are: 1-thio-D-glucose, 5-thio-D-glucose, glucosamine, as well as their salts or hydrates [80–83]. The available literature describes the results of the experimental use of various ^{99m}Tc-labeled glucose derivatives in animal models of a tumor lesion. At the same time, the authors note high efficiency of such drugs [84, 85].

Based on global trends in the production of RPs and our own experience in the development of drugs for radionuclide diagnosis, a team of authors from Cancer Research Institute of Tomsk NRMC in close cooperation with National Research Tomsk Polytechnic University developed a new drug based on a technetium-99m-labeled glucose derivative for radionuclide diagnosis of malignant neoplasms – "^{99m}Tc-1-thio-D-glucose" [86–88]. During phase I clinical trials, both safety and efficacy of ^{99m}Tc-1-thio-D-glucose for imaging of brain tumors by SPECT was demonstrated [89].

In neurology, ^{18}F-FDG PET is also quite effectively used to diagnose various pathological changes in the brain. In recent years, it has been demonstrated that the sensitivity and specificity of ^{18}F-FDG PET in the diagnosis of Alzheimer's disease (AD) reach 94% and 73%, respectively. The role of ^{18}F-FDG PET in predicting the development of cognitive impairments is also great [90]. In this case, an important diagnostic feature is a decrease in the accumulation of ^{18}F-FDG in the association cortex.

AD is characterized by a decrease in ^{18}F-FDG metabolism in the cortex of the temporal and parietal lobes, posterior cingulate gyri, while dementia with Lewy bodies is characterized by hypometabolism in the occipital cortex. Huntington's disease is characterized by changes in glucose metabolism in the lenticular nuclei and the heads of the caudate nuclei. Post-stroke dementia, which is characterized by multiple foci of hypometabolism in the cerebral cortex and cerebellum, also has specific presentation in ^{18}F-FDG PET. An early diagnostic sign of Pick's disease and other frontotemporal dementias is a pronounced decrease in glucose metabolism in the cortex of the frontal lobes of the brain [91–93].

PET is used quite successfully for the differential diagnosis of various types of dementia. It is known that dementia with Lewy bodies is accompanied by the development of parkinsonism, in contrast to AD. Therefore, when using ^{18}F-DOPA (dihydroxyphenylalanine), in most cases it is possible to differentiate between AD and dementia with Lewy bodies [94].

The possibility of studying the state of the presynaptic dopaminergic system during ^{18}F-DOPA PET allows to diagnose Parkinson's disease (PD) at the preclinical stage. The main diagnostic criterion is a decrease in the metabolic rate of ^{18}F-DOPA in the striatum. This drug reflects the activity of the enzyme dopadecarboxylase and the level of dopamine in neurons of the striatum [95]. Some authors argue that ^{18}F-DOPA PET allows not only to detect a decrease in the number of neurons in the striatonigral system in PD patients, but also to predict the development of this disease. This is possible due to the fact that clinical manifestations of PD occur when about 60–70% of dopaminergic neurons die [96]. ^{18}F-DOPA PET is also used to assess the effectiveness of PD treatment; in addition, the level of ^{18}F-DOPA accumulation depends on the severity of motor impairments, but does not in any way reflect the severity of cognitive impairments in such patients.

Currently, the most promising RPs for diagnosing AD and other neurodegenerative disorders are markers of β-amyloid peptide (Aβ), the increased deposition of which is the main component in the pathogenesis of AD. The carbon-11-labeled RP substance B-[^{11}C] PiB, proposed by the Pittsburgh scientists, became the first Aβ-selective radioligand for PET imaging of amyloid deposits in the association cortex. Additionally, it was shown in clinical trials that patients without dementia also had accumulation of [^{11}C] PiB in the association cortex, which, in turn, further confirmed the predictive role of [^{11}C] PiB PET in the diagnosis of AD. Currently, the development of specific RPs for the radionuclide diagnosis of neurodegenerative disorders is being actively pursued.

It is known that the second generation of PET markers of Aβ has been developed – benzofuran, benzoxazole, imidazobenzothiazole derivatives, etc. Some compounds have been labeled with ^{18}F, which will simplify their clinical use. Most of the compounds have shown their effectiveness in preclinical studies and are at the stage of clinical trials. Therefore, there is still no comprehensive information on the effectiveness of such compounds in the available literature.

CONCLUSION

Therefore, analyzing the information presented, it can be stated that high-tech nuclear medicine techniques have integrated into the development trends in modern neurology and neuro-oncology. Not a single clinic dealing with the problems of treating tumors of the central nervous system or various types of dementia can do without methods of radionuclide diagnosis in its clinical practice. It is also important that the research teams of the Russian scientific schools of physics, oncology, and nuclear medicine manage to keep up with the times in their scientific research and in some areas are ahead of the world-class leaders.

REFERENCES

1. Katsanos A.H., Alexiou G.A., Fotopoulos A.D., Jabbour P., Kyritsis A.P., Sioka C. Performance of 18F-FDG, 11C-Methionine, and 18F-FET PET for Glioma Grading: A Meta-analysis. *Clin. Nucl. Med.* 2019; 44 (11): 864–869. DOI: 10.1097/RLU.0000000000002654.
2. Horky L.L., Treves S.T. PET and SPECT in brain tumors and epilepsy. *Neurosurg. Clin. N. Am.* 2011; 22 (2): 169–1884. DOI: 10.1016/j.nec.2010.12.003.
3. Roelcke U. Imaging brain tumors with PET, SPECT, and ultrasonography. *Handb. Clin. Neurol.* 2012; 104: 135–142. DOI: 10.1016/B978-0-444-52138-5.00010-4.
4. Inubushi M., Tatsumi M., Yamamoto Y. et al. European research trends in nuclear medicine. *Ann. Nucl. Med.* 2018; 32 (9): 579–582. DOI: 10.1007/S12149-018-1303-7.
5. Lotan E., Friedman K.P., Davidson T., Shepherd T.M. Brain 18F-FDG-PET: Utility in the Diagnosis of Dementia and Epilepsy. *Isr. Med. Assoc. J.* 2020; 22 (3): 178–184.
6. Karpuz M., Silindir-Gunay M., Ozer A.Y. Current and future approaches for effective cancer imaging and treatment. *Cancer Biother. Radiopharm.* 2018; 33 (2): 39–51. DOI: 10.1089/CBR.2017.2378.
7. Terada H., Kamata N. Contribution of the combination of (201)Tl SPECT and (99m)T(c)O(4)(–) SPECT to the differential diagnosis of brain tumors and tumor-like lesions. A preliminary report. *J. Neuroradiol.* 2003; 30 (2): 91–94.
8. Le Jeune F.P., Dubois F., Blond S., Steinling M. Sestamibi technetium-99m brain single-photon emission computed tomography to identify recurrent glioma in adults: 201 studies. *J. Neurooncol.* 2006; 77 (2): 177–183. DOI: 10.1007/s11060-005-9018-8.
9. Cecchin D., Chondrogiannis S., Della Puppa A. et al. Presurgical 99mTc-sestamibi brain SPET/CT versus SPET: a comparison with MRI and histological data in 33 patients with brain tumours. *Nucl. Med. Commun.* 2009; 30 (9): 660–668. DOI: 10.1097/MNM.0b013e32832ea9b7.
10. Shibata Y., Yamamoto T., Takano S. et al. Direct comparison of thallium-201 and technetium-99m MIBI SPECT of a glioma by receiver operating characteristic analysis. *J. Clin. Neurosci.* 2009; 16 (2): 264–269. DOI: 10.1016/J.JOCN.2008.04.010.
11. Choi J.Y., Kim S.E., Shin H.J., Kim B.T., Kim J.H. Brain tumor imaging with 99mTc-tetrofosmin: comparison with 201Tl, 99mTc-MIBI, and 18F-fluorodeoxyglucose. *J. Neurooncol.* 2000; 46 (1): 63–70. DOI: 10.1023/a:1006391701818.
12. Alexiou G.A., Fotopoulos A.D., Tsiouris S., Voulgaris S., Kyritsis A.P. 99mTc-tetrofosmin SPECT for the evaluation of cerebral lesions. *Eur. J. Nucl. Med. Mol. Imaging*. 2010; 37 (12): 2403–2004. DOI: 10.1007/s00259-010-1602-2.
13. Fan Y.X., Luo R.C., Li G.P., Huang K. Di Yi Jun Yi Da Xue Xue Diagnostic value of 99mTc-MIBI brain SPECT for brain glioma. *Bao.* 2004; 24 (10): 1184–1185.
14. Bleichner-Perez S., Le Jeune F., Dubois F., Steinling M. 99mTc-MIBI brain SPECT as an indicator of the chemotherapy response of recurrent, primary brain tumors. *Nucl. Med. Commun.* 2007; 28 (12): 888–894. DOI: 10.1097/MNM.0b013e3282f1646c.
15. Palumbo B., Lupattelli M., Pelliccioli G.P., Chiarini P., Moschini T.O., Palumbo I., Siepi D., Buoncristiani P., Nardi M., Giovenali P., Palumbo R.Q. Association of 99mTc-MIBI brain SPECT and proton magnetic resonance spectroscopy (1H-MRS) to assess glioma recurrence after radiotherapy *J. Nucl. Med. Mol. Imaging.* 2006; 50 (1): 88–93.
16. Langen K.J., Coenen H.H., Roosen N., Kling P., Muzik O., Herzog H., Kuwert T., Stöcklin G., Feinendegen L.E. SPECT studies of brain tumors with L-3-[123I] iodo-alpha-methyl tyrosine: comparison with PET, 124IMT and first clinical results. *J. Nucl. Med.* 1990; 31 (3): 281–286.
17. Hellwig D., Ketter R., Romeike B.F., Sell N., Schaefer A., Moringlane J.R., Kirsch C.M., Samnick S. Validation of brain tumour imaging with p-[123I]iodo-L-phenylalanine and SPECT. *Eur. J. Nucl. Med. Mol. Imaging.* 2005; 32 (9): 1041–1049. DOI: 10.1007/s00259-005-1807-y.
18. Pauleit D., Floeth F., Tellmann L., Hamacher K., Hautzel H., Müller H.W., Coenen H.H., Langen K.J. Comparison of O-(2-18F-fluoroethyl)-L-tyrosine PET and 3-123I-iodo-alpha-methyl-L-tyrosine SPECT in brain tumors. *J. Nucl. Med.* 2004; 45 (3): 374–381.
19. Langen K.J., Roosen N., Coenen H.H., Kuikka J.T., Kuwert T., Herzog H., Stöcklin G., Feinendegen L.E. Brain and brain tumor uptake of L-3-[123I]iodo-alpha-methyl tyrosine: competition with natural L-amino acids. *J. Nucl. Med.* 1991; 32 (6): 1225–1229.
20. Rainer E., Wang H., Traub-Weidinger T., Widhalm G., Fueger B., Chang J., Zhu Z., Marosi C., Haug A., Hacker M., Li S. The prognostic value of [123I]-vascular endothelial growth factor ([123I]-VEGF) in glioma. *Eur. J. Nucl. Med. Mol. Imaging.* 2018; 45 (13): 2396–2403. DOI: 10.1007/s00259-018-4088-y.
21. Hellwig D., Ketter R., Romeike B.F., Schaefer A., Farmakis G., Grgic A., Moringlane J.R., Steudel W.I., Kirsch C.M., Samnick S. Prospective study of p-[123I]-iodo-L-phenylalanine and SPECT for the evaluation of newly diagnosed cerebral lesions: specific confirmation of glioma. *Eur. J. Nucl. Med. Mol. Imaging.* 2010; 37 (12): 2344–53. DOI: 10.1007/s00259-010-1572-4.
22. Sun D., Liu Q., Liu W., Hu W. Clinical application of 201Tl SPECT imaging of brain tumors. *J. Nucl. Med.* 2000; 41 (1): 5–10.
23. Kahn D., Follett K.A., Bushnell D.L., Nathan M.A., Piper J.G., Madsen M., Kirchner P.T. Diagnosis of recurrent brain tumor: value of 201Tl SPECT vs 18F-fluorodeoxyglucose PET. *Am. J. Roentgenol.* 1994; 163 (6): 1459–1465. DOI: 10.2214/ajr.163.6.7992747.
24. Sugo N., Yokota K., Kondo K., Harada N., Aoki Y., Miyazaki C., Nemoto M., Kano T., Ohishi H., Seiki Y. Early dynamic 201Tl SPECT in the evaluation of brain tumours. *Nucl. Med. Commun.* 2006; 27 (2): 143–149. DOI: 10.1097/01.mnm.0000191853.34574.3f. PMID: 16404227.
25. Otsuka H., Shinbata H., Hieda M., Yamashita K., Kitamura H., Senba T., Kashihara K., Tagashira H. The retention indices of 201Tl-SPECT in brain tumors. *Ann. Nucl. Med.* 2002; 16 (7): 455–459. DOI: 10.1007/BF02988641.
26. Choi J.Y., Kim S.E., Shin H.J., Kim B.T., Kim J.H. Brain tumor imaging with 99mTc-tetrofosmin: comparison with 201Tl, 99mTc-MIBI, and 18F-fluorodeoxyglucose. *J. Neurooncol.* 2000; 46 (1): 63–70. DOI: 10.1023/a:1006391701818.

27. Nose A., Otsuka H., Nose H., Otomi Y., Terazawa K., Harada M. Visual and semi-quantitative assessment of brain tumors using (201)Tl-SPECT. *J. Med. Invest.* 2013; 60 (1-2): 121–126. DOI: 10.2152/jmi.60.121.
28. Suchorska B., Tonn J.C., Jansen N.L. PET imaging for brain tumor diagnostics. *Curr. Opin. Neurol.* 2014; 27 (6): 683–688. DOI: 10.1097/WCO.0000000000000143.
29. How Does It Work? Positron emission tomography. *BMJ.* 2003; 28: 326 (7404): 1449. DOI: 10.1136/bmj.326.7404.1449.
30. Inubushi M., Tatsumi M., Yamamoto Y. et al. European research trends in nuclear medicine. *Ann. Nucl. Med.* 2018; 32 (9): 579–582. DOI: 10.1007/S12149-018-1303-7.
31. Karpuz M., Silindir-Gunay M., Ozer A.Y. Current and future approaches for effective cancer imaging and treatment. *Cancer Biother. Radiopharm.* 2018; 33 (2): 39–51.
32. Di Chiro G., Brooks R.A., Patronas N.J. Issues in the *in vivo* measurement of glucose metabolism of human central nervous system tumor. *Ann. Neurol.* 1984; 15: 138–146. DOI: 10.1002/ana.410150727.
33. La Fougere C., Suchorska B., Bartenstein P. et al. Molecular imaging of gliomas with PET: opportunities and limitations. *Neuro Oncol.* 2011; 13 (8): 806–819. DOI: 10.1093/neuonc/nor054.
34. Chen W., Silverman D.H., Delaloye S. et al. 18F-FDOPA PET imaging of brain tumours: comparison study with 18F-FDG PET and evaluation of diagnostic accuracy. *J. Nucl. Med.* 2006; 47 (6): 904–911.
35. Kosaka N., Tsuchida T., Uematsu H. et al. 18F-FDG PET of common enhancing malignant brain tumors. *Am. J. Roentgenol.* 2008; 190 (6): 365–369. DOI: 10.2214/AJR.07.2660.
36. Yamashita K., Yoshiura T., Hiwatashi A. et al. Differentiating primary CNS lymphoma from glioblastoma multiforme: assessment using arterial spin labeling, diffusion-weighted imaging, and (18)F-fluorodeoxyglucose positron emission tomography. *Neuroradiology.* 2013; 55 (2): 135–143. DOI: 10.1007/s00234-012-1089-6.
37. Omuro A.M., Leite C.C., Mokhtari K. et al. Pitfalls in the diagnosis of brain tumours. *Lancet Neurol.* 2006; 5 (11): 937–948. DOI: 10.1016/S1474-4422(06)70597-X.
38. Prieto E., Marti-Climent J.M., Dominguez-Prado I. et al. Voxel-based analysis of dual-time-point 18F-FDG PET images for brain tumor identification and delineation. *J. Nucl. Med.* 2011; 52 (6): 865–872.
39. Lee J.W., Kang K.W., Park S.H., Lee S.M., Paeng J.C., Chung J.K., Lee M.C., Lee D.S. 18F-FDG PET in the assessment of tumor grade and prediction of tumor recurrence in intracranial meningioma. *European Journal of Nuclear Medicine and Molecular Imaging.* 2009; 36 (10): 1574–1582. dOI: 10.1007/s00259-009-1133-x.
40. Koç Z.P., Kara P.Ö., Dağtekin A. Detection of unknown primary tumor in patients presented with brain metastasis by F-18 fluorodeoxyglucose positron emission tomography/computed tomography. *CNS Oncol.* 2018; 7 (2): CNS12. DOI: 10.2217/cns-2017-0018.
41. Colavolpe C., Metellus P., Mancini J. et al. Independent prognostic value of pre-treatment 18-FDG-PET in high-grade gliomas. *J. Neurooncol.* 2012; 107 (3): 527–535. DOI: 10.1007/s11060-011-0771-6.
42. Colavolpe C., Chinot O., Metellus P. et al. FDG-PET predicts survival in recurrent high-grade gliomas treated with bevacizumab and irinotecan. *Neuro Oncol.* 2012; 14 (5): 649–657. DOI: 10.1093/neuonc/nos012.
43. Spence A.M., Muzi M., Graham M.M. et al. 2-[(18)F]Fluoro-2-deoxyglucose and glucose uptake in malignant gliomas before and after radiotherapy: correlation with outcome. *Clin. Cancer Res.* 2002; 8 (4): 971–979.
44. Charnley N., West C.M., Barnett C.M. et al. Early change in glucose metabolic rate measured using FDG-PET in patients with high-grade glioma predicts response to temozolomide but not temozolomide plus radiotherapy. *Int. J. Radiat. Oncol. Biol. Phys.* 2006; 66 (2): 331–338. DOI: 10.1016/J.IJROBP.2006.04.043.
45. Caroline I., Rosenthal M.A. Imaging modalities in high-grade gliomas: pseudoprogression, recurrence, or necrosis? *J. Clin. Neurosci.* 2012; 19 (5): 633–637. DOI: 10.1016/j.jocn.2011.10.003.
46. Nihashi T., Dahabreh I.J., Terasawa T. Diagnostic accuracy of PET for recurrent glioma diagnosis: a meta-analysis. *Am. J. Neuroradiol.* 2013; 34 (5): 944–950. DOI: 10.3174/ajnr.A3324.
47. Basu S., Alavi A. Molecular imaging (Pet) of brain tumors? *Nneuroimaging. Clin. N. Amer.* 2009; 19 (4): 625–646. DOI: 10.1016/j.nic.2009.08.012.
48. Katsanos A.H., Alexiou G.A., Fotopoulos A.D., Jabbour P., Kyritsis A.P., Sioka C. Performance of 18F-FDG, 11C-Methionine, and 18F-FET PET for Glioma Grading: A Meta-analysis. *Clinical Nuclear Medicine.* 2019; 44 (11): 864–869. DOI: 10.1097/RLU.0000000000002654.
49. Glaudemans A.W., Enting R.H., Heesters M.A. et al. Value of 11C-methionine PET in imaging brain tumours and metastases. *Eur. J. Nucl. Med. Mol. Imaging.* 2013; 40 (4): 615–635. DOI: 10.1007/s00259-012-2295-5.
50. He Q., Zhang L., Zhang B., Shi X., Yi C., Zhang X. Diagnostic accuracy of ^{13}N-ammonia PET, ^{11}C-methionine PET and ^{18}F-fluorodeoxyglucose PET: a comparative study in patients with suspected cerebral glioma. *BMC Cancer.* 2019: 19 (1): 332. DOI: 10.1186/s12885-019-5560-1.
51. Takenaka S., Asano Y., Shinoda J. et al. Comparison of (11)C-methionine, (11)C-choline, and (18)F-fluorodeoxyglucose-PET for distinguishing glioma recurrence from radiation necrosis. *Neurol. Med. Chir. (Tokyo)*. 2014; 54 (4): 280–289. DOI: 10.2176/nmc.oa2013-0117.
52. Filss C.P., Galldiks N., Stoffels G., Sabel M., Wittsack H.J., Turowski B., Antoch G., Zhang K., Fink G.R., Coenen H.H. et al. Comparison of 18F-FET PET and perfusion-weighted MR imaging: a PET/MR imaging hybrid study in patients with brain tumors. *J. Nucl. Med.* 2014; 55 (4): 540–545. DOI: 10.2967/jnumed.113.129007.
53. Kebir S., Weber M., Lazaridis L. et al. Hybrid 11C-MET PET/MRI combined with «machine learning» in glioma diagnosis according to the revised glioma WHO classification 2016. *Clin. Nucl. Med.* 2019; 44 (3): 214–220. DOI: 10.1097/RLU.0000000000002398.
54. Jung T.Y., Jung S., Ryu H.S. et al. The application of magnetic resonance imaging-deformed 11c-methionine-positron emission tomography images in stereotactic radiosurgery.

Stereotact. Funct. Neurosurg. 2019; 97 (4): 217–224. DOI: 10.1159/000503732.
55. Hotta M., Minamimoto R., Miwa K. 11C-methionine-PET for differentiating recurrent brain tumor from radiation necrosis: radiomics approach with random forest classifier. *Sci. Rep.* 2019; 9 (1): 156–166. DOI: 10.1038/s41598-019-52279-2.
56. Skvortsova T.Y., Gurchin A.F., Savintseva Z.I. C-methionine PET in assessment of brain lesions in patients with glial tumors after combined treatment. *Zh. Vopr. Neirokhir. im. N. N. Burdenko.* 2019; 83 (2): 27–36. DOI: 10.17116/neiro20198302127.
57. Dandois V., Rommel D., Renard L. et al. Substitution of 11C-methionine PET by perfusion MRI during the follow-up of treated high-grade gliomas: preliminary results in clinical practice. *J. Neuroradiol.* 2010; 37 (2): 89–97. DOI: 10.1016/J.NEURAD.2009.04.005.
58. Schinkelshoek M., Lopci E., Clerici E. et al. Impact of 11C-methionine positron emission tomography/computed tomography on radiation therapy planning and prognosis in patients with primary brain tumors. *Tumori.* 2018; 104 (6): 480. DOI: 10.1700/1778.19268.
59. Goldbrunner R., Ruge M., Kocher M., Lucas C.W., Galldiks N., Grau S. The treatment of gliomas in adulthood. *Dtsch. Arztebl. Int.* 2018; 115 (20-21): 356–364. DOI: 10.3238/arztebl.2018.0356.
60. Borbély K., Nyáry I., Tóth M., Ericson K., Gulyás B. Optimization of semi-quantification in metabolic PET studies with 18F-fluorodeoxyglucose and 11C-methionine in the determination of malignancy of gliomas. *J. Neurol. Sci*. 2006; 246 (1-2): 85–94. DOI: 10.1016/j.jns.2006.02.015.
61. Xu W., Gao L., Shao A., Zheng J., Zhang J. The performance of 11C-Methionine PET in the differential diagnosis of glioma recurrence. *Oncotarget.* 2017; 8 (53): 91030–91039. DOI: 10.18632/oncotarget.19024.
62. Muoio B., Giovanella L., Treglia G. Recent Developments of 18F-FET PET in Neurooncology. *Curr. Med. Chem.* 2018; 25 (26): 3061–3073. DOI: 10.2174/0929867325666171123202644.
63. Jansen N.L., Suchorska B., Wenter V. et al. Dynamic 18F-FET PET in newly diagnosed astrocytic low-grade glioma identifies high-risk patients. *J. Nucl. Med.* 2014; 55 (2): 198–203. DOI: 10.2967/JNUMED.113.122333.
64. Jansen N.L., Suchorska B., Wenter V. et al. Prognostic significance of dynamic 18F-FET PET in newly diagnosed astrocytic high-grade glioma. *J. Nucl. Med.* 2015; 56 (1): 9–15. DOI: 10.2967/jnumed.114.144675.
65. Jansen N.L., Graute V., Armbruster L. et al. MRI-suspected low-grade glioma: is there a need to perform dynamic FET PET? *Eur. J. Nucl. Med. Mol. Imaging.* 2012; 39 (6): 1021–1029. DOI: 10.1007/s00259-012-2109-9.
66. Galldiks N., Langen K.J., Holy R. et al. Assessment of treatment response in patients with glioblastoma using O-(2-18F-fluoroethyl)-L-tyrosine PET in comparison to MRI. *J. Nucl. Med.* 2012; 53 (7): 1048–1057. DOI: 10.2967/jnumed.111.098590.
67. Suchorska B., Jansen N.L., Linn J. et al. Biological tumor volume in 18FET-PET before radiochemotherapy correlates with survival in GBM. *Neurology.* 2015; 84 (7): 710–719. DOI: 10.1212/WNL.0000000000001262.
68. Floeth F.W., Pauleit D., Sabel M. et al. Prognostic value of O-(2-18F-fluoroethyl)-L-tyrosine PET and MRI in low-grade glioma. *J. Nucl. Med.* 2007; 48 (4): 519–527. DOI: 10.2967/jnumed.106.037895.
69. Walter F., Cloughesy T., Walter M.A. et al. Impact of 3,4-dihydroxy-6-18F-fluoro-L-phenylalanine PET/CT on managing patients with brain tumors: the referring physician's perspective. *J. Nucl. Med.* 2012; 53 (3): 393–398. DOI: 10.2967/jnumed.111.095711.
70. Fueger B.J., Czernin J., Cloughesy T. et al. Correlation of 6-18F-fluoro-L-dopa PET uptake with proliferation and tumor grade in newly diagnosed and recurrent gliomas. *J. Nucl. Med.* 2010; 51 (10): 1532–1538. DOI: 10.2967/jnumed.110.078592.
71. Shen G., Ma H., Pang F., Ren P., Kuang A. Correlations of 18F-FDG and 18F-FLT uptake on PET with Ki-67 expression in patients with lung cancer: a meta-analysis. *Acta Radiol.* 2018; 59 (2): 188–195. DOI: 10.1177/0284185117706609.
72. Chen W., Delaloye S., Silverman D.H.S. et al. Predicting treatment response of malignant gliomas to bevacizumaband irinotecan by imaging proliferation with [18F] fluoro-thymidine positron emission tomography: a pilot study. *J. Clin. Oncol.* 2007; 25 (30): 4714–4721. DOI: 10.1200/JCO.2006.10.5825.
73. Bekaert L., Valable S., Lechapt-Zalcman E. et al. [18F]-FMISO PET study of hypoxia in gliomas before surgery: correlation with molecular markers of hypoxia and angiogenesis. *Eur. J. Nucl. Med. Mol. Imaging.* 2017; 44 (8): 1383–1392. DOI: 10.1007/s00259-017-3677-5.
74. Van Dongen G.A., Huisman M.C., Boellaard R. et al. 89Zr-immuno-PET for imaging of long circulating drugs and disease targets: why, how and when to be applied? *Q. J. Nucl. Med. Mol. Imaging.* 2015; 59 (1): 18–38.
75. Kim H., Lee S.J., Davies-Venn C. et al. 64Cu-DOTA as a surrogate positron analog of Gd-DOTA for cardiac fibrosis detection with PET: pharmacokinetic study in a rat model of chronic MI. *Nucl. Med. Commun.* 2016; 37 (2): 188–196. DOI: 10.1097/MNM.0000000000000417.
76. Siitonen R., Peuhu E., Autio A. et al. ^{68}Ga-DOTA-E[c(RGDfK)]$_2$ PET imaging of SHARPIN-regulated integrin activity in mice. *J. Nucl. Med.* 2019; 60 (10): 1380–1387. DOI: 10.2967/jnumed.118.222026.
77. Soldevilla-Gallardo I., Medina-Ornelas S.S., Davanzo J., Pedrero-Piedras R. ^{68}Ga-DOTA-E-[c(RGDfK)]2 positron emission tomography-computed tomography in the evaluation of hepatic hemangioendothelioma epithelioid. *Rare Tumors.* 2019; 11: 2036361319831097. DOI: 10.1177/2036361319831097
78. Lee H.-K., Moon D.-H., Ryu J.-S. et al. Radioisotope-labeled complexes of glucose derivatives and kits for the preparation thereof. *Patent United States.* 2003. Pub. No. 2003/0120046 A.
79. Cheng D., Rusckowski M., Wang Y.., Liu Y., Liu G., Liu X., Hnatowich D. A brief evaluation of tumor imaging in mice with 99mTc-glucarate including a comparison with 18F-FDG. *Curr. Radiopharm.* 2011; 4 (1): 5–9. DOI: 10.2174/1874471011104010005.
80. Chen X., Li L., Liu F., Liu B. Synthesis and biological evaluation of technetium-99m-labeled deoxyglucose derivatives as imaging agents for tumor. *Bioorg. Med. Chem. Lett.* 2006; 16 (21): 5503–5506. DOI: 10.1016/j.bmcl.2006.08.050.

81. Chen Y., Huang Z.W., He L., Zheng S.L. et al. Synthesis and evaluation of a technetium-99m-labeled diethylentriamine-pentaacetate-deoxyglucose complex ^{99m}Tc-DTPA-DG as a potential imaging modality for tumors. *Fppl. Radiat. and Isot.* 2006; 64 (3): 342–347. DOI: 10.1016/j.apradiso.2005.08.004.
82. Seidensticker M., Ulrich G., Muehlberg F.L., Pethe A., Grosser O.S., Steffen I.G., Stiebler M., Goldschmidt J., Smalla K.H., Seidensticker R., Ricke J., Amthauer H., Mohnike K. Tumor cell uptake of 99mTc-labeled 1-thio-β-D-glucose and 5-thio-D-glucose in comparison with 2-deoxy-2-[18F]fluoro-D-glucose in vitro: kinetics, dependencies, blockage and cell compartment of accumulation. *Mol. Imaging Biol.* 2014; 16 (2): 189–198. DOI: 10.1007/s11307-013-0690-3.
83. Dapueto R., Aguiar R.B., Moreno M., Machado C.M., Marques F.L., Gambini J.P., Chammas R., Cabral P., Porcal W. Technetium glucose complexes as potential cancer imaging agents. *Bioorg. Med. Chem. Lett.* 2015; 25 (19): 4254–4259. DOI: 10.1016/j.bmcl.2015.07.098.
84. Chernov V.I., Triss S.V., Skuridin V.S., Lishmanov Yu.B. Thallium-199: a new radiopharmaceutical for myocardial perfusion imaging. *The International Journal of Cardiovascular Imaging.* 1996; 12 (2): 119–126. DOI: 10.1007/bf01880743.
85. Chernov V.I., Sinilkin I.G., Zelchan R.V., Medvedeva A.A., Lyapunov A.Yu., Bragina O.D., Varlamova N.V., Skuridin V.S. Experimental Study of 99mTc-Aluminum Oxide Use for Sentinel Lymph Nodes Detection. *AIP Conference Proceedings. 2*016; 1760: 020012. DOI: 10.1063/1.4960231
86. Zeltchan R., Medvedeva **A**., Sinilkin I., Bragina O., Chernov V., Stasyuk E., Rogov A., Il'ina E., Larionova L., Skuridin V., Dergilev A. Experimental study of radiopharmaceuticals based on technetium-99m labeled derivative of glucose for tumor diagnosis. *IOP Conf. Series: Materials Science and Engineering.* 2016; 135: 012054. DOI: 10.1088/1757-899X/135/1/012054.
87. Zeltchan R., Chernov V., Medvedeva **A**., Sinilkin I., Stasyuk E., Rogov A., Il›ina E., Skuridin V., Bragina O. Study of a Glucose Derivative Labeled with Technetium-99m as Potential Radiopharmaceutical for Cancer Diagnosis. Congress of the European Association of Nuclear Medicine, Barcelona, Spain in October 15–19, 2016. *Eur. J. Nucl. Med. Mol. Imaging*. 2016; 43 (Suppl. 1): 466.
88. Bragina O., von Witting E., Garousi J., Zelchan R., Sandström M., Medvedeva A., Orlova A., Doroshenko A., Vorobyeva A., Lindbo S., Borin J., Tarabanovskaya N., Sorensen J., Hober S., Chernov V., Tolmachev V. Phase I study of 99mTc-ADAPT6, a scaffold protein-based probe for visualization of HER2 expression in breast cancer. *Journal of Nuclear Medicine.* 2020; DOI: 10.2967/jnumed.120.248799. URL: http://jnm.snmjournals.org/content/early/2020/08/13/jnumed.120.248799.abstract.
89. Stasyuk E., Sкuridin V., Rogov A., Zelchan R., Sadkin V., Varlamova N., Nesterov E. 99mTc-labeled monosaccharide kits: Development methods and quality control. *Scientific Reports*. 2020; 10 (1): 5121 DOI: 10.1038/s41598-020-61707-7. URL: https://www.nature.com/articles/s41598-020-61707-7.pdf.
90. Shivamurthy V.K., Tahari A.K., Marcus C., Subramaniam R.M. Brain FDG PET and the diagnosis of dementia. *Am. J. Roentgenol.* 2015; 204 (1): 76–85. DOI: 10.2214/AJR.13.12363.
91. Nestor P.J., Altomare D., Festari C., Drzezga A., Rivolta J., Walker Z., Bouwman F., Orini S., Law I., Agosta F., Arbizu J., Boccardi M., Nobili F., Frisoni G.B. EANM-EAN task force for the prescription of fdg-pet for dementing neurodegenerative disorders. Clinical utility of FDG-PET for the differential diagnosis among the main forms of dementia. *Eur. J. Nucl. Med. Mol. Imaging.* 2018; 45 (9): 1509–1525. DOI: 10.1007/s00259-018-4035-y.
92. Wilson H., Pagano G., Politis M. Dementia spectrum disorders: lessons learnt from decades with PET research. *Journal of Neural. Transmission*. 2019; 126 (3): 233–251. DOI: 10.1007/s00702-019-01975-4.
93. Zukotynski K., Kuo P.H., Mikulis D., Rosa-Neto P., Strafella A.P., Subramaniam R.M., Black S.E. PET/CT of Dementia. *Am. J. Roentgenol.* 2018; 211 (2): 246–259. DOI: 10.2214/AJR.18.19822.
94. Masdeu J.C. Neuroimaging of diseases causing dementia. *Neurol. Clin.* 2020; 38 (1): 65–94. DOI: 10.1016/j.ncl.2019.08.003.
95. Pagano G., Niccolini F., Politis M. Imaging in Parkinson's disease. *Clin. Med. (Lond.).* 2016; 16 (4): 371–375. DOI: 10.7861/clinmedicine.16-4-371.
96. Uzuegbunam B.C., Librizzi D., Hooshyar Yousefi B. PET Radiopharmaceuticals for Alzheimer's disease and Parkinson's disease diagnosis, the current and future landscape. *Molecules.* 2020; 25 (4): 977. DOI: 10.3390/molecules25040977.

Authors contribution

Zelchan R.V., Chernov V.I. – conception and design, analysis and interpretation of data, substantiation of the manuscript and critical revision of the manuscript for important intellectual content, final approval of the manuscript for publication. Medvedeva A.A., Bragina O.D., Rybina A.N., Ryabova A.I., Choinzonov E.L. – substantiation of the manuscript and critical revision of the manuscript for important intellectual content.

Authors information

Zelchan Roman V., Cand. Sci. (Med.), Senior Researcher, Department of Radionuclide Diagnostics, Cancer Research Institute, Tomsk NRMC, Tomsk, Russian Federation. ORCID 0000-0002-4568-1781.

Medvedeva Anna A., Cand. Sci. (Med.), Senior Researcher, Department of Radionuclide Diagnostics, Cancer Research Institute, Tomsk NRMC, Tomsk, Russian Federation. ORCID 0000-0002-5840-3625.

Rybina Anastasia N., Cand. Sci. (Med.), Radiologist, Department of Radionuclide Diagnostics, Cancer Research Institute, Tomsk NRMC, Tomsk, Russian Federation. ORCID 0000-0002-6488-0647.

Bragina Olga D., Cand. Sci. (Med.), Senior Researcher, Department of Radionuclide Diagnostics, Cancer Research Institute, Tomsk NRMC, Tomsk, Russian Federation. ORCID 0000-0001-5281-7758.

Ryabova Anastasia I., Cand. Sci. (Med.), Researcher, Head and Neck Cancer Unit, Cancer Research Institute, Tomsk NRMC, Tomsk, Russian Federation. ORCID 0000-0002-7171-8728.

Chernov Vladimir I., Dr. Sci. (Med.), Professor, Deputy Director for Research and Innovation of the Tomsk NRMC; Head of the Department of Radionuclide Diagnostics, Cancer Research Institute, Tomsk NRMC, Tomsk, Russian Federation. ORCID 0000-0001-8753-7916.

Choynzonov Evgeny L., Dr. Sci. (Med.), Professor, Academician of RAS, Director of the Cancer Research Institute, Tomsk NRMC, Tomsk, Russian Federation. ORCID 0000-0002-3651-0665.

(✉) **Zelchan Roman V.,** e-mail: r.zelchan@yandex.ru

Received 26.01.2021
Accepted 25.05.2021

УДК 616.211-002-056.3-092
https://doi.org/10.20538/1682-0363-2021-4-143-152

Synaptic transmission molecules and their role in the pathogenesis of allergic rhinitis

Klimov A.V.[1], Kalyuzhin O.V.[2], Klimov V.V.[1], Naidina O.A.[1]

[1] Siberian State Medical University (SSMU)
2, Moscow Trakt, Tomsk, 634050, Russian Federation

[2] I.M. Sechenov First Moscow State Medical University (Sechenov University)
8/2, Trubetskaya Str., Moscow, 119991, Russian Federation

ABSTRACT

Immune cells and molecules, as well as synaptic transmission molecules play a regulatory role in the communication pathways of the entire body when it is necessary to engage all body resources in the fight against infections or tumor cells wherever they appear. In potential allergy, the neuroimmune network controls allergen tolerance maintenance at both local and systemic levels.

The review focuses on different neurotransmitters and our understanding of a balance and imbalance between the immune system and the nervous system in allergic inflammation, including allergic rhinitis. However, the pathogenesis of the two endotypes of rhinitis (conventional allergic rhinitis and local allergic rhinitis) and the impact of the neuroimmune network on it remain unresolved.

Key words: allergic rhinitis, neurotransmitters, neurohormones, neuropeptides, receptors for neuro molecules.

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: Klimov A.V., Kalyuzhin O.V., Klimov V.V., Naidina O.A. Synaptic transmission molecules and their role in the pathogenesis of allergic rhinitis. *Bulletin of Siberian Medicine.* 2021; 20 (4): 143–152. https://doi.org/10.20538/1682-0363-2021-4-143-152.

Синаптические нейромолекулы и их роль в патогенезе аллергического ринита

Климов А.В.[1], Калюжин О.В.[2], Климов В.В.[1], Найдина О.А.[1]

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

[2] Первый Московский медицинский государственный университет им. И.М. Сеченова (Сеченовский университет)
Россия, 119991, г. Москва, ул. Трубецкая, 8/2

РЕЗЮМЕ

Иммунные клетки и молекулы, а также синаптические нейромолекулы играют регуляторную роль в путях коммуникации на уровне всего организма, когда возникает необходимость максимального вовлечения ресурсов для отражения инфекций и подавления опухолей. При потенциальной аллергии нейроиммунная сеть контролирует поддержание аллергенной толерантности и на системном, и на локальном уровнях.

✉ *Klimov Vladimir V.,* e-mail: klimov@mail.tomsknet.ru

Данный обзор фокусируется на рассмотрении разных нейромолекул и нашем понимании баланса и дисбаланса иммунной и нервной систем при аллергическом воспалении, включая аллергический ринит. Однако все еще остается нерешенным вопрос о механизмах патогенеза двух эндотипов ринита, классического аллергического ринита и локального аллергического ринита, и степени влияния на него нейроиммунной сети.

Ключевые слова: аллергический ринит, нейротрансмиттеры, нейрогормоны, нейропептиды, рецепторы для нейромолекул.

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: Климов А.В., Калюжин О.В., Климов В.В., Найдина О.А. Синаптические нейромолекулы и их роль в патогенезе аллергического ринита. *Бюллетень сибирской медицины.* 2021; 20 (4): 143–152. https://doi.org/10.20538/1682-0363-2021-4-143-152.

INTRODUCTION

Due to a narrow focus of disciplines, biology and biomedical sciences used to develop in isolation from each other in the past. In particular, immunology was isolated from neurobiology. Currently, there is clear evidence for production and use of immune factors by the central nervous system and production and use of neuroendocrine mediators by the immune system. Alterations in communication pathways between these two systems can account for many pathological conditions that used to be considered isolated diseases of certain organs [1]. The role of neurotransmitters in the pathogenesis of allergic inflammation, including different endotypes of allergic rhinitis [2], made this problem very relevant.

INNERVATION OF TARGET ORGANS IN ALLERGIC INFLAMMATION

Target organs in allergic inflammation are innervated differently and, consequently, are exposed to effects of a distinct set of synaptic transmission molecules [3] (Table). The skin is innervated with somatosensory neurons with their cell bodies located in the trigeminal ganglion and dorsal root ganglion, which have central brainstem and spinal cord projections. The gastrointestinal tract is innervated with:

(1) extrinsic sensory neurons originating in the dorsal root ganglia;

(2) vegetative neurons which are divided into parasympathetic neurons (vagus nerve), whose cell bodies reside in the nodose and jugular ganglia and brainstem, and sympathetic neurons, whose cell bodies reside in the paravertebral ganglia;

(3) self-contained autonomic nervous system called the enteric nervous system, which consists of intrinsic primary afferent neurons, interneurons, and myenteric and submucosal plexuses. The enteric nervous system can accept vegetative signals, regulate gut microbiota, mucus production, and peristalsis, and respond to food consumption. This system appears to maintain allergen tolerance in the intestine.

In contrast to the skin and gastrointestinal tract, innervation of the unified airway is characterized by distinctive features. These organs are innervated by (1) somatosensory neurons with their cell bodies located in the thoracic dorsal root ganglia, and (2) autonomic nervous system via parasympathetic and sympathetic fibers. However, the unified airway has no self-contained nervous system [3] that can matter for the development of local forms of allergy, such as asthma [4, 5], local allergic rhinitis (LAR) [6, 7], dual allergic rhinitis [8, 9], and local allergic conjunctivitis [10].

Neurons of all types produce different neuromolecules (Table), which act on the neurons and target organs and cells, including cells of the immune system [11]. Within the neuroimmune network, cells of the immune system acquire more functional plasticity. On the other hand, all types of neuronal activity are modulated by cells of the immune system and modified depending on receptors expressed on the target cells [12].

NEUROTRANSMITTERS, NEUROHORMONES, AND NEUROPEPTIDES

For a neuromolecule to belong to neurotransmitters, it must meet some criteria:

(1) it must be produced by neurons;

(2) it should be present in the presynaptic membrane of the first neuron and released in amounts sufficient to exert a defined action on the postsynaptic membrane of the second neuron or target cells in effector organs;

(3) exogenous administration should mimic the effect of an endogenously synthesized neurotransmitter;

(4) intrinsic mechanisms must exist to remove neurotransmitters from their site of action [13, 14].

Neurotransmitters are stored in small synaptic vesicles, but also found in the blood and target organs. On the one hand, neurotransmitters influence innate and adaptive immune responses. On the other hand, immune cells send signals to the brain through cytokines and are present in the brain to influence neuronal processes [12]. Some neuromolecules, including dopamine, L-glutamate, serotonin, and substance P, are crucial in the classical neuroimmune network [15]. The neuroimmune network is closely associated with the hypothalamic-pituitary-adrenal (HPA) axis, vagus nerve, sympathetic nervous system, and synapse from the vagus nerve to the spleen [15]. Depending on their defined action, all neurotransmitters may be categorized as excitatory (*proimmunogenic*), inhibitory (*protolerogenic*), and modulatory (*immunomodulatory*). Destroyed bidirectional communication between the nervous and immune systems is a prerequisite in immunopathological disorders [15, 16] (Table).

Table

Types of synaptic transmission molecules

Category	Characterization of signal transmission	Impact on immune system in the context of allergic inflammation	Molecules
Excitatory neuro-transmitters	For a short time, they've managed to increase the electrical excitability on the postsynaptic membrane due to ion flow that leads to the facilitation of signal transmission	Proimmunogenic, proinflammatory (except for norepinephrine)	Acetylcholine* Norepinephrine* Dopamine* L-glutamate* Histamine
Inhibitory neuro-transmitters	For a short time, they've managed to decrease the electrical excitability on the postsynaptic membrane due to ion flow that results in the reduction of signal transmission	Protolerogenic, anti-inflammatory	Serotonin* γ aminobutyric acid (GABA) Dopamine* Glycine
Modulatory neurotransmitters	They spend for a long time in the cerebrospinal fluid that affects the activity of other neurons, and target cells	Immunomodulatory	Acetylcholine Norepinephrine Dopamine L-glutamate Serotonin
Neurohormones	They act in the whole body	Immunomodulatory	Oxytocin Vasopressin Melatonin
Neuropeptides	They are slow-onset long-lasting modulatory synaptic neuro molecules packaged in large granular vesicles	Immunomodulatory	Substance P Calcitonin gene-related peptide (CGRP) Neuromedin U Vasoactive intestinal peptide
Atypical neuro-transmitters (neurochemicals)	They are synthesized «on- demand» and released from the postsynaptic membrane	Immunomodulatory	Nitric oxide Carbon monoxide Hydrogen sulfide Lipid mediators Adenosine Angiotensin-converting enzyme (ACE) Endocannabinoids

* also immunomodulatory effects.

The brain also synthesizes molecules, neurochemicals, neurohormones, and neuropeptides, which act on various receptors of immune cells but do not meet the criteria for neurotransmitters [17–20]. So far, only 12 small molecule neurotransmitters and over 100 neuropeptides have been identified [11]. During crosstalk between the nervous system and the immune system, most neuromolecules exploit membrane vesicles,

ligand- and voltage-gated ion channels, transporters for extracellular transport and entry into cells, as well as G protein-coupled receptors for signaling [15].

IMPACT OF NEUROMOLECULES ON CELLS OF THE IMMUNE SYSTEM

In response to environmental allergens (Figure), nasal epitheliocytes produce alarmins, IL(interleukin)-25, IL-33, and thymic stromal lymphopoietin (TSLP), which, together with neuromedin U [21], upregulate group 2 innate lymphoid cells (ILC2) [22], dendritic cells (DCs), and type 2 helper T (Th2) cells. These cytokines are essential regulators of type 2 immunity, as they lead to the production of IL-13 and IL-5.

Allergens that pass through the unified airway epithelial barriers are processed by DCs, which, in turn, migrate to draining lymph nodes, where they present allergen-derived peptides on HLA class II molecules to naïve T cells. The naïve T cells can differentiate into Th2 cells and follicular helper T (Tfh) cells. Th2 cells produce type 2 cytokines, such as IL-4, IL-5, IL-9, and IL-13 and function as effector cells that drive many aspects of allergic inflammation. Tfh cells produce IL-21, IL-4, and IL-13, which promote IgE class switch recombination in B cells, plasma cell maturation, and allergen-specific IgE production. Allergen-specific IgE antibodies bind to FcεRI molecules on mast cells and basophils, resulting in their degranulation and allergic inflammation development due to histamine and other mediators [23, 24]. In theory, proimmunogenic neuromediators must upregulate allergen tolerance breakdown, whereas protolerogenic neuromolecules should inhibit the process.

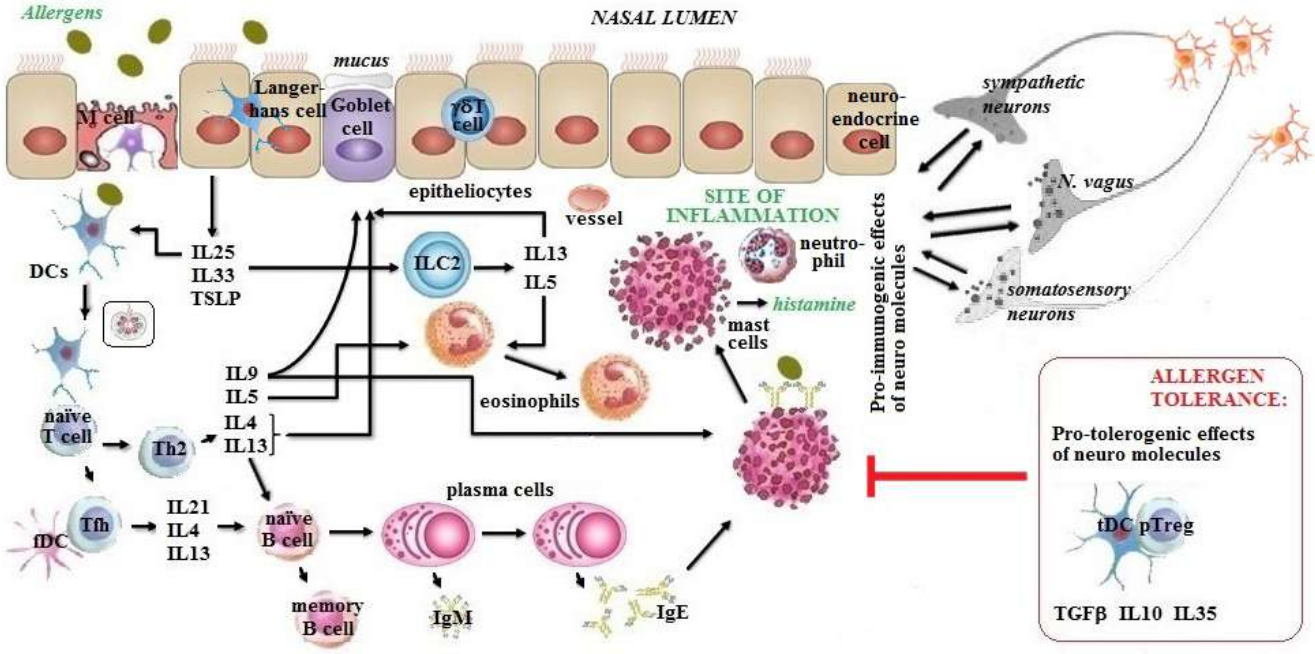


Fig. Allergen tolerance breakdown in allergic rhinitis in the context of the role of neuromolecules

Acetylcholine

Acetylcholine is the main neurotransmitter of the parasympathetic nervous system synthesized in neurons from choline and acetyl-coenzyme A by choline acetyltransferase. A deficit of acetylcholine in the cerebral cortex and hippocampus progressively leads to Alzheimer's disease [25]. Commonly, immune cells, such as T cells and monocytes, can interact with cholinergic nerves associated with lymphatic vessels or acetylcholine synthesized by immune cells themselves [13]. Functioning via muscarinic and nicotinic receptors, acetylcholine promotes differentiation of Th2 and degranulation of mast cells and basophils, but paradoxically downregulates ILC2 proliferation and upregulates T-regulatory (Treg) cells. Acetylcholine can bind to the muscarinic receptors M1AchR, M2AchR

and nicotinic receptor a7nAchR on the ciliated epithelial cells, resulting in mucus secretion [3, 12].

On the whole, a decrease in acetylcholine in target organs leads to reduction of cholinergic modulation promoting allergic inflammation [26]. Neuromedin U secretion by cholinergic neurons triggers ILC2 proliferation and expression of Th2 cytokines, including IL-5 and IL-13 [27]. Solitary chemosensory cells in the nasal cavity can use cholinergic neurotransmission to induce the neurogenic inflammation pathway [28].

Norepinephrine

Norepinephrine (noradrenaline) is a sympathetic neurotransmitter of the catecholamine family that mediates the fight-or-flight response and is produced in the brain neurons, especially inside the pons, sympathetic ganglia near the spinal cord, and adrenal medulla [13, 24]. Adrenergic receptors are expressed on immune cells, including T cells, B cells, macrophages, and natural killer (NK) cells. This neurotransmitter has a modulatory effect on the immune system. Norepinephrine mainly exerts anti-inflammatory effects by interacting with the adrenoreceptors expressed on lymphocytes and macrophages and inhibiting the production of TNFα, IL1β, and IFNγ and migration of lymphocytes from the lymph nodes to inflamed tissues. Norepinephrine binds to the β_2-adrenergic receptor on Th2 cells to suppress T cell activation [3]. Additionally, norepinephrine upregulates the production of IL-10 [13], limits ILC2-dependent type 2 inflammation, and counterbalances the effects of neuromedin U to prevent overactivation of ILC2s [29]. However, it may lower the activity of Treg cells. Interestingly, norepinephrine can promote inflammation in the initial phase of immune responses, whereas it downregulates inflammation in later phases [30].

Dopamine

Dopamine is a critical neurotransmitter of the catecholamine family, associated with emotions, pleasure, reward system, and gamble. A decrease in dopamine in the substantia nigra promotes Parkinson's disease, whereas its excess in the frontal lobes may result in schizophrenic episodes [25]. Dopamine is synthesized in the brain from L-tyrosine by tyrosine hydroxylase. It was identified in cells of the immune system, such as Treg cells, macrophages, granulocytes, T cells, and B cells. It functions via D_1–D_5 receptors promoting Th2 cell differentiation through D_4 [15]. D_1 activation on DCs upregulates Th2 and Th17 polarization, whereas signaling via this receptor expressed on Treg cells slows down the above-mentioned effect.

The communication between dopamine and CD4+ T cells is provided by an age-related mechanism underlying susceptibility to Th2-mediated allergic inflammation at an early age [31]. During B cell-mediated responses, the activity of dopamine in the brain is markedly elevating. However, direct effects of dopamine on the immune cells are contradictory, as they may also be immunosuppressive. Dopamine signaling through D_4 is known to suppress lymphocyte function by inhibiting a set of tyrosine kinases and transcription factors. Activation of D_1 and D_5 on Treg cells reduces their protolerogenic activity. Moreover, dopamine released from T cells can enhance intracellular reactive oxygen species (ROS) production, leading to oxidative stress and apoptosis in peripheral lymphocytes. In T cell-dependent responses, dopamine simultaneously displays increased production of TNF and IL-10 by naïve T cells [13, 24].

L-glutamate

L-glutamate is a critical neurotransmitter synthesized from glutamine in the brain by glutaminase and from a-ketoglutaric acid in the citric acid cycle [15]. L-glutamate influences the ability of learning and memory functioning through two groups of receptors: metabotropic (mGluRs) and ionotropic (iGluRs) glutamate receptors. In the brain, this neurotransmitter can contribute to neurotoxicity in multiple sclerosis and amyotrophic lateral sclerosis [25]. L-glutamate prevents apoptosis in activated T cells, facilitates TCR signaling, and promotes Th1 differentiation. Conversely, in some situations, L-glutamate can contribute to immunosuppression and resolution of chronic inflammation [15].

Serotonin

Serotonin (5-hydroxytryptamine, 5-HT) is a critical neurotransmitter synthesized from L-tryptophan in enterochromaffin cells of the gastrointestinal tract, epithelial neuroendocrine cells, and neurons of the central nervous system and taken up by platelets, basophils, and mast cells. On the one hand, serotonin transmission between neurons in the brain is responsible for mood, pleasure, sleep, and appetite. Serotonin may play an essential role in behavioral and psychological manifestations in depression, anxiety, obsessive-compulsive disorder, impulse control disorder, autism spectrum disorder, and attention deficit hyperactivity disorders [25].

On the other hand, it predominanly promotes the immunosuppressive effects and in some cases – immunostimulatory effects [32, 33]. Serotonin functions

through 5-HT1-5-HT7 receptors as a protolerogenic neurotransmitter inhibiting the production of proinflammatory cytokines, such as TNFα and IL-12, and canceling Th1 and Th17 polarization in immunopathology. Additionally, serotonin inhibits CXCL10 production, maturation of proinflammatory DCs, and promotes differentiation of tolerogenic DCs and synthesis of IL-10 [34]. During B cell-mediated responses, the activity of serotonin in the brain markedly decreases. However, only via the 5-HT2B receptor, serotonin has proimmunogenic effects in the context of Th1 and Th17 polarization [35].

GABA

Gamma aminobutyric acid (GABA) is a major inhibitory neurotransmitter in the central nervous system displaying anti-anxiety effects. Loss of GABA in the brain may be a prerequisite of an epileptic attack [13]. GABA is produced by glutamic acid decarboxylase from L-glutamate in the brain and spinal cord neurons and other cells (e.g., epithelial neuroendocrine cells) in many organs, including the unified airway. There are two main GABA receptors: $GABA_A$ and $GABA_B$, which mediate the activity of this neurotransmitter [36–38]. To date, GABAergic mechanisms have been demonstrated in many parts of the body, including synthesis in epithelial neuroendocrine cells [12]. In the immune system, GABA exhibits anti-inflammatory and immunosuppressive effects inhibiting Th1 and Th17 cells differentiation. However, the amount of GABA in the brain during inflammation in the body increases.

Oxytocin

Oxytocin is a neurohormone of the posterior pituitary mediating stress resilience, well-being, social interaction, growth, feeling of love, childbirth, and regeneration [39]. It is synthesized as an inactive precursor protein encoded by the *OXT* gene, undergoes a series of enzymes, and is released into the bloodstream. Oxytocin maintains immune homeostasis, allergen tolerance, and immune defense, acting via oxytocin receptors. Moreover, oxytocin downregulates allergic inflammation, autoimmune processes, and stress-associated immune disorders [40].

Melatonin

Melatonin, a hormone of the pineal gland (or epiphysis), has multiple effects. It acts as a biorhythmic regulator, modulates many of the immune processes related to stress responses, and regulates the level of glucose and cholesterol in the blood. Regarding the immune system, melatonin is exhibits proimmunogenic and anti-tumor effects. It has proinflammatory effect in asthma, leading to bronchoconstriction [41]. However, it disables inflammasome NLRP3 [42] and limits oxidative stress.

Substance P

Substance P is a critical neuropeptide of the neuroimmune network released from the terminals of specific somatosensory nerves in the brain, regulating emotions, as well as in most peripheral regions of the nervous system. It is synthesized in many immune cells [13, 15, 43]. Substance P exerts its biological effect through neurokinin receptors, which are found in close proximity to cells containing serotonin and norepinephrine. Substance P amplifies Th1- or Th17-mediated inflammatory responses, secretion of proinflammatory cytokines by T cells and macrophages, and production of immunoglobulins by plasma cells. However, CD8+T cells and NK cells show reduced activity in the presence of substance P [15].

Calcitonin gene-related peptide (CGRP)

Calcitonin gene-related peptide (CGRP) exists in two isoforms, α and β [13], encoded by separate genes and synthesized due to alternative splicing. α-CGRP is released from sensory neurons of the central nervous system, spinal cord, and trigeminal ganglion. In contrast, β-CGRP is mainly produced by organs of the immune system, immune cells, and epithelial neuroendocrine cells.

CGRP receptors are found throughout the body, suggesting that this neuropeptide may modulate a wide range of physiological functions and pathological reactions, including neurogenic inflammation. CGRP is responsible for transmission of pain (migraine), decreased appetite, and increased heart rate. Additionally, CGRP inhibits activation of ILC2 [44] and differentiation of Th2 [45]. Therefore, CGRP acts as an anti-inflammatory mediator responsible for preventing tissue damage during allergic and other types of inflammation.

Neuroimmune regulation in allergic rhinitis

It was shown that allergic rhinitis induced anxiety-like behavior in humans and altered social interaction in rodents, along with the increased expression of Th2 cells [46]. Interestingly, the immunosuppressive effect of tryptophan, a precursor to the neurotransmitter serotonin, was demonstrated. Increased serum tryptophan concentrations were reported in patients with seasonal rhinitis and found only outside the pollen season and not during it. Besides, the association

of elevated tryptophan concentrations with a poor response to allergen-specific immunotherapy was demonstrated [47].

The well-studied GABAergic system exists not only in the brain but also in airway epithelial cells playing a protolerogenic role. In experiments, the protolerogenic neurotransmitter GABA inhibits overproduction of mucus and synthesis of IL-13 in mice with respiratory allergic reactions induced by ovalbumin [48]. It is known that the proimmunogenic neurotransmitter L-glutamate is a precursor of GABA. Interestingly, L-glutamate concentration in the nasal mucosa in patients with allergic rhinitis is significantly higher, whereas GABA corresponds to the control level [49].

In a pilot study [50], patients with allergic rhinitis were exposed to a standardized Trier Social Stress Test (TSST), followed by allergy skin tests. Stress responders were estimated based on salivary cortisol concentrations, anxiety scale, and serum norepinephrine and oxytocin levels. The baseline concentrations, independent of TSST, were significantly higher in allergic individuals. Therefore, it indicates that patients with allergic rhinitis are less resistant to stress. In another study, mast cells and macrophages in the mucosa expressed oxytocin receptors against the background of elevated oxytocin concentrations, which could indicate the presence of local allergic responses, linking neuron-mediated emotions and inflammation [51]. Unfortunately, similar clinical studies in selected groups with conventional allergic rhinitis and LAR in humans have not yet been carried out.

CONCLUSION

The accumulation of knowledge about synaptic transcription molecules led to the concept that neuronal signaling can produce neurogenic inflammation [52]. It has become clear that neuronal regulation of immunity plays an essential role in the context of allergic inflammation [3]. Mast cells, which take part in inflammation, are in close contact with nerves in the nasal mucosa [53]. Eosinophils, another key innate effector cell type in allergic reactions, were also found to be localized close to cholinergic nerves in allergic rhinitis [54]. Allergic inflammation in the respiratory tract involves a complex crosstalk between neurons and immune cells that could play a critical role in mediating disease progression. The nervous system could be a novel and exciting target in this process [3].

Neurons secrete mediators, including neurotransmitters and neuropeptides, which act on their cognate receptors on cells engaged in inflammation to drive or regulate immunity. These bidirectional neuroimmune interactions occur early and significantly influence the onset and development of allergic inflammation. On the whole, the molecular mechanisms of neurogenic inflammation are not completely understood. In the presence of nasal allergic inflammation, the neuronal function can also be chronically upregulated depending on stimulation of nociceptors and neurotrophins, such as nerve growth factor (NGF) [55].

In the context of allergic inflammation in allergic rhinitis associated with the neuroimmune network, we considered the neuromolecules with predominant proimmunogenic effects (such as acetylcholine, dopamine, L-glutamate, melatonin, and substance P) and protolerogenic effects (such as serotonin, GABA, norepinephrine, oxytocin, and CGRP). At the local (nasal) level, allergen tolerance is mainly associated with the peculiarities of the innervation and an appropriate set of neurotransmitters in the nasal cavity [3, 13]. When systemic allergen tolerance maintenance is still available, but the appropriate neurotransmitter imbalance occurs in the nose, the autonomic nervous system may be responsible for autonomous allergen tolerance breakdown that results in LAR.

We proposed a hypothesis to be tested that the autonomous allergen tolerance breakdown in the nose may be caused by an imbalance of proimmunogenic and protolerogenic neurotransmitters with a lower concentration of the latter [56]. The neurotransmitter imbalance paradigm seems to be among possible explanations of the pathogenesis of LAR in individuals with atopic predisposition, but requires further study and discussion.

REFERENCES

1. Dantzer R. Neuroimmune interactions: From the brain to the immune system and vice versa. *Physiol. Rev.* 2018; 98: 477–504. DOI: 10.1152/physrev.00039.2016.
2. Klimov A.V., Isaev P.Yu., Klimov V.V., Sviridova V.S. Endotypes of allergic rhinitis and asthma accompanying food allergy. *Bull. Sib. Med.* 2019; 18 (2): 287–289. DOI: 10.20538/1682-0363-2019-2-287-289.
3. 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.
4. Campo P., Eguíluz-Gracia I., Plaza-Serón M., Salas M., Rodríguez M.J., Pérez-Sánchez N., González M., Molina A., Mayorga C., Torres M.J., Rondón C. Bronchial asthma triggered by house dust mites in patients with local allergic rhinitis. *Allergy.* 2019; 74 (8): 1502–1510. DOI: 10.1111/all.13775.
5. Kılıç E., Kutlu A., Hastalıkları G. et al. Does local allergy (entopy) exists in asthma? *J. Clin. Anal. Med.* 2016. Letters to editors from 01.02.2016. DOI: 10.4328/JCAM.3272.

6. Campo P., Eguiluz-Gracia I., Bogas G., Salas M., Plaza Seron C., Perez N., Mayorga C., Torres M.J., Shamji M.H., Rondón C. Local allergic rhinitis: implications for management. *Clin. Exp. Allergy.* 2019; 49 (1): 6–16. DOI: 10.1111/cea.13192.
7. Klimov A.V., Kalyuzhin O.V., Klimov V.V., Sviridova V.S. Allergic rhinitis and the phenomenon of entopy. *Bull. Sib. Med.* 2020; 3: 137–143. DOI: 10.20538/1682-0363-2020-3-137-143.
8. Eguíluz-Gracia I., Fernandez-Santamaria R., Testera-Montes A., Ariza A., Campo P., Prieto A., Pérez-Sánchez N., Salas M., Mayorga C., Rondón C. Coexistence of nasal reactivity to allergens with and without IgE sensitization in patients with allergic rhinitis. *Allergy.* 2020; 1: 1689–1698. DOI: 10.1111/all.14206.
9. Maoz-Segal R., Machnes-Maayan D., Veksler-Offengenden I., Frizinsky S., Hajyahia S., Agmon-Levin N. Local allergic rhinitis: An old story but a new entity. In: Gendeh B.S., Turkalj M. (eds.). Rhinosinusitis. London: IntechOpen, 2019: 1–9. DOI: 10.5772/intechopen.86212.
10. Yamana Y., Fukuda K., Ko R., Uchio E. Local allergic conjunctivitis: A phenotype of allergic conjunctivitis. *Int. Ophthalmol.* 2019; 39: 2539–2544. DOI: 10.1007/s10792-019-01101-z.
11. Cuevas J. Neurotransmitters and their life cycle. 2019. DOI: 10.1016/B978-0-12-801238-3.11318-2.
12. Kabata H., Artis D. Neuro-immune crosstalk and allergic inflammation. *J. Clin. Invest.* 2019; 129 (4): 1475–1482. DOI: 10.1172/JCI124609.
13. 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.
14. Hampel L., Lau T. Neurobiological principles: neurotransmitters. In: Riederer P., Laux G., Mulsant B., Le W., Nagatsu T. (ed.) NeuroPsychopharmacotherapy. *Cham: Springer,* 2020: 1–21. DOI: 10.1007/978-3-319-56015-1_365-1.
15. Hodo T.W., de Aquino M.T.P., Shimamoto A., Shanker A. Critical neurotransmitters in the neuroimmune network. *Front. Immunol.* 2020; 11: 1869. DOI: 10.3389/fimmu.2020.01869.
16. Wilkinson M., Brown R.E. Chapter 5 – Neurotransmitters. In: Wilkinson M., Brown R.E. (ed.) An introduction to neuroendocrinology. Cambridge: Cambridge University Press, 2015: 78–119. DOI: 10.1017/CBO9781139045803.006.
17. Meriney S.D., Fanselow E.E. Chapter 20 - Gaseous neurotransmitters. In: Meriney S.D., Fanselow E.E. (ed.) Synaptic Transmission. Cambridge: Academic Press, 2019: 435–447. DOI: 10.1016/B978-0-12-815320-8.00020-X.
18. Elphick M.R., Mirabeau O., Larhammar D. Evolution of neuropeptide signalling systems. *J. Exp. Biol.* 2018; 221 (3): 1–27. DOI: 10.1242/jeb.151092.
19. Silva-Vilches C., Ring S., Mahnke K. ATP and its metabolite adenosine as regulators of dendritic cell activity. *Front. Immunol.* 2018; 9: 2581. DOI: 10.3389/fimmu.2018.02581.
20. Fogaça M.V., Lisboa S.F., Aguilar D.C., Moreira F.A., Gomes F.V., Casarotto P.C., Guimarães F.S.Fine-tuning of defensive behaviors in the dorsal periaqueductal gray by atypical neurotransmitters. *Braz. J. Med. Biol. Res.* 2011; 45 (4): 357–365. DOI: 10.1590/S0100-879X2012007500029.
21. Ren X., Dong F., Zhuang Y., Wang Y., Ma W. Effect of neuromedin U on allergic airway inflammation in an asthma model (Review). *Exp. Ther. Med.* 2020; 19 (2): 809–816. DOI: 10.3892/etm.2019.8283.
22. Pasha M.A., Patel G., Hopp R., Yang Q. Role of innate lymphoid cells in allergic diseases. *Allergy Asthma Proc.* 2019; 40: 138–145. DOI: 10.2500/aap.2019.40.4217.
23. Schoos A.-M.M., Bullens D., Chawes B.L., De Vlieger L., DunnGalvin A., Epstein M.M., Johan Garssen J., Hilger C., Knipping K., Kuehn A., Mijakoski D., Munblit D., Nekliudov N.A., Ozdemir C., Patient K, Peroni D., Stoleski S., Stylianou E., Tukalj M., Verhoeckx K., Mihaela Zidarn M., van de Veen W. Immunological outcomes of allergen-specific immunotherapy in food allergy. *Front. Immunol.* 2020; 11: 568598. DOI: 10.3389/fimmu.2020.568598.
24. Klimov V.V. From basic to clinical immunology. Cham: Springer Nature, 2019: 378. DOI: 10.1007/978-3-030-03323-1.
25. Choudhury A., Sahu T., Ramanujam P.L., Banerjee A.K., Chakraborty I., Kumar A.R., Arora N. Neurochemicals, behaviours and psychiatric perspectives of neurological diseases. *Neuropsychiatry (London).* 2018; 8 (1): 395–424. DOI: 10.4172/Neuropsychiatry.1000361.
26. Bosmans G., Bassi G.S., Florens M., Gonzalez-Dominguez E., Matteoli G., Boeckxstaens G.E. Cholinergic modulation of type 2 immune responses. *Front. Immunol.* 2017; 8: 1873. DOI: 10.3389/fimmu.2017.01873.
27. Chen C.-S., Barnoud C., Scheiermann C. Peripheral neurotransmitters in the immune system. *Curr. Opin. Physiol.* 2021; 19: 73–79. DOI: 10.1016/j.cophys.2020.09.009.
28. Saunders C.J., Christensen M., Finger T.E., Tizzano M. Cholinergic neurotransmission links solitary chemosensory cells to nasal inflammation. *PNAS.* 2014; 111 (16): 6075–6080. DOI: 10.1073/pnas.1402251111.
29. Moriyama S., Brestoff J.R., Flamar A.L., Moeller J.B., Klose C.S.N., Rankin L.C., Yudanin N.A., Monticelli L.A., Putzel G.G., Rodewald H.-R., Artis D. Beta2-adrenergic receptor-mediated negative regulation of group 2 innate lymphoid cell responses. *Science.* 2018; 359: 1056–1061. DOI: 10.1126/science.aan4829.
30. Pongratz G., Straub R.H. The sympathetic nervous response in inflammation. *Arthritis Res. Ther.* 2014; 16 (6): 504–510. URL: http://arthritis-research.com/content/16/6/504
31. Wang W., Cohen J.A., Wallrapp A., Trieu K.G., Barrios J., Shao F., Krishnamoorthy N., Kuchroo V.K., Jones M.R., Fine A., Bai Y., Ai X. Age-related dopaminergic innervation augments T helper 2-type allergic inflammation in the postnatal lung. *Immunity.* 2019; 51: 1102–1118. DOI: 10.1016/j.immuni.2019.10.002.
32. Herr N., Bode C., Duerschmied D. The effects of serotonin in immune cells. *Front. Cardiovasc. Med.* 2017; 4: 48. DOI: 10.3389/fcvm.2017.00048.
33. Shajib M.S., Khan W.I. The role of serotonin and its receptors in activation of immune responses and inflammation. *Acta. Physiol. (Oxford).* 2015; 213: 561–574.
34. Švajger U., Rožman P. Induction of tolerogenic dendritic cells by endogenous biomolecules: An Update. *Front. Immunol.* 2018; 9: 2482. DOI: 10.3389/fimmu.2018.02482.
35. Szabo A., Gogolak P., Koncz G., Foldvari Z., Pazmandi K.,

Miltner M., Poliska S., Bacsi A., Djurovic S., Rajnavolgyi E. Immunomodulatory capacity of the serotonin receptor 5-HT2B in a subset of human dendritic cells. *Sci. Rep.* 2018; 8: 1765. DOI: 10.1038/s41598-018-20173-y.
36. Barragan A., Weidner J.M., Jin Z., Korpi E.R., Birnir B. GABAergic signalling in the immune system. *Acta. Physiol. (Oxford).* 2015; 213 (4): 819–827. DOI: 10.1111/apha.12467.
37. Jin Z., Mendu S.K., Birnir B. GABA is an effective immunomodulatory molecule. *Amino Acids.* 2013; 45 (1): 87–94. DOI: 10.1007/s00726-011-1193-7.
38. Dionisio L., De Rosa M.J., Bouzat C., Esandi M.D.C. An intrinsic GABAergic system in human lymphocytes. *Neuropharmacology.* 2011; 60 (2-3): 513–519. DOI: 10.1016/j.neuropharm.2010.11.007.
39. Moberg K.U., Handlin L., Kendall-Tackett K., Petersson M. Oxytocin is a principal hormone that exerts part of its effects by active fragments. *Medical Hypotheses.* 2019; 133: 1–9. DOI: 10.1016/j.mehy.2019.109394.
40. Li T., Wang P., Wang S.C., Wang Y.-F. Approaches mediating oxytocin regulation of the immune system. *Front. Immunol.* 2017; 7: 693. DOI: 10.3389/fimmu.2016.00693.
41. Marseglia L., D'Angelo G., Manti S., Salpietro C., Arrigo T., Barberi I., Reiter R.J., Gitto E. Melatonin and atopy: Role in atopic dermatitis and asthma. *Int. J. Mol. Sci.* 2014; 15 (8): 13482–13493. DOI: 10.3390/ijms150813482.
42. Hardeland R. Melatonin and inflammation – Story of a double-edged blade. *J. Pineal. Res.* 2018; 65 (4); e12525. DOI: 10.1111/jpi.12525.
43. Mashaghi A., Marmalidou A., Tehrani M., Grace P.T., Pothoulakis C., Dana R. Neuropeptide substance P and the immune response. *Cell. Mol. Life Sci.* 2016; 73 (22): 4249–4264. DOI: 10.1007/s00018-016-2293-z.
44. Wallrapp A., Burkett P.R., Riesenfeld S.J., Kim S.J., Christian E., Abdulnour R.E., Thakore P.I., Schnell A., Lambden C., Herbst R., Khan P., Tsujikawa K., Ramnik J. Xavier R.J., Chiu I.M., Levy B.D., Regev A., Kuchroo V.K. Calcitonin gene-related peptide negatively regulates alarmin-driven type 2 innate lymphoid cell responses. *Immunity.* 2019; 51: 709–723. DOI: 10.1016/j.immuni.2019.09.005.
45. Nagashima H., Mahlakoiv T., Shih H.Y., Davis F.P., Meylan F., Huang Y., Harrison O.J., Yao C., Mikami Y., Urban Jr. J.F., Caron K.M., Belkaid Y., Kanno Y., Artis D., O'Shea J.J. Neuropeptide CGRP limits group 2 INNATE LYMPHOID CELL responses and constrains type 2 inflammation. *Immunity.* 2019; 51: 682–695. DOI: 10.1016/j.immuni.2019.06.009.
46. Tonelli L.H., Katz M., Kovacsics C.E., Gould T.D., Joppy B., Hoshino A., Hoffman G., Komarow H., Postolache T.T. Allergic rhinitis induces anxiety-like behavior and altered social interaction in rodents. *Brain Behav. Immun.* 2009; 23 (6): 784–793. DOI: 10.1016/j.bbi.2009.02.017.
47. Gostner J.M., Becker K., Kofler H., Strasser B., Fuchs D. Tryptophan metabolism in allergic disorders. *Int. Arch. Allergy. Immunol.* 2016; 169: 203–215. DOI: 10.1159/000445500.
48. Xiang Y.-Y., Wang S., Liu M., Hirota J.A., Li J., Ju W., Fan Y., Kelly M.M., Ye B., Orser B., O'Byrne P.M., Inman M.D., Yang X., Lu W.-Y. A GABAergic system in airway epithelium is essential for mucus overproduction in asthma. *Nature Med.* 2007; 3 (7): 862–867. DOI: 10.1038/nm1604.
49. Lee H.-S., Goh E.-K., Wang S.-G., Chon K.-M., Kim H.-K, Roh H.-J. Detection of amino acids in human nasal mucosa using microdialysis technique: Increased glutamate in allergic rhinitis. *Asian Pac. J. Allergy.* 2006; 23 (4): 213–219. Access: https://www.researchgate.net/publication/7206172
50. Gotovina J., Pranger C.L., Jensen A.N., Palme R., Larenas-Linnemann D., Singh J., Mösges R., Felnhofer A., Glenk L.-M., Jensen-Jarolim E. Elevated oxytocin and noradrenaline indicate higher stress levels in allergic rhinitis patients: Implications for the skin prick diagnosis in a pilot study. *PLoS One.* 2018; 13 (5): e0196879. DOI: 10.1371/ journal.pone.0196879.
51. Szeto A., Nation D.A., Mendez A.J., Dominguez-Bendala J., Brooks L.G., Schneiderman N., Philip M., McCabe P.M. Oxytocin attenuates NADPH-dependent superoxide activity and IL-6 secretion in macrophages and vascular cells. *Am. J. Physiol. Endoc. M.* 2008; 295 (6): E1495–1501. DOI: 10.1152/ajpendo.90718.2008.
52. Carlton S.M. Nociceptive primary afferents: they have a mind of their own. *J. Physiol.* 2014; 592 (16): 3403–3411. DOI: 10.1113/jphysiol.2013.269654.
53. Le D.D., Schmit D., Heck S., Omlor A.J., Sester M., Herr C., Schick B., Daubeuf F., Fähndrich S., Bals R., Frossard N., Al Kadah B., Dinh Q.T. Increase of mast cell-nerve association and neuropeptide receptor expression on mast cells in perennial allergic rhinitis. *Neuroimmunomodulation.* 2016; 23: 261–270. DOI: 10.1159/000453068.
54. Thornton M.A., Akasheh N., Walsh M.T., Moloney M., Sheahan P.O., Smyth C.M., Walsh R.M., Morgan R.M., Curran D.R., Walsh M.T., Gleich G.J., Costello R.W. Eosinophil recruitment to nasal nerves after allergen challenge in allergic rhinitis. *Clin. Immunol.* 2013; 147: 50–57. DOI: 10.1016/j.clim.2013.02.008.
55. Sarin S., Undem B., Sanico A., Togias A. The role of the nervous system in rhinitis. *J. Allergy Clin. Immunol.* 2006; 118 (5); 999–1014. DOI: 10.1016/j.jaci.2006.09.013.
56. Klimov V.V., Klimov A.V. Autonomous breakdown of the allergen tolerance in the nose. *Authorea*. 2020. June 13. DOI: 0.22541/au.159204964.43963580.

Authors information

Klimov Andrew V., Cand. Sci. (Med.), Assistant, ENT Division, Siberian State Medical University, Tomsk, Russian Federation. ORCID 0000-0002-2776-5834.

Kalyuzhin Oleg V., Dr. Sci. (Med.), Professor, Clinical Immunology and Allergy Department, I.M. Sechenov First Moscow State Medical University (Sechenov University), Moscow, Russian Federation. ORCID 0000-0003-3628-2436.

Klimov Vladimir V., Dr. Sci. (Med.), Professor, Head of the Immunology and Allergy Division, Siberian State Medical University, Tomsk, Russian Federation. ORCID 0000-0001-6673-7556.

Naidina Oxana A., Cand. Sci. (Med.), Assistant, Immunology and Allergy Division, Siberian State Medical University, Tomsk, Russian Federation. ORCID 0000-0002-1407-2086.

(✉) **Klimov Vladimir V.,** e-mail: klimov@mail.tomsknet.ru

Received 10.02.2021
Accepted 25.05.2021

УДК 616.98:578.825]:616-006.52]-06:616.65:616.62]-006.6-092
https://doi.org/10.20538/1682-0363-2021-4-153-161

The role of herpes and human papillomavirus infection in prostate and bladder carcinogenesis

Mikhaleva L.M.[1, 2], Kamalov A.A.[3, 4], Maryin G.G.[4], Karpov V.K.[4], Akopyan E.P.[1, 2], Osmanov O.A.[4], Pechnikova V.V.[1, 2]

[1] *A.P. Avtsyn Research Institute of Human Morphology*
3, Tsyrupy Str., Moscow, 117418, Russian Federation

[2] *City Clinical Hospital No. 31*
42, Lobachevskogo Str., Moscow, 119415, Russian Federation

[3] *Lomonosov Moscow State University*
1, Leninskiye Gory, Moscow, 119991, Russian Federation

[4] *Russian Medical Academy of Postgraduate Education*
3–14, Solyanka Str., Moscow, 109240, Russian Federation

ABSTRACT

Human papillomavirus (HPV) is a small epithelial, non-enveloped, double-stranded DNA virus that belongs to the Papillomaviridae family. HPV infection is one of the most common sexually transmitted infections, and certain types of HPV are known to be carcinogenic to humans. According to the scientific literature, there is reliable information about the role of highly oncogenic HPV types in the development of cervical, anal, vulvar, vaginal, penile, and oropharyngeal cancer.

Currently, a relevant and promising research area is the study of the role of HPV infection in prostate cancer (PC) and bladder cancer (BC), but scientific data on the potential pathogenetic relationship between these phenomena remain contradictory. An in-depth study of the question how herpes and human papillomavirus affect the origin of malignant tumors of the prostate and bladder, as well as the course of these diseases, and the prognosis of their development can become a source of information for development of new approaches to their diagnosis, prevention, and monitoring of morbidity. This literature review analyzes the results of modern studies on the role of oncogenic HPV types in the carcinogenesis of PC and BC.

Key words: human papillomavirus, prostate cancer, bladder cancer, carcinogenesis.

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: Mikhaleva L.M., Kamalov A.A., Maryin G.G., Karpov V.K., Akopyan E.P., Osmanov O.A., Pechnikova V.V. The role of herpes and human papillomavirus infection in prostate and bladder carcinogenesis. *Bulletin of Siberian Medicine.* 2021; 20 (4): 153–161. https://doi.org/10.20538/1682-0363-2021-4-153-161.

✉ *Mikhaleva Liudmila M.,* e-mail: mikhalevalm@yandex.ru

Роль инфекции, вызванной вирусами герпеса и папилломы человека, в канцерогенезе предстательной железы и мочевого пузыря

Михалева Л.М.[1, 2], Камалов А.А.[3, 4], Марьин Г.Г.[4], Карпов В.К.[4], Акопян Э.П.[1, 2], Османов О.А.[4], Печникова В.В.[1, 2]

[1] Научно-исследовательский институт морфологии человека (НИИМЧ) имени академика А.П. Авцына
Россия, 117418, г. Москва, ул. Цюрупы, 3

[2] Городская клиническая больница (ГКБ) № 31
Россия, 119415, г. Москва, ул. Лобачевского, 42

[3] Московский государственный университет (МГУ) им. М.В. Ломоносова
Россия, 119991, г. Москва, Ленинские Горы, 1

[4] Российская медицинская академия непрерывного профессионального образования (РМАНПО)
Россия, 109240, г. Москва, ул. Солянка, 14/3

РЕЗЮМЕ

Вирус папилломы человека (ВПЧ) – это небольшой эпителиотропный, безоболочечный, двухцепочечный ДНК-вирус, который относится к семейству Papillomaviridae. Инфекция, вызванная ВПЧ, является одной из наиболее распространенных инфекций, передаваемых половым путем. Известно, что определенные типы ВПЧ относятся к канцерогенам для человека. По данным научной литературы, имеется достоверная информация о роли высокоонкогенных типов ВПЧ в развитии рака шейки матки, анального канала, вульвы, влагалища, полового члена и ротоглотки.

Актуальным и перспективным направлением исследования в настоящее время является изучение роли ВПЧ-инфекции в раке предстательной железы (РПЖ) и раке мочевого пузыря (РМП). Однако научные данные о потенциальной патогенетической связи между этими явлениями остаются противоречивыми. Углубленное изучение вопроса о том, как вирусы герпеса и папилломы человека влияют на происхождение злокачественных опухолей предстательной железы и мочевого пузыря, течение данных заболеваний и прогноз их развития, может стать источником информации для разработки новых подходов к их диагностике, профилактике и мониторингу заболеваемости. В данном обзоре проанализированы результаты современных исследований по проблеме участия онкогенных типов ВПЧ в канцерогенезе ПЖ и МП.

Ключевые слова: вирус папилломы человека, рак предстательной железы, рак мочевого пузыря, канцерогенез.

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: Михалева Л.М., Камалов А.А., Марьин Г.Г., Карпов В.К., Акопян Э.П., Османов О.А., Печникова В.В. Роль инфекции, вызванной вирусами герпеса и папилломы человека, в канцерогенезе предстательной железы и мочевого пузыря. *Бюллетень сибирской медицины.* 2021; 20 (4): 153–161. https://doi.org/10.20538/1682-0363-2021-4-153-161.

INTRODUCTION

The study of the etiological role of infectious agents (primarily viral) in carcinogenesis of various tumors is one of the most relevant problems in modern medicine. Currently, there is reliable information about the role of highly oncogenic types of human papillomavirus (HPV) in the development of cervical, anal, vulvar, vaginal, penile, and oropharyngeal cancers.

In foreign and Russian literature, there is a growing number of publications that confirm the influence of HPV and herpes on the emergence of prostate and bladder cancer. This fact proves the need for a more profound and thorough study of the role of these viruses in the emergence, course, and prognosis of

these cancer types. This review analyzes the current research data on this topic.

THE ROLE OF HPV INFECTION IN THE CARCINOGENESIS OF PROSTATE CANCER

Prostate cancer (PC) is the second most common cancer and the fifth leading cause of cancer death in men [1]. According to the World Health Organization, 1.2 million new cases and 358.000 deaths were identified in 2018 [2]. More than 550 thousand new cases of PC are diagnosed in the world annually. USA, Canada, and some European countries have the highest rates of PC incidence, where it ranks first [3].

According to researchers' forecasts, in 2030, the number of PC cases in the world will be 1.7 million and the morbidity rate will reach about 500.000 [4]. In the Russian Federation, there has been a steady increase in the incidence of PC. In 2018, 42,518 new cases of PC were diagnosed, and the standardized incidence rate was 41.45 new cases per 100, 000 population. The increase in morbidity from 2008 to 2018 was 87.70%, with an average growth rate of 5.92% for 2018 [5].

RISK FACTORS

PC is traditionally considered a disease of the elderly. The disease is quite rare in men under 45 years of age. However, after that age, an increase in the incidence of PC is observed, with a maximum in the 65–74 age group [6]. Risk factors also include genetic predisposition, ethnicity (Black and Hispanic populations), obesity, alcohol consumption, and high testosterone levels [7].

In addition to these factors, infectious agents are of great importance in the pathogenesis of PC [8]. According to clinical and epidemiological studies, infections can lead to chronic inflammation, which induces an inflammatory microenvironment, promotes malignant cell proliferation, angiogenesis, and metastasis, disrupts adaptive immune responses, and alters the response to hormonal and chemotherapeutic agents [9, 10].

HPV infection is one of the most common sexually transmitted infections (STIs) worldwide [11]. HPV is a small, epitheliotropic, non-enveloped, double-stranded DNA virus that belongs to the Papillomaviridae family. According to numerous epidemiological studies, the International Agency for Research on Cancer (IARC) identified the types of HPV that are related to human carcinogens. These types include HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59 [8]. Highly oncogenic types of HPV can cause cervical, anal, vulvar, vaginal, penile, and oropharyngeal cancer [12–15].

In the study of HPV-associated carcinogenesis, it was found that the most well-known studies are those on HPV-induced cervical cancer. Epithelial cells become malignant with prolonged exposure to oncogenic strains of HPV. Inactivation of tumor growth suppressors *p53* and *RB* (retinoblastoma gene) occurs under the influence of the expressed viral oncogenes *E6* and *E7*. Due to changes in the normal functions of these suppressor genes, the cell begins to divide uncontrollably, which leads to tumor formation. Suppression of the antitumor properties of these proteins depends on the viral activity: in viruses with high activity, the formed E6–p53 and E7–rRb complexes remain stable. On the contrary, infected cells become malignant [16].

HPV infection is one of the causes of intraprostatic inflammation, and there is evidence that chronic inflammation is involved in the regulation of cellular events in prostate carcinogenesis [17–20].

In 1990, P.J. McNicol and J.G. Dodd were the first to identify HPV DNA in the prostate tissue by polymerase chain reaction (PCR) [21]. Today, more studies are being conducted to investigate the association between HPV infection and PC.

In 2017, G. M. Volgareva et al. conducted a study of the surgical material from 17 patients with PC for the presence of the HPV 16 *E7* oncogene by PCR. The microscopic analysis of the surgical material confirmed PC in 16 of the 17 patients. In all these cases, except one, acinar adenocarcinoma was detected, and in 1 patient, moderately differentiated adenocarcinoma was observed. PCR revealed the presence of HPV 16 *E7* DNA in lysates obtained from 7 specimens of 17 examined patients. The result was positive in five cases of cancer and two cases of prostatic intraepithelial neoplasia (PIN) [22].

In 2019, G. M. Volgareva et al. analyzed the same group of patients to detect HPV 18. The *E7* oncogene was found in the tissues removed from patients with PC in 2 out of 17 cases. Together with the previously obtained data, this result suggested that HPV 16 and HPV 18 are the main types of HPV responsible for the development of cervical cancer and are often present in the prostate glands of patients with PC [16].

In 2016, F. Atashafrooz et al. conducted a study that evaluated the incidence of various types of HPV in PC and benign prostatic hyperplasia (BPH) in Kerman province (Iran) using the PCR method. The aim of the study was to identify the association of HPV infection with the occurrence of PC. PCR showed that HPV DNA was detected in 20% of 100 PC samples ($n = 20$), 80% (16 patients) of which had highly oncogenic HPV types, 40% (8 patients) had HPV 16 and 18, 30% (6 patients) had HPV 31 and 33, and 10% (2 patients) had HPV 54. The DNA of highly oncogenic HPV types was found in only 2% of prostate samples with benign hyperplasia.

This study confirmed the role of highly oncogenic HPV types in prostate diseases in Iranian patients and the correlation between the presence of HPV DNA and PC. In particular, HPV 16 and 18 may play an important role in the development of PC [23].

In the same year, L. Huang et al. examined prostate tissue samples from 75 patients with PC and 73 patients with BPH. The immunohistochemical study revealed positive staining for HPV 16 and 18 in 16 PC cases (21.3%) and in 7 benign hyperplasia samples (9.5%) with a statistically significant difference between the two groups ($p = 0.049$). PCR combined with western blotting showed the presence of HPV 16 in 17 (22.6%) cases and HPV 18 (17.8%) in 13 cases, including four positive episodes of HPV 16 and 18 in the PC group.

In the control group of BPH, six samples were infected with HPV 16 (8.2 %) and three samples – with HPV 18 (4.1%), while there were no patients with positive staining for HPV 16 / 18, which is significantly lower than in the group with PC. Additionally, no significant differences were found between the results of immunohistochemical testing and PCR in combination with western blotting ($p = 0.069$). The authors also found that HPV infection of types 16 and 18 correlated with the clinical stage and the Gleason score in PC ($p < 0.05$), but not with the patient's age, the level of prostate-specific antigen (PSA), and the presence of metastases in the lymph nodes ($p > 0.05$) [24].

In 2017, W.K. Glenn et al. carried out a study in which HPV was detected by PCR in prostate biopsy samples of 52 men with BPH who later developed PC after 1–10 years. HPV screening using the PCR method was performed among 28 of the 52 samples. HPV *L1* genes were detected in 13 patients with BPH and in 8 patients with PC. HPV *E7* genes were identified in 23 cases (82%) of BPH and in 19 (68%) cases of PC. The same types of HPV were present in patients with both BPH and PC, detected later in 9 episodes [25].

HPV 16 was detected in 15% and 3% of cases of BPH and PC, respectively. HPV 18 was detected in 26% of prostate samples with BPH and in 16% cases with PC. High reliability of the sequenced RNA data for HPV 16 and 18 was identified in 12 (2%) of the 502 transcriptomes of PC in The Cancer Genome Atlas (TCGA). The oncoprotein *E7* was positive in 23 (82%) of the 28 benign hyperplasia samples and only in 8 (29%) cases of the 28 PC samples. PSA expression was more prominent in 26 (50%) of the 52 PC samples compared with BPH samples in the same patients.

This study confirms that highly oncogenic HPV types are present in prostate tissues with BPH prior to the development of HPV-positive PC in the same patients. In addition, much more pronounced expression of the oncoprotein *E7* in BPH samples indicates that the oncogenic activity of HPV is an early phenomenon in prostate carcinogenesis [25].

In 2018, O. Medel-Flores et al. conducted a study aimed at identifying the relationship between the occurrence of PC and HPV in the Mexican population. 356 paraffin blocks from unrelated men with PC or BPH were studied, with the latter serving as a control group. HPV detection was performed by PCR using universal primers; viral genotypes were determined by sequencing or multiplex PCR [26].

The microscopic analysis revealed koilocytes in the material which was subsequently analyzed by *in situ* PCR for the presence of HPV, as well as by the immunohistochemical method for detecting the expression of p16-INK4A. *In situ* PCR is a modification of classical PCR, which has similar sensitivity, but at the same time allows to visualize infected cells and assess their relative number. The results showed that highly oncogenic HPV types were detected in 37 of the 189 (19.6%) PC samples, compared with 16 of the 167 (9.6%) BPH samples ($p = 0.01$).

These findings suggest that highly oncogenic HPV types may contribute to the development of PC. HPV 52 and 58 were the most common genotypes (33% and 17%, respectively) found in the studied population. Koilocytes, representing a pathognomonic sign of infection, were found in all *in situ* PCR HPV-positive samples. The researchers also observed increased expression of p16-INK4A

in HPV-positive samples compared with HPV-negative samples, indirectly confirming the presence of the oncoprotein E7.

These results demonstrate that HPV plays an important role in the development of PC. Detection of highly oncogenic HPV types amounted to 81.4% (83% in the BPH group and 79% in the PC group), and detection of low-risk HPV was four times lower – only 19% (17% in the BPH group and 21% in the PC group). The virus genotypes observed in the samples in order of decreasing prevalence were distributed as follows: HPV 52 (33.3%), HPV 58 (17.17%), HPV 11 (12.7%), HPV 18 (10.8%), HPV 16 (7.8%), HPV 33 (6.9%), HPV 6 (5.9%), and HPV 31 (4.0%) [26].

In 2020, G.I. Russo et al. conducted a meta-analysis of 30 studies examining the relationship between HPV 16 and 18 and an increase in PSA values in 6,321 individuals. All men with elevated PSA values ($p < 0.01$) tested positive for HPV 16. There were seven studies involving 2,391 patients with elevated PSA in the blood serum and 4,059 patients in the control group. All studies investigated the relationship between HPV 18 and an increase in the PSA value. The results of the studies did not reveal an increase in the PSA value ($p = 0.49$) in men with positive HPV 18. This meta-analysis suggests that HPV 16 may be a risk factor for an increase in the PSA value, whereas no similar association was found for HPV 18 [27].

In 2019, M. Moghoofei et al. analyzed the results of 24 studies conducted from January 1990 to December 2016, which included 5,546 patients with PC, to assess the heterogeneity of the main parameters, reflecting the study area, sample type, HPV DNA source, detection method, publication calendar period, and Gleason score. The odds ratio (OR) and the corresponding 95% confidence interval (CI) were calculated to identify the relationship between the prevalence of HPV and the risk of developing PC. A significant positive correlation was found between HPV infection and the risk of developing PC (OR = 1.281).

The HPV16 genotype was more common in patients with PC, which significantly increased the risk of developing cancer (OR= 1.60). The risk of developing PC increased significantly at the age of 65 years and older (OR = 3.564). The results of this meta-analysis support a potential pathogenetic association between HPV infection and an increased risk of developing PC, confirming that HPV infection may contribute to the risk of developing PC [28].

THE ROLE OF HPV INFECTION IN THE CARCINOGENESIS OF BLADDER CANCER

Bladder cancer (BC) is the seventh most common type of cancer in the world. The prevalence of BC is the highest among men, where it is the fourth most common cancer type [29]. In the Russian Federation, the incidence of BC increases every year. In 2018, 13,479 new cases of BC were diagnosed, and the standardized incidence rate was 13.20 new cases per 100, 000 population. The increase in morbidity from 2008 to 2018 was 28.12%, with an average growth rate of 2.44 % in 2018 [5].

Histologically, 94% of BC cases are urothelial carcinomas. The remaining cases include squamous cell carcinoma (2%), adenocarcinoma (2%), and mesenchymal and other tumors (2%) [30]. Squamous cell carcinoma and urothelial carcinoma with squamous cell differentiation are often high-grade tumors associated with poor prognosis and worse outcomes after surgery, radiation, and chemotherapy, compared with urothelial carcinomas [31].

Well-known risk factors for BC are chronic urologic diseases, the presence of co-morbidities that reduce immune response (COPD, asthma, autoimmune thyroiditis, etc.), occupational hazards, cigarette smoking, alcohol consumption, radiation exposure, as well as a family history of cancer. Risk factors specifically associated with squamous cell BC encompass chronic bladder irritation caused by prolonged use of catheters and previous schistosomiasis [32]. Recently, HPV has been considered as a causative agent of squamous cell BC [33]. In this regard, more research has been conducted in this area lately.

In 2019, B. Javanmard et al. studied HPV DNA in the tumor tissue and urine at various stages of BC. The average age of 110 patients was 61.6 ± 10 years, 14 patients were female (12.7%). The authors believe that the selection of urine samples for HPV detection is as reliable as the selection of tumor tissue, which can be considered as a prognostic marker. PCR for the common HPV primer in the bladder tumor tissue was positive in 3 (9.4%), 22 (38.6%), and 15 (71.4%) Ta, T1, and T2 stage bladder tumors, respectively ($p < 0.001$).

PCR for HPV 16 in the bladder tumor tissue was positive in 2 (6.3%), 10 (17.5%), and 13 (61.9%)

cases, and PCR for HPV 18 in the bladder tumor tissue was positive in 1 (3.1%), 14 (24.6%), and 12 (57.1%) Ta, T1, and T2 stage tumors, respectively ($p < 0.001$, $p < 0.001$). 37 (33.6%) urine samples were positive for HPV using PCR, and HPV 16, 18 subtypes were positive in 17 (15.5%) and 14 (12.7%) urine samples, respectively. This study suggests that HPV infection may be associated with the development of late-stage BC [34].

In 2018, K.R. Børgensen et al. conducted a study in which the relationship between HPV, oncoprotein p16INK4a, and squamous cell BC was evaluated. The patients were divided into three groups based on the histological evaluation. A study included 100 patients: 50 patients with squamous cell BC, 25 patients with urothelial carcinomas, and 25 patients with urothelial carcinoma with squamous cell differentiation. Overall, HPV was found in 12 of 100 (12%) patients and in 9 of 50 (18%) patients with squamous cell carcinoma.

Overall, overexpression of p16INK4a was observed in 52/100 (52%) patients. However, concomitant HPV and p16INK4a overexpression were observed in only 4/100 (4%) patients. The study demonstrated the presence of HPV in one-fifth of the patients with squamous cell carcinoma, which may significantly contribute to the carcinogenesis of squamous cell carcinoma [35].

In 2018, U.K. Mete et al. (India) studied material from 50 patients with urothelial BC. The control group included ten people who were hospitalized for transurethral resection of the prostate for BPH and/or ureterorenoscopy for urolithiasis. The average age of patients was 54.1 years. Tissue samples were analyzed for the presence of HPV 16 and 18 with PCR. Histological examination of the tumor tissue was performed to assess the degree of differentiation of the tumor.

A total of 28 (56%) patients were diagnosed with low-grade tumors and 22 (44%) patients – with high-grade tumors. 18 (36%) patients had T2 or higher stage of the disease. All tumor biopsies and control samples were HPV-negative. The prevalence of HPV in the urothelium was very low, regardless of the stage and degree of the disease, and, therefore, it is unlikely that HPV is the causative agent of urothelial BC in the Indian population. However, the role of other types of HPV in the etiology of BC requires clarification and further research on this topic [36].

The role of herpesviruses in the carcinogenesis of prostate and bladder cancer

The role of viruses of the Herpesviridae family in the etiology of bladder and prostate cancer is currently being discussed. Herpesviridae is a large group of big viruses with linear genomic DNA up to 20 thousand nucleotide pairs in size. With more than 25 groups, only 6 can reliably cause diseases in humans: herpes simplex virus I and II, herpesvirus type 3 (chickenpox virus), herpesvirus type 4 (Epstein-Barr virus), herpesvirus type 5 (Cytomegalovirus), and herpesvirus type 6. Data on the carcinogenic effect of cytomegalovirus infection (CMV), herpes simplex virus (HSV), and Epstein-Barr virus (EBV) are increasingly appearing in the literature.

In recent years, more research has been conducted on the role of various viruses in the carcinogenesis of PC. According to the study by T.T. Andabekov et al. (2010), low-grade PC was observed in patients with a positive test for CMV ($p < 0.05$). Moreover, in this category of patients, the five-year survival rate after radical prostatectomy without relapse was lower (38.1%) compared with CMV-negative men (96.3%) ($p < 0.05$). The average life expectancy of deceased patients with CMV who underwent combination treatment was less than in uninfected patients: 27.71 and 76.6 months, respectively ($p < 0.05$). The data of the conducted study show that CMV is an important risk factor for the relapse of the disease, which should be taken into account when choosing surgical treatment for patients with PC [37].

In the study by O.B. Laurent et al. (2015), 54 patients (44 men and 10 women) with BC and one patient with urothelial papilloma were examined. The histological evaluation revealed high-grade urothelial cancer in 72.2% of cases (39 patients), low-grade cancer in 25.9% (14 patients), and urothelial papilloma in 1 case (1.8%). Fifteen patients (27.8%) had a relapse of the disease. All patients participating in the study also underwent blood tests for IgG and IgM antibodies to herpes simplex virus (HSV) type I and II, CMV, and Epstein–Barr virus (EBV). The results of the study revealed high titers of anti-CMV IgG in patients with BC. Moreover, the level of these antibodies in patients with recurrent tumors, a high degree of anaplasia, and high grade of the disease was much higher. There was a statistically significant correlation between the presence of CMV DNA in the tumor and the level of anti-CMV IgG, as well as the stage of the disease, the level of early EBV

antibodies, and antibodies to the nuclear antigen of EBV. In addition, the relationship between the level of anti-CMV IgG and the stage of the disease, tumor recurrence, the level of early antibodies to EBV, as well as a significant change in the level of anti-HSV I and II IgG was revealed [38].

In 2018, I. V. Kosova et al. examined and analyzed 100 patients (72 men and 28 women) aged 38 to 90 years with BC. This study was performed using molecular and genetic and ELISA methods for diagnosing the presence of viral infections (HSV I and II, CMV, EBV, and human papillomavirus). Additionally, histological (evaluation of lymphocytic infiltrate, inflammatory activity, cytopathic changes) and immunohistochemical (CD31, EGFR, Ki67, p63, p53, CD44, Bcl-2) methods were used.

In the course of this study, a relationship was revealed between the studied viral infection parameters in patients with BC. EGFR expression and the level of anti-EBV Ig-VCA ($p = 0.032$), proliferative activity ($p = 0.05$), and p53 ($p = 0.025$) correlated in patients with the presence of viral DNA in the tumor tissue, and the presence of CMV was associated with focal hyperplasia ($p = 0.012$), koilocytosis ($p = 0.028$), the presence of leukocytes ($p = 0.012$) and eosinophils ($p = 0.012$). Infection of tumor tissues with highly oncogenic HPV strains affected proliferative activity ($p = 0.05$), koilocytosis, and neoangiogenesis ($p = 0.008$). Increased proliferative activity, expression of apoptotic factors, growth factors, and neoangiogenetic factors in patients with the presence of viral DNA in the tumor tissue indicates an unfavorable course of the tumor process [39].

CONCLUSION

An in-depth study of how human herpes and papillomaviruses affect the origin of malignant tumors of the prostate and bladder, as well as the course and prognosis of these diseases, can become a source of information for the development of new approaches to their diagnosis, prevention, and monitoring of morbidity. In addition, the data obtained can be used in practical medicine, which can significantly improve the five-year survival rate in patients.

The analysis of available literature has shown the importance of viral infection testing for herpes and human papillomaviruses in the male population for timely treatment. It will definitely become one of the links in the prevention of prostate and bladder cancer development.

REFERENCES

1. Ferlay J., Soerjomataram I., Dikshit R., Eser S., Mathers C., Rebelo M., Parkin D.M., Forman D., Bray F. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int. J. Cancer.* 2015; 136 (5): e359–386. DOI: 10.1002/ijc.29210.
2. The Global Cancer Observatory. URL: http://gco.iarc.fr
3. Prilepskaya E.A., Maltsev E.G., Kolontarev K.B., Govorov A.V., Rasner P.P., Vasilyev A.O., Sadchenko A.V., Dyakov V.Ya., Pushkar D.Yu. Comparative analysis of functional and oncological findings of retropubic, laparoscopic and robot-assisted radical prostatectomy. *Urologic Oncology.* 2015; 11 (4): 54–58 (in Russ.). DOI: 10.17650/1726-9776-2015-11-4-54-58.
4. Schröder F.H., Roobol M.J. Prostate cancer epidemic in sight? *Eur. Urol.* 2012; 61 (6): 1093–1095. DOI: 10.1016/j.eururo.2012.03.019.
5. Malignant tumors in the Russian Federation in 2018; ed. by A.D.Kaprin, V.V. Starinskiy, G.V.Petrova. M.: Herzen Moscow Oncology Research Institute – branch of the National Medical Research Radiological Center of the Ministry of Health of Russia, 2019: 250 (in Russ.).
6. Delongchamps N.B., Singh A., Haas G.P. The role of prevalence in the diagnosis of prostate cancer. C*ancer Control.* 2006; 13 (3): 158–168. DOI: 10.1177/107327480601300302.
7. Siraziev A.M., Khasanova G.R., Ulyanin M.Yu. Risk factors for prostate cancer. *Medical Almanac*. 2019; 3-4 (60): 110–114 (in Russ.). DOI: 10.21145/2499-9954-2019-3-110-114.
8. Chen C.J., Hsu W.L., Yang H.I., Lee M.H., Chen H.C., Chien Y.C., You S.L. Epidemiology of virus infection and human cancer. *Recent Results Cancer Res.* 2014; 93: 11–32. DOI: 10.1007/978-3-642-38965-8_2.
9. Coussens L.M., Werb Z. Inflammation and cancer. *Nature.* 2002; 420 (6917): 8607. DOI: 10.1038/nature01322.
10. Mantovani A., Allavena P., Sica A., Balkwill F. Cancer-related inflammation. *Nature.* 2008; 454 (7203): 436–444. DOI: 10.1038/nature07205.
11. Heidegger I., Borena W., Pichler R. The role of human papilloma virus in urological malignancies. *Anticancer Res.* 2015; 35 (5): 2513–2519.
12. Gillison M.L., Chaturvedi A.K., Anderson W.F., Fakhry C. Epidemiology of human papillomavirus-positive head and neck squamous cell carcinoma. *J. Clin. Oncol.* 2015; 33 (29): 3235–3242. DOI: 10.1200/JCO.2015.61.6995.
13. Gao G., Smith D.I. Human papillomavirus and the development of different cancers. *Cytogenet. Genome Res*. 2016; 150 (3-4): 185–193. DOI: 10.1159/000458166.
14. Stratton K.L., Culkin D.J. A contemporary review of HPV and penile cancer. *Oncology (Williston Park).* 2016; 30 (3): 245–249.
15. Nelson V.M., Benson A.B. 3rd. Epidemiology of anal canal cancer. *Surg. Oncol. Clin. N. Am.* 2017 26 (1): 9–15. DOI: 10.1016/j.soc.2016.07.001.
16. Volgareva G.M., Ermilova V.D. Detecting the E7 oncogene of human papillomavirus 18 in patients with prostate cancer. *Russian Journal of Biotherapy.* 2019; 18 (1): 37–41 (in Russ.). DOI: 10.17650/1726-9784-2019-18-1-37-41.
17. Jiang J., Li J., Yunxia Z., Zhu H., Liu J., Pumill C. The role of prostatitis in prostate cancer: meta-analysis. *PLoS One.* 2013; 8 (12): e85179. DOI: 10.1371/journal.pone.0085179.

18. Sfanos K.S., Isaacs W.B., De Marzo A.M. Infections and inflammation in prostate cancer. A*m. J. Clin. Exp. Urol.* 2013; 1 (1): 3–11.
19. Caini S., Gandini S., Dudas M., Bremer V., Severi E., Gherasim A. Sexually transmitted infections and prostate cancer risk: a systematic review and meta-analysis. *Cancer Epidemiol.* 2014; 38 (4): 329–338. DOI: 10.1016/j.canep.2014.06.002.
20. Taverna G., Pedretti E., Di Caro G., Borroni E.M., Marchesi F., Grizzi F. Inflammation and prostate cancer: friends or foe? *Inflamm. Res.* 2015; 64 (5): 275–286. DOI: 10.1007/s00011-015-0812-2.
21. McNicol P.J., Dodd J.G. Detection of human papillomavirus DNA in prostate gland tissue by using the polymerase chain reaction amplification assay. *J. Clin. Microbiol.* 1990; 28 (3): 409–412. DOI: 10.1128/JCM.28.3.409-412.1990.
22. Volgareva G.M., Ermilova V.D., Khachaturyan A.V., Tatarskiy V.V., Matveev V.B., Pavlova L.S. Detecting the genetic material of human papillomavirus 16 in the surgical material of prostate cancer patients using polymerase chain reaction. *Urologic Oncology*. 2017; 3 (4): 51–54 (in Russ.). DOI: 10.17650/1726-9776-2017-13-4-51-54/.
23. Atashafrooz F., Rokhbakhsh-Zamin F. Frequency and type distribution of human papilloma virus in patients with prostate cancer, Kerman, Southeast of Iran. *Asian Pac. J. Cancer Prev.* 2016; 17 (8): 3953–3958.
24. Huang L., Wu M.G., He J., Wei Z.S., Lü W.X., Song X.J., Zhang Y., Wu S.X., Yin Y.L., Fan Y.Y. Correlation of high-risk HPV 16/18 infections with prostate cancer. *Zhonghua Nan Ke Xue.* 2016; 22 (6): 501–505. PMID: 28963837.
25. Glenn W.K., Ngan C.C., Amos T.G., Edwards R.J., Swift J., Lutze-Mann L., Shang F., Whitaker N.J., Lawson J.S. High risk human papilloma viruses (HPVs) are present in benign prostate tissues before development of HPV associated prostate cancer. *Infect Agent Cancer.* 2017; 12: 46. DOI: 10.1186/s13027-017-0157-2.
26. Medel-Flores O., Valenzuela-Rodríguez V.A., Ocadiz-Delgado R., Castro-Muñoz L.J., Hernández-Leyva S., Lara-Hernández G., Silva-Escobedo J.G., Vidal P.G., Sánchez-Monroy V. Association between HPV infection and prostate cancer in a Mexican population. *Genet. Mol. Biol.* 2018; 41 (4): 781–789. DOI: 10.1590/1678-4685-GMB-2017-0331.
27. Russo G.I., Calogero A.E., C21ondorelli R.A., Scalia G., Morgia G., La Vignera S. Human papillomavirus and risk of prostate cancer: a systematic review and meta-analysis. *Aging Male.* 2020; 23 (2): 132–138. DOI: 10.1080/13685538.2018.1455178.
28. Moghoofei M., Keshavarz M., Ghorbani S. et al. Association between human papillomavirus infection and prostate cancer: A global systematic review and meta-analysis. *Asia Pac. J. Clin. Oncol*. 2019; 15 (5): e59–e67. DOI: 10.1111/AJCO.13124.
29. Jørgensen K.R., Jensen J.B. Human papillomavirus and urinary bladder cancer revisited. *APMIS.* 2020 Feb.; 128 (2): 72–79. DOI: 10.1111/apm.13016.
30. National clinical guidelines for treatment of bladder tumors in Denmark. Denmark: Danish Bladder Cancer Group (DaBlaCa); 2017. URL: https://www.sundhed.dk/content/cms/86/15686_dablacadata_-aarsrapport_2017_v2_endelig.pdf
31. Huang W., Williamson S.R., Rao Q., Lopez-Beltran A., Montironi R., Eble J.N., Grignon D.J., Idrees M.T., Emerson R.E., Zhou X.J., Zhang S., Baldridge L.A., Hahn N.M., Wang M., Koch M.O., Cheng L. Novel markers of squamous differentiation in the urinary bladder. *Hum. Pathol.* 2013; 44 (10): 1989–1997. DOI: 10.1016/j.humpath.2013.04.005.
32. Westenend P.J., Stoop J.A., Hendriks J.G. Human papillomaviruses 6/11, 16/18 and 31/33/51 are not associated with squamous cell carcinoma of the urinary bladder. *BJU Int.* 2001; 88 (3): 198–201. DOI: 10.1046/j.1464-410x.2001.02230.x.
33. Kim S.H., Joung J.Y., Chung J., Park W.S., Lee K.H., Seo H.K. Detection of human papillomavirus infection and p16 immunohistochemistry expression in bladder cancer with squamous differentiation. *PLoS One.* 2014; 9 (3): e93525. DOI: 10.1371/journal.pone.0093525.
34. Javanmard B., Barghi M.R., Amani D., Fallah Karkan M., Mazloomfard M.M. Human papilloma virus DNA in tumor tissue and urine in different stage of bladder cancer. *Urol. J.* 2019; 16 (4): 352–356. DOI: 10.22037/uj.v0i0.4181.
35. Jørgensen K.R., Høyer S., Jakobsen J.K., Jensen T.K., Marcussen N., Wrist Lam G., Hasselager T., Thind P.O., Toft B.G., Steiniche T., Jensen J.B. Human papillomavirus and squamous cell carcinoma of the urinary bladder: DaBlaCa-10 study. *Scand. J. Urol.* 2018; 52 (5-6): 371–376. DOI: 10.1080/21681805.2018.1531920.
36. Mete U.K., Shenvi S., Singh M.P., Chakraborti A., Kakkar N., Ratho R.K., Mandal A.K. Human Papillomavirus in Urothelial Carcinoma of Bladder: An Indian study. *Int. J. Appl. Basic Med. Res.* 2018; 8 (4): 217–219. DOI: 10.4103/ijabmr.IJABMR_91_18.
37. Andabekov T.T. The impact of cytomegalovirus infection on the clinical course of prostate cancer: Candidate of Medical Sciences thesis. St-Pet., 2010: 98 (in Russ.).
38. Loran O.B., Sinyakova L.A., Gundorova L.V., Kosov V.A., Kosova I.V., Kolbasov D.N., Dolnov I.A., Kononkov I.V., Kandinova Yu.V. The role of some viruses in the carcinogenesis of bladder cancer. *Experimental and Clinical Unrology.* 2015; 3: 32–37 (in Russ.).
39. Kosova I.V., Loran O.B., Sinyakova L.A. et al. Associated viral infection in bladder cancer. *Consilium Medicum.* 2018; 20 (7): 30–36 (in Russ.). DOI: 10.26442/2075-1753_2018.7.30-36.

Authors information

Mikhaleva Liudmila M., Dr. Sci. (Med.), Professor, Director of Avtsyn Research Institute of Human Morphology; Head of the Pathologic Anatomy Department, City Clinical Hospital No.31, Moscow, Russian Federation. ORCID 0000-0003-2052-914X.

Kamalov Armais A., Dr. Sci. (Med.), Professor, Academician of the Russian Academy of Sciences, Head of the Urology and Andrology Department, Director of Medical Research and Education Center, Lomonosov Moscow State University, Moscow, Russian Federation. ORCID 0000-0003-4251-7545.

Maryin German G., Dr. Sci. (Med.), Professor, Department of Epidemiology, Russian Medical Academy of Postgraduate Education, Moscow, Russian Federation. ORCID 0000-0003-2179-8421.

Karpov Valeriy K., Cand. Sci. (Med.). Associate Professor, Urology and Andrology Department , Doctor of the Highest Qualification Category, Honored Doctor of the Russian Federation, Lomonosov Moscow State University, Moscow, Russian Federation. ORCID 0000-0001-7644-4263.

Akopyan Emma P., Researcher, Clinical Morphology Laboratory, Avtsyn Research Institute of Human Morphology; Pathologist, City Clinical Hospital No. 31, Moscow, Russian Federation. ORCID 0000-0002-1826-9169.

Osmanov Omar A., Post-Graduate Student, Urology and Andrology Department, Lomonosov Moscow State University, Moscow, Russian Federation. ORCID 0000-0003-1453-9771.

Pechnikova Valentina V., Post-Graduate Student, Clinical Morphology Laboratory, Avtsyn Research Institute of Human Morphology; Pathologist, City Clinical Hospital No. 31, Moscow, Russian Federation. ORCID 0000-0001-5896-4556.

(✉) **Mikhaleva Liudmila M.,** e-mail: mikhalevalm@yandex.ru

Received 08.12.2020
Accepted 25.05.2021

УДК 617.58-005.96-08-039.73
https://doi.org/10.20538/1682-0363-2021-4-162-170

Possibilities of conservative treatment of patients with lymphedema of the extremities (literature review)

Myshentsev P.N., Yarovenko G.V., Katorkin S.E.

Samara State Medical University
89, Chapaevskaya Str., Samara, 443099, Russian Federation

ABSTRACT

The literature review describes various methods for treating patients with lymphedema of the extremities. Statistics show an increase in the incidence and disability of patients with this pathology. However, the possibilities of therapeutic measures in lymphedema are far from perfect.

The analysis of literature data showed that the basis of treatment for lymphedema of the extremities is comprehensive conservative therapy with the use of pathogenetically grounded physical, mechanical, and medical methods. In complex schemes of conservative treatment for lymphedema, physiotherapy methods occupy a prominent place. The most common technique among them is regular combined decongestive therapy. This method is recognized by leading experts as the main one in treating patients with lower extremity lymphedema. Commitment of patients to treatment and their social and psychological counseling are of great importance. The choice of the volume and method of surgical intervention requires a difficult and individual assessment of pathological changes developing throughout the course of the disease.

Despite certain improvements in treatment methods, lower extremity lymphedema is still an unsolved issue. The experience of most specialists involved in lymphedema treatment demonstrates a reasonable balance between basic conservative and surgical treatment methods. Undoubtedly, results of evaluation of these methods will improve the choice of an optimal technique for treating patients with lymphedema of the extremities.

Key words: lymphedema of the extremities, conservative treatment, physiotherapy.

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: Myshentsev P.N., Yarovenko G.V., Katorkin S.E. Possibilities of conservative treatment of patients with lymphedema of the extremities (literature review). *Bulletin of Siberian Medicine.* 2021; 20 (4): 162–170. https://doi.org/10.20538/1682-0363-2021-4-162-170.

Возможности консервативного лечения пациентов с лимфедемой конечностей (обзор литературы)

Мышенцев П.Н., Яровенко Г.В., Каторкин С.Е.

Самарский государственный медицинский университет (СамГМУ)
Россия, 443099, г. Самара, ул. Чапаевская, 89

РЕЗЮМЕ

Представлен обзор литературных источников, посвященных различным методам лечения пациентов с лимфедемой конечностей. Статистические данные свидетельствуют о росте заболеваемости и инвалидизации

✉ *Myshentsev Pavel N.,* e-mail: pnmy63@rambler.ru

пациентов с этой патологией. Вместе с тем возможности лечебных мероприятий при лимфедеме оставляют желать лучшего.

Анализ литературных данных показал, что основу лечения лимфедемы конечностей составляют комплексные консервативные мероприятия, которые должны обеспечить применение патогенетически обоснованных физических, механических и медикаментозных методов. В комплексных схемах консервативного лечения лимфатических отеков значительное место занимают физиотерапевтические методы. Ведущим среди них является регулярная комплексная противоотечная терапия. Этот метод признается ведущими специалистами в качестве основного в лечении пациентов с лимфедемой. Большое значение имеют приверженность пациентов к лечению, их социальная и психологическая поддержка. Выбор объема и метода оперативного вмешательства требует осуществления трудной, индивидуальной оценки патологических изменений, развивающихся при заболевании.

Несмотря на определенные достижения, результаты такого лечения далеки до удовлетворительных значений. Опыт большинства специалистов, занимающихся лимфедемой, свидетельствует о разумном балансе между базовой консервативной терапией и хирургическими способами лечения. Несомненно, результаты оценки этих методов позволят улучшить выбор оптимального метода лечения пациентов с лимфедемой конечностей.

Ключевые слова: лимфедема конечностей, консервативное лечение, физиотерапия.

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: Мышенцев П.Н., Яровенко Г.В., Каторкин С.Е. Возможности консервативного лечения пациентов с лимфедемой конечностей (обзор литературы). *Бюллетень сибирской медицины.* 2021; 20 (4): 162–170. https://doi.org/10.20538/1682-0363-2021-4-162-170.

INTRODUCTION

The current level of care for patients with lymphedema of the extremities is quite high. New methods of conservative and surgical treatment are being developed and implemented in clinical practice. At the same time, observations show that with an obvious increase in the incidence of lymphedema associated with cancer, inflammatory diseases, and malformations, the effectiveness of therapeutic measures is far from perfect [1, 2]. The peculiarities of the pathogenesis and clinical course of lymphedema result in a dubious prognosis and the opinion of some doctors about the futility of treating patients with lymphedema [3]. Undoubtedly, lymphedema of the extremities is a chronic disease, but its effective treatment is a difficult, but quite solvable task [4–7].

The main principles of the treatment strategy are complexity and phasing. Experience shows better results when treatment starts early. In treatment of patients, it necessary to take into account medical, psychological, and social rehabilitation [8]. Patient's commitment to treatment and systematic cooperation between the doctor and the patient are of great importance. A special role is attributed to the system of training patients to fight against their disease [9].

Currently, conservative therapeutic measures occupy a prominent place in the treatment of patients with lymphedema of the extremities [2, 3, 6, 10]. They should ensure correction of all interrelated pathogenetic links of this disease and be complex and step-by-step, including preoperative preparation and postoperative care [3, 5].

CONSERVATIVE TREATMENT OF LYMPHEDEMA

Compression therapy. Compression therapy is one of the basic methods recommended for daily use in treatment of patients with lymphedema of the extremities [1, 2, 4]. Using elastic compression garments leads to an increase in pressure in the interstitial space with increased reabsorption of fluid and proteins into lymphatic capillaries. A decrease in the diameter of superficial and deep veins of the limb results in acceleration of venous blood flow and lymph

outflow in the supra- and subfascial spaces with a moderate fibrinolytic effect [4, 6, 7].

When using elastic bandages, short-stretch bandages are recommended, as they create a more pronounced increase in the pressure during motor activity, contributing to the work of the skeletal muscle pump and providing a high decongestant effect. The structure of elastic bandages, which is constantly being improved, is of great importance. So, one of the studies shows that using single-layer silicone bandages with stretchability of 35% provides optimal pressure not only in the horizontal, but also in the vertical body position and during movements of patients. This reduces the volume and consistency of the limb tissues by up to 40% [11].

The use of therapeutic compression garments has a number of advantages. It is advisable to use flat-knit compression garments, which creates sufficient working pressure, has increased strength and wear resistance, and prevents formation of folds [4, 12]. For stage I lymphedema, class 2 compression is recommended. Class 3 compression garments are indicated for stage II lymphedema, class 4 compression (pressure over 49 mm Hg) is indicated for stage III–IV lymphedema with fibredema.

Inelastic compression in lymphedema is more effective than elastic one, as it improves emptying of veins and outflow of lymph by creating a stiff shell around the limb and high working pressure. For example, the CIRCAID device consists of a series of nylon strips wrapping a leg and connected together via a Velcro system, which allows the patient to dose pressure on the limb. Products are sold ready-made or are tailor-made to a given size for the entire limb or for its individual segments [13].

Recently, there have been reports on using special Kinesio tapes on the limbs in patients with lymphedema [14, 15]. According to studies, fan cut taping can be used as additional treatment for lymphedema in order to reduce edema and pain by stimulating proprioception, blood flow, and lymph outflow. However, despite the fact that this technique gives positive results, its use requires detailed scientific justification.

Massage. Lymphatic massage or manual lymphatic drainage in comprehensive treatment of patients with lymphedema of the extremities in recent decades has received universal recognition due to its practical effectiveness and scientific confirmation of its usefulness [1–4, 12]. The main effects of manual lymphatic drainage are to improve the transport function of the lymphatic system by stimulating lymphangions, increasing collateral lymphatic outflow, and allowing for lymphatic vessel and lymphovenous anastomoses. The effect of fluid reabsorption, and most importantly, removal of proteins from the interstitial space, which is essential for reducing fibrotic changes in soft tissues in lymphedema, is of great importance [3, 7, 12, 16].

Manual lymphatic drainage is very different from traditional types of massage techniques. Massage movements should be slow, eliminating the friction of the skin. The pressure exerted on the tissues by the hands is much lower than in other types of massage (no higher than 30–40 mm Hg), since the main area of influence is the lymphatic network of the skin and subcutaneous tissue. The sequence of massage movements is of particular importance. Initial movements are performed in the area of regional lymph nodes with a gradual movement to the peripheral areas of the limb. Each movement in a certain zone is carried out in the proximal direction, based on the principle that the lymph should move to the area from where it is displaced [12, 16].

Recently, new, clinically proven and objective markers have been developed to assess the effectiveness and scientific validity of manual lymphatic drainage. The data of bioimpedance spectroscopy, tonometry, and short-wave diathermy determine with sufficient accuracy movement of fluid on different segments of the limb, depending on the applied massage manipulations.

Course application of professional manual lymphatic massage creates a temporary effect, although with a certain aftereffect. Therefore, patient's mastering this method for its regular use in complex self-treatment is of great importance [4]. Reports on introduction in recent years of devices that allow for dosed lymph massage are worth noting. Thus, the Linforoll device consists of a handpiece with a roller attached to the tip and connected to a computerized system that allows for real-time optimization of pressure exerted by the roller on the main tissues of the limb and improves results of treatment [17, 18].

Exercise therapy. Various physical exercises are aimed at activating extralymphatic stimulation of lymph outflow, since a significant increase in lymph transport in the limb has been proven during active and passive muscle movements and respiratory gymnastics [2, 3, 6]. In the horizontal body po-

sition, light-intensity movements in the joints of the extremities are performed with alternating vertical walking on the spot, toe lift, and performing 'swallow' position. Swimming has a significant positive effect on lymphedema. With severe forms of lymphedema, being in a water pool becomes problematic for patients. In this regard, classes in a dry pool are essential, which is a tank filled with special plastic balls. In the dry pool, the patient can perform any movement, including simulated swimming. Unlike in a water pool, the patient does not need to remove the compression bandage [12]. Recently, there have been reports on the positive effect of dosed Nordic walking in the comprehensive treatment of patients with lower extremity lymphedema [19].

A combination of manual lymphatic drainage, compression bandaging, and physical therapy forms the basis for combined decongestive therapy. This method is recognized by leading experts as the main one in the treatment of patients with lymphedema [2, 3, 9, 10, 12].

PHYSIOTHERAPY METHODS

In the complex schemes of conservative treatment for lymphedema, physiotherapy methods occupy a prominent place [2, 3, 4, 9].

Intermittent pneumatic compression. The method of intermittent pneumatic compression (IPC) or pneumatic massage of the extremities is widely used [2, 6, 20, 21]. The effect of regional pneumatic compression mainly consists in significant reabsorption of fluid and, to a lesser extent, proteins from the edema into the lymphatic bed. There is also a decrease in hydrostatic pressure and tissue tension. Standard equipment allows for regular IPC sessions from distal to proximal parts of the limb in the modes from 30 to 50 mm Hg, lasting 25–30 minutes [9]. Recently, to increase the efficiency of this method, IPC devices with retrograde velocity signals with constant pressure parameters of 40 mm Hg have been introduced [22].

The study of tissue fluid pressure during IPC in patients with stage II-IV lymphedema guided by electronic manometry and plethysmography was carried out. It was noted that pressure in the tissue fluid in IPC reached 40–100 mm Hg, and the volume of displaced tissue fluid varied from 10 to 30 ml in proximal parts of the limb during the compression cycle, and in some cases – up to 100 ml [23, 24]. Studies using fluorescence lymphography showed that IPC accelerates lymph flow in the superficial lymph vessels of the extremity with faster and more efficient flow during relaxation than during compression.

In the treatment of patients with lymphedema, a positive effect is provided by a combination of IPC and manual lymphatic drainage, exercise therapy, and respiratory gymnastics [2, 3, 7]. One of the advantages of IPC is a possibility of using this method by the patient at home adhering to all indications and contraindications. For this purpose, small-sized and inexpensive devices have been developed [4, 7].

Laser therapy. Among physiotherapy methods, laser therapy has become a common method of treating patients with lymphedema in the outpatient setting [25]. Under the influence of low-intensity laser radiation, changes occur at all levels of a living organism. At the subcellular level, a stereochemical rearrangement of molecules and activation of redox processes occur. At the cellular level, a change in the membrane potential and an increase in proliferative activity are observed. At the tissue and organ levels, blood and lymph circulation are activated. At the systemic and organizational levels, adaptive neuro-reflex and neuro-humoral reactions take place [26, 27]. For treatment with low-intensity laser radiation, helium-neon lasers with a wavelength of 0.63 μm and power of 20 mW and semiconductor infrared lasers with a wavelength of 0.89 μm, a frequency of 70–100 Hz, and radiation power of 10–11 mW are used.

Photodynamic therapy is a special method of laser therapy based on the photochemical reaction of an injected photosensitizer that selectively accumulates in cancer or microbial cells and exposure to laser radiation of a certain wave for damaging pathological formations [25]. In patients with lower extremity lymphedema, complicated by recurrent erysipelas, antimicrobial photodynamic therapy has been recognized. After a course of photodynamic therapy, histological studies show a significant decrease in the number of lymphocytes and histiocytes, reduction of lymphatic lacunation, and, most importantly, almost complete elimination of microbial cells. This contributes not only to relief of exacerbations of erysipelas, but also to stabilization of the clinical presentation of lymphedema [25].

Magnetic therapy. Currently, magnetic fields are widely used as a powerful physiotherapy method in various pathologies. The therapeutic effect of

magnetic fields is primarily associated with positive changes in the morphofunctional state of vessels in the microcirculatory bed: vasodilation, increased blood flow and lymph outflow, improved blood rheology. Clinically, this is manifested through anti-inflammatory, trophotropic, antispasmodic, decongestant, and disaggregating effects [25].

With lymphedema of the lower extremities, using of a constant magnetic field at initial stages of the disease is the most effective. So, patients subjectively report a decrease in the feeling of heaviness and pressure sensations in the lower extremities. In lymphedema, characterized by significant fibrotic changes in the soft tissues of the limb, the effect of magnetic therapy is not observed [25]. The obtained data confirm reasonability of the therapeutic use of pulsed low-frequency magnetic fields in treatment of patients with lymphedema [26]. The effectiveness of the method is due to various synergistic actions (diamagnetic force acting on a liquid medium, thermal effect and stimulation of transport of macromolecular compounds).

Good results were noted both according to clinical and instrumental studies. In particular, in the experimental group, the authors observed clinical improvement compared with the control group, as evidenced by improved quality of life on the clinical severity scale, as well as results of echographic studies [26].

Electrophoresis with enzymes and calcium chloride. Anti-edematous and anti-inflammatory effects of electrophoresis with these substances are mainly effective at stages I and II of lymphedema, which is due to stimulation of human lymphatic pacemaker and an increase in contractility of lymphangions [28, 29]. Disadvantages of the standard electrophoresis technique are the superficial effect on tissues, inability to create a drug depot in this lymphatic region, and rapid elimination of the drug from the focus.

Ultrasound therapy. Using low-frequency ultrasound in combination with thermocontrast absorption of drugs (hyaluronidase, ronidase) increases permeability of the skin and reduces density of the subcutaneous fat due to loose connective tissue and depolymerization of hyaluronic acid [7]. In addition, direct and indirect effects of ultrasound lead to emergence of acoustic flows, cavitation, and variable sound pressure, which increases the functional activity of the cell and ultimately leads to improved drainage function of lymphatic vessels and increased lymphatic and venous outflow.

Electrical stimulation of lymphatic vessels. This method can significantly enhance the drainage function of lymphatic vessels by normalizing their tone, restoring and accelerating rhythm of their contractile activity, and stimulating additional lymph outflow pathways in order to influence the crucial link in the pathogenesis of lymphedema – impaired lymphangion function [2, 4, 7, 28, 29]. Developments on the application of electrical stimulation in treatment of lymphedema using the Lymphavision stimulator are of great interest. The device is based on generation of currents similar to impulses of human nervous system [7].

Follow-up of patients using anthropometry, rheolymphovasography, and thermal imaging showed significant reduction of edema at all levels of the affected limb by an average of 12%, a decrease in thermal symmetry of healthy and affected limbs by 48.2%, and an increase in the rate of lymphatic outflow on the affected side by more than 2 times. There is successful experience of using the Body Drain device for treatment of lower extremity lymphedema, designed to provide a combined effect of electrical stimulation of lymphatic and venous systems and vacuum therapy [7].

The effect on lymphatic outflow in the region of the pathological process is achieved due to electrical stimulation of smooth muscles in the lymphatic vessels and striated muscles in the extremities. Creation of negative pressure in projection of main lymph nodes leads to stimulation of their drainage function and an increase in the intensity of extravascular fluid movement.

Ultraviolet blood irradiation. In clinical practice, ultraviolet blood irradiation (UBI) has a powerful biostimulating effect on the immune system, tissue regeneration, and improvement of blood rheology. This method is of great importance for patients with lymphedema of the extremities, complicated by recurrent erysipelas [4, 7, 25, 28]. The most pronounced effect of using UBI is observed in exacerbation of the pathology, and its cycle application prevents development of relapses of erysipelas. Results of using UBI show a good immediate result in 32.6% of patients, satisfactory result in in 59.1% of patients, and unsatisfactory result in 8.3% of patients [27, 28].

Gravity therapy. The main mechanism of the therapeutic effect of this method is the action of centrifugal forces of craniocaudal direction, created by a special artificial gravity unit [30]. Moderate hypergravity (1.5 G) causes an increase in pressure in the interstitial space and increases lymphatic pumping. Increased lymph outflow stimulates lymph formation and activity of preserved lymphangions. Improvement of microcirculation under the gravitational influence with activation of metabolic processes in cells reduces the degree of dystrophic changes and growth of connective tissue. Stimulation of lymphatic drainage in tissues promotes destruction and removal of macromolecular substances from the interstitial space and reduces the degree of fiber rearrangement of soft tissues [31]. The rotation speed of the artificial gravity unit is 29–34 revolutions per minute, the number of sessions is 10–12, the duration of each session is 8–10 minutes [31].

Observations indicate that in comprehensive treatment of patients with lymphedema, the use of gravity therapy is advisable at the initial stages of the disease. According to volumetric data obtained at the end of a treatment cycle, a decrease in the volume of the limbs on average by 14% is observed mainly in the distal parts in patients with stage I lymphedema, by 12% – in stage II lymphedema, and by 8% – in patients with stage III lymphedema. According to computed tomography performed in patients after completion of the comprehensive treatment, there is a decrease in the thickness of the subcutaneous tissue to an average of 12.7±1.22 mm and a decrease in its density with the value of 123.46 ± 3.03 HU [30].

Shockwave therapy. A report on using shockwave therapy is of interest [32]. Electro-pneumatically generated radial shock waves stimulate metabolism, accelerate neoangiogenesis, have an anti-inflammatory effect, and increase transfer of interstitial fluid. In the course of treatment in patients with lymphedema, a decrease in edema density upon palpation and reduction of limb circumference are identified. Ultrasound examination records a decrease in the epifascial thickness of tissues with a decrease in their hyperechogenicity. It should be noted that prescription of certain physical factors (pneumatic compression, shockwave therapy, electrical stimulation) is a contraindication in patients with suspected acute venous thrombosis and acute erysipelas of extremities.

DRUG TREATMENT

Possibilities of medical treatment of lower limb lymphedema are limited. Different groups of drugs affect individual links in the pathogenesis of the disease and its complications with different, often insignificant, degrees of effectiveness. Benzopyrones (coumarin), stimulating the activity of macrophages, promote removal of proteins from tissues in protein-rich lymphatic edema [33, 34].

Along with this, benzopyrones increase lymph outflow and reduce capillary fragility [35, 36]. Flavonoid derivatives (diosmin, hesperidin) affect three main components of interstitial space drainage disorders in edema: microcirculation and venous and lymphatic outflow. One of the most effective drugs according to numerous studies and a large body of evidence is detralex. Its micronized purified flavonoid fraction has certain advantages that increase the pathophysiological orientation of this drug [4, 5, 7].

Feasibility of systemic enzyme therapy (wobenzym, phlogenzym) in lymphedema is determined by its positive effect on blood rheology, reduction of platelet aggregation, increase in fibrinolysis, modulation of the activity of monocytes and macrophages, and reduction of tissue damage. Clinically, this is mainly manifested through immunomodulatory, anti-inflammatory, and decongestant effects, improvement of reparative processes, and reduction of thrombotic complications [4, 6, 37].

Additionally, segmental lymphotropic injections are of particular interest. This method consists in a sequential, step-by-step lymphostimulating effect at various levels of the limb in conditions of lymphatic edema. Administration of drugs with lymphostimulating properties should help restore normal passage of lymph in the affected limb. A course of segmental lymphotropic lymphostimulating injections includes 3 injections with an interval of 48 hours. The composition of injections is the following: lidase 32 units, lidocaine 100 mg, actovegin 200 mg, tramal 50 mg, 40% glucose solution as a filler and solvent [7].

Recurrent erysipelas often leads to formation of persistent lymphedema, and severity of edema is always directly related to the frequency and severity of recurrent erysipelas. [2, 4]. In case of exacerbations of erysipelas, various groups of broad-spectrum antibiotics are used in clinical practice. Lymphotropic and endolymphatic methods of administration of antibiotics provide a more long-term bactericidal

concentration of the drug in the lymphatic vessels and lymph nodes, which reduces the total dose of the drug and side effects of antibiotic therapy [4, 6, 24, 28]. Implementation of long-term preventive antibacterial therapy in the outpatient setting using penicillins (bicillin-1, bicillin-5, retarpen) for 6–12 months, is of great importance.

The combined use of physiotherapy methods and drugs is worth noting. Thus, ultratonotherapy for lymphatic edema in combination with the application of chitosan-based gel allows to optimize non-specific activation of local defense mechanisms of the body and contributes to strengthening of the barrier function of the skin. Chitosan promotes activation of macrophages and can also be used as an adjuvant for immunostimulating agents in order to increase production of antibodies [7].

Long-term experience of leading specialists shows that patients' adherence to independent therapeutic measures recommended by doctors is an indispensable part of comprehensive conservative treatment of lymphedema. The therapy includes skin care, self-massage, wearing compression garments, physical and breathing exercises, creating an elevated position of the limb, and, if possible, using portable pneumatic compression devices. [38–40]. Follow-up of 348 patients with lymphedema showed that self-treatment is an essential and important part in management of this group of patients. The methods are easy to use at home and, therefore, allow to solve difficulties in availability of specialized, systematic, and frequent care, saves time, and strengthens patient's awareness of their own responsibility for participating in results of comprehensive treatment.

CONCLUSION

Thus, conservative treatment of patients with lymphedema of the extremities, taking into account a chronic course of the disease, is indicated for all types and stages of the disease, should be carried out constantly, and be comprehensive. Monotherapy for lymphedema is ineffective. It is advisable to step-by-step combine several methods for treatment simultaneously [2, 3, 7, 38, 41]. In general, the success of treatment in patients with lymphedema of the extremities depends on its early diagnosis and, consequently, early use of preventive and therapeutic measures. These measures, primarily of a conservative nature, are aimed at reducing the accumulation of tissue fluid and lymph and preserving and maintaining the function of lymphatic vessels and designed to slow down the progression of the disease to the greatest extent, achieve a stable positive effect, and improve the quality of life of the patient.

REFERENCES

1. Diagnosis and treatment of primary lymphedema. Consensus document of the International Union of Phlebology. *International Angiology*. 2010; 29 (5): 454–470.
2. The diagnosis and treatment of peripheral lymphedema: 2020 Consensus document of the International Society of Lymphology. *Lymphology*. 2020; 53 (1): 3–19.
3. Rubio-Maicas C., Langhaus-Nixon J., Duarte Alfonso E., Alabajos Cea A., Forner-Cordero I. Maintenance treatment in lymphedema: review. *The European Journal of Lymphology*. 2013; 24 (67-68): 52–54.
4. Potashov L.V., Bubnova N.A., Orlov R.S., Borisov A.V., Borisova R.P., Petrov S.V. Surgical lymphology. St-Pet.: LETI, 2002: 272 (in Russ.).
5. Zolotukhin I.A. Lower extremity lymphedema in the clinical practice: opportunities for diagnosis and treatment. *Reference Book for a Hospital Physician (Spravochnik Polikninicheskogo Vracha)*. 2006; 3: 243–254 (in Russ.).
6. Myshentsev P.N., Katorkin S.E. Treatment strategy in secondary lymphedema of lower extremities. *Surgical News.* 2014; 22 (2): 239–243 (in Russ.). DOI: 10.18484/2305-0047.2014.2.239.
7. Borodin Yu.I., Lyubarskiy M.S., Morozov V.V. Guidelines on clinical lymphology. M.: Medical Information Agency, 2010: 208 (in Russ.).
8. Pereira de Godoy J. M. Quality of life and peripheral lymphedema. *Lymphology*. 2002; 35 (2): 72–75.
9. Torralba-Puebla T., Ortiz-Fernandez L., Zammaripa-Cuesta M. Patient education programm: school of lymphedema prevention. *The European Journal of Lymphology*. 2015; 27 (73): 25–27.
10. Boccardo F. An overview of the treatment of primary and secondary lymphatic diseases: the effort of the ESL to put some order. *The European Journal of Lymphology*. 2017; 29 (77): 1–10.
11. Caldirola R., Conti E., Bordoni M., Famoso L., Cestari M. Experience with siliconed bandages. *The European Journal of Lymphology*. 2017; 29 (77): 10–16.
12. Makarova V.S., Marakov I.G., Spiridonov V.K. Conservative treatment for lymphedema: guidelines for doctors. M.: LIMFA, 2011: 50 (in Russ.).
13. Pastouret F., Gilbert C., Colle M., Zirak C. CIRCAID®: pressures reproducibility with BPS® guide card (built-in pressure system). *The European Journal of Lymphology*. 2016; 28 (74): 66–69.
14. Rel-Monzo P., BayarriI-Garcia V., Alabajos-Cea A., Tortosa-Soriano G., Sevilla-Perez B. Lymph Taping, complementary lymphoedema treatment? *The European Journal of Lymphology*. 2013; 24 (67-68): 56–58.
15. Ozsoy-Unubol T., Sanal-Toprak C., Bahar-Ozdemir Y., Akyuz G. Efficacy of kinesio taping in early stage breast

cancer associated lymphedema: a randomized single blinded study. *Lymphology*. 2019; 52 (4): 166–176.
16. Piller N. Mld and other treatments; how do we know they are working? *The European Journal of Lymphology.* 2013; 24 (67): 24–27.
17. Michelini S., Caldirola R., Forner Cordero I., Olszewski W., Pissas A., Dimakakos E., Michelotti L. Linforoll: a new device for lymphoedema treatment. Preliminary experience. *The European Journal of Lymphology.* 2013; 23 (67-68): 25–29.
18. Olszewski W.L., Zaleska M., Michelini S.: A new method for treatment of lymphedema of limbs: standardized manual massage with a new device linforoll in conservative and surgical therapy protocols. *Lymphat. Res. Biol*. 2016 Dec; 14 (4): 226–232. DOI: 10.1089/lrb.2015.0034.
19. Gonzales-Castro C. Brief overview of the benefits of Nordic walking in the treatment of primary and secondary lymphedema. *The European Journal of Lymphology*. 2013; 24 (69): 28–30.
20. Malinin A.A. Modern concept of a conservative and combined treatment strategy for lymphedema of the extremities. *Angiology and Vascular Surgery*. 2005; 11 (2): 61–69 (in Russ.).
21. Wigg J. A pilot randomized control trial to compare a new intermittent pneumatic compression device and 12-chamber garment with current best practice in the management of limb lymphedema. *The European Journal of Lymphology*. 2009; 20 (58): 16–23.
22. Theys S., Hennequart T., Aguillar Ferrandiz M. E, Deltombe T. Press pneumatic drainage versus manual drainage In upper limb secondary lymphedema. Same compression, same benefit? *The European Journal of Lymphology*. 2015; 27 (73): 6–8.
23. Olszewski W. L., Pradeep Jain , Zaleska M., Cacala M., Gradalski T., Szopinska S. Hydraulics of the tissue fluid during pneumatic compression in the lymphedema of lower limbs. *The European Journal of Lymphology*. 2011; 22 (64): 14–19.
24. Zaleska M., Olszewski W. L., Cakala M. Five-ears of intermittent pneumatic compression in postinflammatory, posttraumatic and post-cancer- therapy edema of lower limbs. *The European Journal of Lymphology.* 2017; 29 (76): 34–38.
25. Zhukov B.N. Pathophysiological aspects of chronic lymphovenous insufficiency of the lower extremities. Samara: Ofort, 2008: 279 (in Russ.).
26. Izzo M., Napolitano L., Coscia V., La Gatta A., Mariani F., Gasparro V. The role of diamagnetic pump (CTU mega 18) in the physical treatment of limbs lymphoedema. A clinical study. *The European Journal of Lymphology*. 2010; 21 (61): 3–8.
27. Zhukov B.N., Lysov N.A., Anisimov V.I. Laser technologies in medicine. Samara, 2001: 224 (in Russ.).
28. Bubnova N.A., Borisova R.P., Borisov A.V. The theory of lymphangions and modern approaches to the pathogenesis, diagnosis, and treatment of lower extremity lymphedema. *Angiology and Vascular Surgery.* 2003; 9 (2): 66–70 (in Russ.).
29. Fionik O.V., Bubnova N.A., Petrov S.V., Erofeev N.P., Ladozhskaya-Gapeenko E.E., Semenov A.Yu. Lower extremity lymphedema: strategy for diagnosis and treatment. *Surgical News.* 2009; 17 (4): 49–64 (in Russ.).
30. Kotelnikov G.P., Yashkov A.V., Makhova A.N., Makarov I.V., Kotelnikov M.G. Experimental substantiation of gravity therapy. M.: Medicine, 2005: 277 (in Russ.).
31. Myshentsev P.N., Zhukov B.N., Katorkin S.E., Yarovenko G.V., Shishkina A.A. Comprehensive treatment of patients with lower extremity lymphedema using a gravity therapy unit. *Bulletin of Lymphology*. 2011; 3: 37–41 (in Russ.).
32. Bordoni M., Cestari M., Conti E., Famoso L. Lymphedema and lipedema: usefulness of shock иwaves. *The European Journal of Lymphology*. 2014; 25 (71): 49–53.
33. Casley-Smith J.R., Piller N.B. The mode of action of coumarin and related compounds in the treatment of lymphedema. Stuttgart: Thieme, 1977: 33–41.
34. Clodius L., Piller N.B. The conservative treatement of postmastectomy lymphedema patients with coumarine results in a marked continious reduction in arm swelling. Advances in lymphology: V. Bartos (ed.). Prague: Avicenum, 1982: 471–474.
35. Piller N.B. Consrevative treatement of acute chronic lymphedema with benzopyrones. *Lymphology*. 1976; 9 (4): 132–139.
36. Michelini S., Fiorentino A., Cardone M. Melilotus, rutin and bromelain in primary and secondary lymphedema. *Lymphology.* 2019; 52 (4): 177–186.
37. Oliva E., Sarcinella R .An association of immune-modulators and natural lympho-kinetics in the treatment of posmastectomy lymphedema. A observational study. *The European Journal of Lymphology.* 2014; 25 (70): 20–24.
38. Warren A.G. Lymphedema: a comprehensive review. *Ann. Plast. Surg*. 2007; 59 (4): 464–472. DOI: 10.1097/01.SAP.0000257149.42922.7E.
39. Benda K., Bendova M. Patients' self-treatment – Why and How? *The European Journal of Lymphology.* 2008; 19 (54): 6–9.
40. Ridner S.H., Fu M.R., Wanchai A., Stewart B.R., Armer J.M., Cormier J.N. Self-management of lymphedema. A systematic review of the literature from 2004 to 2011. *Nursing Research*. 2012; 61 (4): 291–299. DOI: 10.1097/NNR.0b013e31824f82b2.
41. Tzani I., Tsichlaki M., Zerva E., Papathanasiou G., Dimakakos E. Physiotherapeutic rehabilitation of lymphedema: state-of-the-art. *Lymphology.* 2018; 51 (1): 1–12.

Authors contribution

Katorkin S.E. – conception and design of the study, editing of the article. Myshentsev P.N., Yarovenko G.V. – collection and processing of the material, drafting of the manuscript.

Authors information

Myshentsev Pavel N., Cand. Sci. (Med.), Associate Professor, Department and Clinic of Advanced-Level Surgery, Samara State Medical University, Samara, Russian Federation. ORCID 0000-0001-7564-8168.

Yarovenko Galina V., Dr. Sci. (Med.), Department and Clinic of Advanced-Level Surgery, Samara State Medical University, Samara, Russian Federation. ORCID 0000-0002-5043-7193.

Katorkin Sergey E., Dr. Sci. (Med.), Associate Professor, Department and Clinic of Advanced-Level Surgery, Samara State Medical University, Samara, Russian Federation. ORCID 0000-0001-7473-6692.

(✉) **Myshentsev Pavel N.,** e-mail: pnmy63@rambler.ru

Received 22.06.2020
Accepted 28.12.2020

УДК 616.8-008.64-085.32:577.118
https://doi.org/10.20538/1682-0363-2021-4-171-179

The role of nutrients and probiotics in treatment of depression

Neznanov N.G.[1, 2], Rukavishnikov G.V.[1], Kasyanov E.D.[1], Ganzenko M.A.[1], Leonova L.V.[1], Zhilyaeva T.V.[3], Mazo G.E.[1]

[1] V.M. Bekhterev National Medical Research Center for Psychiatry and Neurology
3, Bekhtereva Str., St. Petersburg, 192019, Russian Federation

[2] I.P. Pavlov First Saint-Petersburg State Medical University
6/8, Lva Tolstogo Str., St. Petersburg, 197022, Russian Federation

[3] Privolzhsky Research Medical University
10/1, Minina and Pozarskogo Sq., Nizhny Novgorod, 603005, Russian Federation

ABSTRACT

Currently, a growing amount of data is emerging on the role of various environmental factors (nutrients, gut microbiota, etc.) on formation of depression. The impact on these factors can be effective not only in treatment of major depressive disorder, but also in its early prevention. Therefore, a more detailed study of environmental factors in depression can lead both to a better understanding of the etiology and pathogenesis of the disorder and to optimization of approaches to its treatment. The aim of the review was to assess the potential role of a number of environmental factors associated with nutritional aspects and characteristics of individual microflora, as well as to review the prospects of a strategy for affecting these factors in treatment and prevention of depression.

Key words: depression, nutrients, vitamins, probiotics, microbiome, environmental factors.

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: Neznanov N.G., Rukavishnikov G.V., Kasyanov E.D., Ganzenko M.A., Leonova L.V., Zhilyaeva T.V., Mazo G.E. The role of nutrients and probiotics in treatment of depression. *Bulletin of Siberian Medicine.* 2021; 20 (4): 123–130. https://doi.org/10.20538/1682-0363-2021-4-123-130.

Роль нутриентов и пробиотических препаратов в терапии депрессии

Незнанов Н.Г.[1, 2], Рукавишников Г.В.[1], Касьянов Е.Д.[1], Ганзенко М.А.[1], Леонова Л.В.[1], Жиляева Т.В.[3], Мазо Г.Э.[1]

[1] Национальный медицинский исследовательский центр психиатрии и неврологии (НМИЦ ПН) имени В.М. Бехтерева
Россия, 192019, г. Санкт-Петербург, ул. Бехтерева, 3

[2] Первый Санкт-Петербургский государственный медицинский университет (ПСПбГМУ) имени академика И.П. Павлова
Россия, 197022, г. Санкт-Петербург, ул. Льва Толстого, 6/8

[3] Приволжский исследовательский медицинский университет (ПИМУ)
Россия, 603005, г. Нижний Новгород, пл. Минина и Пожарского, 10/1

✉ *Rukavishnikov Grigory V.,* e-mail: grigory_v_r@mail.ru

РЕЗЮМЕ

В настоящее время появляется все больше сведений о роли различных экологических факторов (особенностей питания, бактериальной флоры человека и проч.) в формировании депрессии. Воздействие на указанные факторы может быть эффективно не только в лечении депрессивного расстройства, но и его ранней профилактике. Таким образом, более подробное изучение роли экологических факторов в формировании депрессии может способствовать как лучшему пониманию этиопатогенеза данного заболевания, так и оптимизации подходов по борьбе с ним.

Цель обзора: оценить потенциальную роль в формировании депрессии ряда экологических факторов, связанных с нутритивными аспектами и особенностями микрофлоры индивида, а также перспективность стратегии воздействия на данные факторы в лечении и профилактике депрессивного расстройства.

Ключевые слова: депрессия, нутриенты, витамины, пробиотики, микробиом, экологические факторы.

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: Незнанов Н.Г., Рукавишников Г.В., Касьянов Е.Д., Ганзенко М.А., Леонова Л.В., Жиляева Т.В., Мазо Г.Э. Роль нутриентов и пробиотических препаратов в терапии депрессии. *Бюллетень сибирской медицины.* 2021; 20 (4): 171–179. https://doi.org/10.20538/1682-0363-2021-4-171-179.

INTRODUCTION

Depression is a chronic mental disorder with complex etiopathogenesis. Numerous biological and environmental factors are increasing depression risks. According to the World Health Organization (WHO), over 264 million people worldwide suffer from depressive disorders, which is associated with high rates of disability [1].

However, despite the widespread prevalence, clinical diversity, and numerous negative consequences of depression, most studies on its treatment and prevention are now focused on neurotransmitter disorders. As a result, not a single drug has appeared in recent years with a fundamentally new mechanism of action and significant superiority in terms of therapeutic efficacy. Moreover, about one third of depressed patients do not respond to standard therapeutic approaches [2].

The search for and correction of new potential risk factors for depression are not given due attention, although there is more and more information about the role of various environmental factors (dietary habits, human gut microbiota, etc.) in the pathology of this disease [2]. Changes in various nutrients, biologically active substances, and individual microflora can affect synthesis of neurotransmitters, a number of metabolic pathways, and mood regulation [4, 5]. In this regard, the regulation of these factors can be effective not only in the treatment of depressive disorders, but also in their early prevention. Therefore, a more detailed study on the role of environmental factors in depression risks can contribute to both a better understanding of the disease etiopathogenesis and optimization of strategies to oppose it.

The aim of the review was to assess the potential role of a number of environmental factors associated with nutritional aspects and characteristics of individual microflora, as well as to review the prospects of a strategy for affecting these factors in treatment and prevention of depression.

Rationale for nutrient augmentation of antidepressant therapy

Nutritional factors, microbiota and mental functions of an individual (including mood regulation) are in a complex, close relation with one other. Thus, biologically active substances and nutrients are processed by the gut microbiota and are able to affect regulation of hormonal, neurotransmitter, and signaling pathways of both the gastrointestinal tract (GIT) and the central nervous system (CNS) [4]. There are literature data that unhealthy diet in terms of a nutrient content significantly increased the risk of depression [2]. The most detailed information in the scientific literature on these issues is presented about vitamins (folates, vitamin D) and polyunsaturated fatty acids (PUFAs).

FOLATE USE IN THE TREATMENT OF DEPRESSION

Folate (vitamin B9) is a collective term for a class of compounds belonging to the group of water-sol-

uble vitamins [5]. This class of compounds plays an important role in one-carbon metabolism (OCM), including pathways for the biosynthesis of key amino- and nucleic acids. Folate deficiency is associated with a number of cardiovascular problems, neurological disorders, and diseases of the blood and reproductive system. Insufficient folate intake is often reported in populations where the intake of green vegetables and fresh fruits is reduced. Other foods rich in folates are legumes, grains, eggs, and liver.

Dietary folate intake is strongly associated with blood homocysteine levels [6]. In the case of hyperhomocysteinemia, folate supplementation corrects this condition, which has been confirmed by a number of studies [6]. In studies of patients with cardiovascular disease (CVD) and high homocysteine levels, folic acid treatment significantly reduced homocysteine levels, while the administration of B6 and B12 did not have a similar effect [7]. Furthermore, patients with CVD and low folate levels have an increased risk of depression [8].

Several forms of folate were used in depression treatment studies. 5-MTHF – 5-methyltetrahydrofolate (metafolin, methylfolate) is a biologically active form of folate. This compound is the closest to the natural food form of folates, which easily penetrates into the central nervous system (unlike synthetic folic acid), does not require preliminary transformation in the liver with dihydrofolate reductase, and is immediately incorporated into the folate metabolism cycle. Moreover, its further biochemical metabolism (in particular, the delivery of methyl groups to the homocysteine – methionine cycle) does not depend on the genetic polymorphism MTHFR677C> T [5]. In most of the studies on the effect of folate on mood, it was 5-MTHF that was studied.

On the other hand, synthetic folic acid is much more widely studied in obstetrics, and its chemical stability makes it possible to use this particular form of folate as food fortification in several countries (USA, Canada, Brazil, Australia). In this regard, a huge amount of observational information has been accumulated on this folate form [5–7]. It was noted that food fortification with folates decreases the prevalence of depressive symptoms in the population. In men, this is mediated by an increase in plasma folate levels, while in women, this effect is not observed [5].

The effectiveness of several different forms of folate at different doses and in different categories of patients has been studied in association with depression. At the same time, studies with the addition of folate to antidepressant therapy are more numerous and rigorous in design, in contrast to studies of independent folate therapy. In a randomized, placebo-controlled study, the group of women who received the antidepressant fluoxetine (20 mg) along with folic acid (500 mcg / day) had 94% of responders, while the group receiving fluoxetine with placebo had only 61% of responders ($p < 0.005$). In men in this study, the difference in the effectiveness between the groups was less significant, also a less significant decrease in homocysteine levels was observed compared with women. This, according to the authors of the study, indicates the need for large doses of folate in men. At the same time, to select the optimal doses, further studies of folate administration in combination with other antidepressants are required [6].

In another study with the same design ($n = 27$), where the dose of folic acid was 10 mg / day, in the group of patients receiving folate with fluoxetine (20 mg) after 6 weeks of treatment, the average score on the Hamilton Depression Rating Scale (HDRS) was significantly lower than in the placebo group receiving only fluoxetine [7].

L-methylfolate has also shown beneficial effects as adjuvant therapy in addition to traditional antidepressants [6]. In a double-blind, placebo-controlled study, standard psychotropic therapy in patients with depression and schizophrenia was successfully supplemented with methylfolate (15 mg) for 6 months. At the same time, clinical and social recovery was significantly accelerated compared with placebo, and, over time, the differences with the placebo group increased ($n = 123$) [5].

G.I. Papakostas et al. (2014) showed in a clinical study that L-methylfolate (15 mg / day), in addition to therapy in patients ($n = 75$) initially resistant to antidepressants belonging to selective serotonin reuptake inhibitors (SSRIs) contributed to overcoming therapeutic resistance. In this regard, the authors conclude that L-methylfolate at a dose of 15 mg / day can be considered an effective, safe, AND relatively well-tolerated agent for overcoming a partial response or a lack of response when using SSRIs in patients with depressive disorders [9]. In the extended 12-month open phase of this study, there were also positive results ($n = 68$) on sustained remission in patients receiving 15 mg of L-methylfolate a day [9].

Another biologically active form of folate, folinic acid (leucovorin, unlike folic acid, does not require conversion by dihydrofolate reductase in the liver), when additionally prescribed to patients with depres-

sive disorder who are resistant to SSRIs, has shown itself as a poorly effective remedy (the level of folate in patients in this study was initially normal) [2].

Based on a systematic review and meta-analysis of 3 randomized, controlled studies, it was concluded that folate may play a potential role as an adjunct to basic therapy for depression and cannot be used as stand-alone treatment [5, 6]. At the same time, there are suggestions that the addition of folate to antidepressant therapy may be useful for patients with depression, regardless of their initial folate status, as well as regardless of the presence / absence of a response to antidepressant therapy [2]. M. Fava et al. concluded that folates are effective and safe in some patients with depressive disorders, but more information is needed on the dosage and patient populations most suitable for folate therapy [6].

A number of researchers draw attention to the need for further long-term research (from 6 months to a year), because the effect of folate administration is slow and builds up over several months. Low doses for a long time are more preferable than high doses for short or even long term [6]. Large doses of folate can be dangerous for the nervous system in terms of antagonism with vitamin B12 and its deficiency, as well as in terms of provoking seizures. Moreover, it was shown that folic acid at a dose of more than 5 mg / day can cause agitation, hyperactivity, and insomnia and provoke hypomanic states in the case of a predisposition [5–7]. At the same time, there is evidence that unmetabolized folic acid (when prescribed in exceeded doses) can inhibit further folate metabolism and lead to aggravation of OCM disorders [5–7].

THE USE OF VITAMIN D IN THE TREATMENT OF DEPRESSION

Vitamin D is another important vitamin in the risk of depression and its therapy. It is obtained from food in the form of vitamin D2 (vegetables, mushrooms) and vitamin D3 (meat, fish, eggs) [10]. Despite this, very few foods are rich in vitamin D. The highest vitamin D content is found in animal products in the form of vitamin D3 in oily fish, egg yolks, meat, liver, and kidneys. Another important environmental factor in the synthesis of vitamin D by the body is sufficient ultraviolet (UV) exposure of the skin.

While the role of vitamin D in calcium homeostasis and the maintenance of bone metabolism has been studied well enough, its effect on the structure of the central nervous system still raises many questions. Vitamin D receptors are widely presented in the brain structures which are responsible for affective disorders, which suggests the role of vitamin D in the mechanisms of depression [11–13]. Another important aspect was identification of frequent decreases in vitamin D levels in cases of seasonal affective disorder with depressive episodes in winter [13]. However, in subsequent randomized controlled trials, no unambiguous information was obtained on either a natural decrease in vitamin D in depression, or its effectiveness in the treatment of depressive disorder.

In the Iranian study, the use of vitamin D (oral vitamin D3 at a dose of 50,000 IU every two weeks) for 8 weeks in 76 patients with postpartum depression and decreased levels of 25[OH]D (less than 75 nmol / l) led to improvement of depressive symptoms and normalization of vitamin levels in the blood [14]. Therapy effectiveness was evaluated in three subgroups: vitamin D in combination with calcium supplements; vitamin D in combination with a placebo instead of calcium supplements; the placebo group alone. The Edinburgh Depression Scale (EDS) was used to assess the severity of depression. A decrease in the severity of depression was noted for both subgroups where vitamin D was used regardless of calcium augmentation. At the same time, a large-scale study on the use of vitamin D3 (400 IU / day) and calcium carbonate (1,000 mg / day) among 36,282 postmenopausal women did not reveal significant reduction of the risk of depression during therapy [15].

A meta-analysis by U. Gowda et al. did not reveal any positive changes in depression symptoms when using vitamin D either as monotherapy or in combination with antidepressants and other drugs [16].

THE USE OF OMEGA-3 POLYUNSATURATED FATTY ACIDS IN THE TREATMENT OF DEPRESSION

For a long time, polyunsaturated fatty acids (PUFAs) have been studied primarily in the context of their use for prevention of cardiovascular diseases. However, there is a growing amount of data on their role in mood regulation [17]. There are 4 classes PUFAs: ω3, ω6, ω7, and ω9 (this division into classes is based on the chemical structure – the position of the first double bond in relation to the carbon of the terminal methyl group) [17]. The most important PUFAs for the body are ω3 and ω6 [17].

The main representative of the ω6 class is arachidonic acid (AA) [17]. It enters the body with food (vegetable oils, the largest amount is found in flaxseed oil) and is partially synthesized by the body,

which ensures its constant presence in the human body [17]. Such ω3 PUFAs as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) enter the body only with food, their main source is fatty marine fish species [11, 17].

Potential mechanisms are currently being explored that could determine the antidepressant activity of ω3 PUFA. In the last decade, the results of many experimental studies indicated that ω3 PUFAs have a significant effect on inflammation, expression of apoptosis-related genes, oxidative stress, neurotrophic functions, and neurotransmitter systems [18]. EPA plays the most significant role in the anti-inflammatory activity of ω3 PUFA. Clinical studies show that the use of EPA in comparison with placebo and DHA is more effective in a group of patients with biochemical markers of inflammation (elevated C-reactive protein (CRP)) [18].

Various studies have used fatty acids both as monotherapy and as an adjunct to standard antidepressant therapy. Different doses (from 1 to 6 g / day) and different duration of treatment (from several weeks to months) were used. Various sources of fatty acids have been studied: both pure eicosapentaenoic and docosahexaenoic fatty acids in combination or their isolated use, also fish oil and fatty acids obtained from plant seeds. However, dietary information was not analyzed in most studies [11, 17].

The results of studies on the effectiveness of ω3 PUFAs have yielded ambiguous conclusions. At the initial stage in the research of this problem, very encouraging results were obtained. A positive effect was registered both for ω3 PUFA monotherapy and for methods of augmenting the antidepressant effect [19]. Furthermore, it was noted that the use of ω3 PUFA significantly reduced the risk of depression recurrence [20]. However, these studies were conducted in small cohorts without regard to the dose ratio of EPA and DHA. Subsequently, it was shown that therapeutic efficacy was revealed when using drugs containing high doses of EPA and low doses of DHA (2–4: 1 ratio or more), while the opposite combination (high doses of DHA and low doses of EPA) was not therapeutically effective in depression [20]. This result was confirmed by the meta-analysis, which showed that ω3 PUFA preparations containing at least 60% EPA were effective in the treatment of depression and may be used for treatment of comorbid somatic symptom disorders [21].

Studies have been conducted on the use of preparations containing only EPA. EPA monotherapy at a dose of 1 g / day for 8 weeks in patients with depressive disorders did not significantly reduce depressive symptoms as measured by the HDRS [19]. At the same time, the use of large doses of the drug (1.2–4 g per day) was significantly more effective [19]. However, in this study, EPA was used in conjunction with an antidepressant. The use of DHA in isolation at a dose of 2 g / day for 6 weeks had no significant effect on depressive symptoms [19].

Currently, on the basis of meta-reviews, it can be assumed that PUFAs can be considered as an effective method for augmentation of antidepressants. Drugs containing both EPA and DHA should be used, because these compounds are likely to have a synergistic effect on depressive symptoms. The effect of PUFAs on the lipid spectrum is of special interest, which is widely used in patients with depression in cases of metabolic syndrome associated with antidepressant therapy, eating disorders, and high consumption of high-calorie foods containing a large amount of animal fats [11].

THE USE OF ANTI- AND PROBIOTICS IN THE TREATMENT OF DEPRESSION

The study of microbiota-modifying therapeutic approaches is associated with obtaining both experimental and clinical data on the role of the microbiota – gut – brain axis in depression [22, 23]. This axis is mediated by bidirectional communication between the brain and the microbiota, which modulates immune and endocrine functions [24, 25]. The composition of the microbiota is associated with age, environmental factors, and dietary habits [22, 25].

In microbiome studies, the microbiota-depleting effects of antibiotics are used to study the effect of reduced microbial diversity on behavior [25]. Recent studies have identified links between frequent antibiotic exposure, especially during development, and many serious diseases, such as autoimmune pathology and mental health problems [26]. However, the use of antibiotics in experimental studies presents a number of problems. Antibiotics usually affect the body and its microbiome (or the transplanted microbiota) in three ways: depletion of the resident microbiota, subsequent enrichment of the antibiotic-resistant microbiota, and impact on the corresponding host tissues [27]. The effect of antibiotics on the host tissues is especially important when studying the central nervous system and behavior, since some antibiotics themselves can be neuroprotective or neurotoxic [28]. This can be seen as a limitation in behavioral research

using antibiotics to alter the composition of the microbiome.

Minocycline is an antibiotic of the tetracycline class. The pleiotropic activity of this drug, including anti-inflammatory, antioxidant, anti-apoptotic, anti-glutamatergic and monoaminergic effects, is widely discussed in the scientific literature [29]. In this regard, interest in the use of minocycline has expanded significantly, and it is considered as a potential candidate for treatment of affective disorders, both bipolar and unipolar ones. Currently, clinical trials of minocycline as monotherapy are underway [29].

Probiotics have been extensively studied in non-psychiatric populations and associated with improved gastrointestinal health, reduced inflammation, and temporary improvements in cognitive performance [30]. Diseases of the nervous system are already being considered a completely new area of probiotics application [26].

Currently, lactobacilli and bifidobacteria in the center of the probiotic market. They are widely used for correction and prevention of various pathological conditions (bacterial vaginosis, irritable bowel syndrome (IBS), diarrhea, obesity, allergies, urolithiasis).

Probiotics that can affect the severity of mental disorders are currently considered as psychobiotics [31]. The first study to evaluate the therapeutic efficacy of prebiotics or probiotics for depression was conducted over a decade ago, but about half of all existing studies were published in just the past two years, reflecting the rapidly growing interest in this area [32]. Most of the hypotheses associated with the potential therapeutic effect of psychobiotics on affective disorders are based on experimental animal models, behavioral tests, and neurophysiological indicators after courses of probiotics and prebiotics [32], as well as changes in the level of stress hormones, monoamines, and GABA receptors in the brain [32, 33].

While research on the use of probiotics for mental disorders is promising, there are conflicting reports on their effectiveness as augmenting agents [33]. Numerous studies have examined the effects of probiotics on mood in both healthy individuals and patients diagnosed with depression. Recent meta-analysis of data mostly confirms the positive effects of certain probiotics on mood [33]. Based on the results obtained, it is suggested that probiotics affect mood only in depressed patients and do not have an antidepressant effect in healthy individuals. Moreover, the antidepressant effects of probiotics are likely to be limited to young adults and do not appear in those over 65 years of age [32].

The study on the prospects of probiotic use in depressed patients has several limitations. This is primarily due to different doses and duration of drug administration, which makes it difficult to compare studies. A similar problem is research of various species and strains of bacteria. Probiotics, which are likely to have an antidepressant effect, mainly belong to the *Bifidobacterium* and *Lactobacillus* genera. These genera contain many different species and strains, and their properties cannot be generalized [34]. For example, *Lactobacillus rhamnosus* (strain JB-1) did not affect mood or anxiety levels in healthy men, while *Lactobacillus casei* (strain Shirota) demonstrated the ability to improve mood in healthy volunteers with low baseline mood scores [35].

Probiotic bacteria can affect the body through various mechanisms that can be specific for the strain (e.g., lactic acid bacteria) or widespread among a variety of strains, including normalization of disturbed microbiota, inhibition of potential pathogens, production of beneficial metabolites or enzymes, and immunomodulation [32, 34]. However, the results of clinical studies are not convincing. There is very limited evidence for the effectiveness of probiotic or prebiotic interventions in altering microbial composition in mental disorders [36]. Conversely, probiotics led to changes in the microbial composition, but without affecting mood symptoms [37].

DISCUSSION AND CONSLUSION

Recent treatment strategies for depression consider the use of antidepressants as the first-choice tactic [11]. Despite market expansion for these drugs with the emergence of a new generation of antidepressants, including drugs with multi-receptor activity, there is no much progress in their efficiency – only 50% of patients manage to achieve stable remissions [11]. The side effects of antidepressants significantly affect treatment and reduce adherence to therapy [11]. Today, augmentation (enhancing the activity of antidepressants with the use of drugs that do not belong to the group of thymoanaleptics) is the main direction that is widely studied and used for treatment-resistant depressive conditions [11]. For this purpose, psychotropic drugs (antipsychotics, lithium salts, anticonvulsants) and hormonal drugs are used. However, the use of this approach increases the number of side effects due to both side effects of additional drugs and drug combinations.

Despite different mechanisms of functioning of various biologically active compounds, nutrients, and their interaction with microflora in the body, in most cases the key question remains, whether it is advisable to use these compounds in all patients or only in those who initially have their deficiency. Potential limitations in the therapeutic use of these compounds indicate difficulties in determining a deficiency of a particular nutrient in various populations [2, 3]. As a rule, in the population of developed countries, changes in such parameters are not clinically visible, which makes it difficult to diagnose them and determine their relationship with depression. At the same time, with regard to probiotics, it is noted that individuals with different genetic predisposition and variable contacts with microorganisms can react differently to identical drugs [4].

The need for further randomized studies to determine the efficiency, which will consider the difference in effect by subgroups, sex, presence / absence of deficiency, and other factors, is indicated for folates [5, 6], vitamin D [10, 12, 14], and fatty acids [17, 19]. For all these compounds, the study of approaches to therapy is carried out on a wide population of patients with depressive disorders, therefore, the effectiveness may be questionable not due to insufficient effect, but due to the phenomenological and biological heterogeneity of the samples and the absence of clearly defined control criteria (laboratory parameters).

Another promising direction in the context of this strategy is the study of not only individual isolated nutrients, but also complexes of dietary patterns in general [38]. Such an approach can help to establish the role of the mutual impact of various nutritional components on depression, as well as their interaction in the context of a number of body characteristics (such as the composition of gut microbiota). Moreover, modification of the entire diet may be a more effective and physiological method of preventing depressive disorder than correcting individual nutrients.

Therefore, even now, the use of nutrients and probiotics can be seen as a paradigm shift in the treatment of treatment resistant depression. At the same time, it is important to select a specific drug for augmentation based on laboratory screening, which makes it possible to apply a personalized approach to therapy.

REFERENCES

1. URL: https://www.who.int/news-room/fact-sheets/detail/depression
2. Ionescu D., Papakostas G. Experimental medication treatment approaches for depression. *Transl. Psychiatry.* 2017; 7 (3): e1068. DOI: 10.1038/tp.2017.33.
3. Sparling T.M., Henschke N., Nesbitt R.C., Gabrysch S. The Role of Diet and Nutritional Supplementation in Perinatal Depression: a Systematic Review. *Matern. Child Nutr.* 2017; 13 (1): 10.1111/mcn.12235. DOI: 10.1111/mcn.12235.
4. Huang R., Wang K., Hu J. Effect of Probiotics on Depression: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Nutrients.* 2016; 8 (8): pii: E483. DOI: 10.3390/nu808048331.
5. Naderi N., House, J.D. Recent Developments in Folate Nutrition. *Adv. Food Nutr. Res*. 2018; 83: 195–213. DOI: 10.1016/bs.afnr.2017.12.006
6. Fava M., Mischoulon D. Folate in depression: efficacy, safety, differences in formulations, and clinical issues. *J. Clin. Psychiatry*. 2009; 70 (5): 12–17. DOI: 10.4088/JCP.8157su1c.03.
7. Resler G., Lavie R., Campos J. et al. Effect of folic acid combined with fluoxetine in patients with major depression on plasma homocysteine and vitamin B12, and serotonin levels in lymphocytes. *Neuroimmunomodulation.* 2008; 15 (3): 145–152. DOI: 10.1159/000151527.
8. Kasyanov E.D., Mazo G.E. Disorders of one-carbon metabolism in treatment-resistant depressions: two family clinical cases. *Pharmacogenetics and Pharmacogenomics.* 2019; 2: 43–43 (in Russ.). DOI: 10.24411/2588-0527-2019-10064.
9. Papakostas G.I., Shelton R.C., Zajecka J.M., et al. Effect of adjunctivel-methylfolate 15 mg among inadequate responders to SSRIs in depressed patients who were stratified by biomarker levels and genotype. *The Journal of Clinical Psychiatry*. 2014; 75 (8): 855–863. DOI: 10.4088/jcp.13m08947.
10. Wilson L.R., Tripkovic L., Hart K.H., Lanham-New S.A. Vitamin D deficiency as a public health issue: using vitamin D2 or vitamin D3 in future fortification strategies. *Proc. Nutr. Soc*. 2017; 76 (3): 392–399. DOI: 10.1017/S0029665117000349
11. Depression and risk of developing somatic symptom disorders: guidelines for doctors; ed. by N.G.Neznanov, G.E. Mazo, A.O. Kibitov. M.: Specialized Medical Publishing House, 2018: 248 (in Russ.).
12. Dorofeykov V.V., Petrova N.N., Zadorozhnaya M.S. Diagnostic and prognostic value of determining serum vitamin D(OH) in depression. *Medical Alphabet.* 2018; 2 (17): 47–52 (in Russ.).
13. Li G., Mbuagbaw L., Samaan Z., Zhang S., Adachi J.D., Papaioannou A., Thabane L. Efficacy of vitamin D supplementation in depression in adults: a systematic review protocol. *Syst. Rev*. 2013;2: 64. DOI: 10.1186/2046-4053-2-64.
14. Amini S., Amani R., Jafarirad S., Cheraghian B., Sayyah M., Hemmati A.A. The effect of vitamin D and calcium supplementation on inflammatory biomarkers, estradiol levels and severity of symptoms in women with postpartum depression: a randomized double-blind clinical trial. *Nutr. Neurosci*. 2020; Online ahead of print: 1–11. DOI: 10.1080/1028415X.2019.1707396.
15. Bertone-Johnson E.R., Powers S.I., Spangler L. et al. Vitamin D supplementation and depression in the women's health initiative calcium and vitamin D trial. *Am. J. Epidemiol*. 2012; 176 (1): 1–13. DOI: 10.1093/aje/kwr482.

16. Gowda U., Mutowo M.P., Smith B.J., Wluka A.E., Renzaho A.M. Vitamin D supplementation to reduce depression in adults: meta-analysis of randomized controlled trials. *Nutrition*. 2015; 31 (3): 421–429. DOI: 10.1016/j.nut.2014.06.017.
17. Vaskovskiy V.E., Gorbach T.A., Esipov A.V., Svetashev V.I., Yatskova M.A. Omega-3 fatty acids and their application in medicine. *Pacific Medical Journal*. 2010; 40 (2): 15–19 (in Russ.).
18. Rapaport M.H., Nierenberg A.A., Schettler P.J. et al. Inflammation as a predictive biomarker for response to omega-3 fatty acids in major depressive disorder: a proof-of-concept study. *Mol. Psychiatry.* 2016; 21 (1): 71–79. DOI: 10.1038/mp.2015.22.
19. Peet M., Horrobin D.F. A dose-ranging study of the effects of ethyl-eicosapentaenoate in patients with ongoing depression despite apparently adequate treatment with standard drugs. *Arch. Gen. Psychiatry*. 2002; 59(10): 913–919. DOI: 10.1001/archpsyc.59.10.913.
20. Ginty A.T., Conklin S.M. Short-term supplementation of acute long-chain omega-3 polyunsaturated fatty acids may alter depression status and decrease symptomology among young adults with depression: A preliminary randomized and placebo controlled trial. *Psychiatry Res*. 2015; 229 (1-2): 485–489. DOI: 10.1016/j.psychres.2015.05.072.
21. Sublette M.E., Ellis S.P., Geant A.L., Mann J.J. Meta-analysis of the effects of eicosapentaenoic acid (EPA) in clinical trials in depression. *J. Clin. Psychiatry*. 2011; 72 (12): 1577–1584. DOI: 10.4088/JCP.10m06634.
22. Kostryukova E.S., Alifirova V.M., Zhukova N.G. et al. Olfactory dysfunction and changes in the microbiota as early non-motor manifestations of Parkinson's disease. *Bulletin of Siberian Medicine.* 2016; 15 (5): 66–74 (in Russ.). DOI: 10.20538/1682-0363-2016-5-66-74.
23. Cussotto S., Sandhu K.V., Dinan T.G., Cryan J.F. The neuroendocrinology of the microbiota-gut-brain axis: A behavioural perspective. *Front. Neuroendocrinol*. 2018; 51: 80–101. DOI: 10.1016/j.yfrne.2018.04.002.
24. Carabotti M., Scirocco A., Maselli M.A., Severi C. The gut-brain axis: interactions between enteric microbiota, central and enteric nervous systems. *Ann. Gastroenterol*. 2015; 28 (2): 203–209.
25. Ferrer M., Méndez-García C., Rojo D., Barbas C., Moya A. Antibiotic use and microbiome function. *Biochem. Pharmacol*. 2017; 134: 114–126. DOI: 10.1016/j.bcp.2016.09.007.
26. Blaser M.J. Antibiotic use and its consequences for the normal microbiome. *Science*. 2016; 352 (6285): 544–545. DOI: 10.1126/science.aad9358.
27. Morgun A., Dzutsev A., Dong X., et al. Uncovering effects of antibiotics on the host and microbiota using transkingdom gene networks. *Gut.* 2015; 64 (11): 1732–1743. DOI: 10.1136/gutjnl-2014-308820.
28. Champagne-Jorgensen K., Kunze W.A., Forsythe P., Bienenstock J., McVey Neufeld K.A. Antibiotics and the nervous system: More than just the microbes? *Brain Behav. Immun.* 2019; 77: 7–15. DOI: 10.1016/j.bbi.2018.12.014.
29. Savitz J., Preskorn S., Teague T.K., Drevets D., Yates W., Drevets W. Minocycline and aspirin in the treatment of bipolar depression: a protocol for a proof-of-concept, randomised, double-blind, placebo-controlled, 2 × 2 clinical trial. *BMJ Open*. 2012; 2 (1): e000643. DOI: 10.1136/bmjopen-2011-000643.
30. Khalesi S., Bellissimo N., Vandelanotte C., Williams S., Stanley D., Irwin C. A review of probiotic supplementation in healthy adults: helpful or hype? *Eur. J. Clin. Nutr*. 2019; 73 (1): 24–37. DOI: 10.1038/s41430-018-0135-9.
31. Dinan T.G., Stanton C., Cryan J.F. Psychobiotics: a novel class of psychotropic. *Biol. Psychiatry.* 2013; 74 (10): 720–726. DOI: 10.1016/j.biopsych.2013.05.001.
32. Liu R.T., Walsh R.F.L, Sheehan A.E. Prebiotics and probiotics for depression and anxiety: A systematic review and meta-analysis of controlled clinical trials. *Neurosci. Biobehav. Rev.* 2019; 102: 13–23. DOI: 10.1016/j.neubiorev.2019.03.023.
33. Ng Q.X., Peters C., Ho C.Y.X, Lim D.Y., Yeo W.S. A meta-analysis of the use of probiotics to alleviate depressive symptoms. *J. Affect. Disord*. 2018; 228: 13–19. DOI: 10.1016/j.jad.2017.11.063.
34. Butler M.I., Mörkl S., Sandhu K.V., Cryan J.F., Dinan T.G. The gut microbiome and mental health: what should we tell our patients? *Can. J. Psychiatry*. 2019; 64 (11): 747–760. DOI: 10.1177/0706743719874168.
35. Kelly J.R., Allen A.P., Temko A. et al. Lost in translation? The potential psychobiotic Lactobacillus rhamnosus (JB-1) fails to modulate stress or cognitive performance in healthy male subjects. *Brain Behav. Immun*. 2017; 61: 50–59. DOI: 10.1016/j.bbi.2016.11.018.
36. Okubo R., Koga M., Katsumata N. et al. Effect of bifidobacterium breve A-1 on anxiety and depressive symptoms in schizophrenia: A proof-of-concept study. *J. Affect. Disord*. 2019; 245: 377–385. DOI: 10.1016/j.jad.2018.11.011.
37. Flowers S.A., Baxter N.T., Ward K.M. et al. Effects of atypical antipsychotic treatment and resistant starch supplementation on gut microbiome composition in a cohort of patients with bipolar disorder or schizophrenia. *Pharmacotherapy*. 2019; 39 (2): 161–170. DOI: 10.1002/phar.2214.
38. Sanchez-Villegas A., Martínez-González M.A. Diet, a new target to prevent depression? *BMC Med.* 2013; 11: 3. DOI: 10.1186/1741-7015-11-3.

Authors information

Neznanov Nikolay G., Dr. Sci. (Med.), Director, V.M. Bekhterev National Medical Research Center for Psychiatry and Neurology; Head of Psychiatry and Addictions Department, I.P. Pavlov First Saint-Petersburg State Medical University, Saint-Petersburg, Russian Federation. ORCID 0000-0001-5618-4206.

Rukavishnikov Grigory V., Cand. Sci. (Med.), Senior Researcher, Translational Psychiatry Department, V.M. Bekhterev National Medical Research Center for Psychiatry and Neurology, St. Petersburg, Russian Federation. ORCID 0000-0002-5282-2036.

Kasyanov Evgeny D., Junior Researcher, Translational Psychiatry Department, V.M. Bekhterev National Medical Research Center for Psychiatry and Neurology, St. Petersburg, Russian Federation. ORCID 0000-0002-4658-2195.

Ganzenko Maria A., Junior Researcher, Translational Psychiatry Department, V.M. Bekhterev National Medical Research Center for Psychiatry and Neurology, St. Petersburg, Russian Federation.

Leonova Lubov V., Resident, Translational Psychiatry Department, V.M. Bekhterev National Medical Research Center for Psychiatry and Neurology, St. Petersburg, Russian Federation. ORCID 0000-0001-6565-6594.

Zhilyaeva Tatiana V., Dr. Sci. (Med.), Associate Professor, Psychiatry Department, Privolzhsky Research Medical University, Nizhny Novgorod, Russian Federation. ORCID 0000-0001-6155-1007.

Mazo Galina E., Dr. Sci. (Med.), Head of Translational Psychiatry Department, Deputy Director for Innovative Development, V.M. Bekhterev National Medical Research Center for Psychiatry and Neurology, St. Petersburg, Russian Federation. ORCID 0000-0001-7036-5927.

(✉) **Rukavishnikov Grigory V.,** e-mail: grigory_v_r@mail.ru

Received 16.04.2020
Accepted 28.12.2020

УДК 616-056.43
https://doi.org/10.20538/1682-0363-2021-4-180-192

Epicutaneous sensitization. what do we know?

Novik G.A., Zhdanova M.V., Demidova A.S.

Saint-Petersburg State Pediatric Medical University
2, Litovskaya Str., St.Petersburg, 194100, Russian Federation

ABSTRACT

Background. According to the currently existing hypothesis, epicutaneous sensitization is one of the leading mechanisms in the development of food allergy.

The aim of this review was to analyze immune mechanisms in epicutaneous sensitization and the role of skin barrier impairment.

We performed a literature search using PubMed, UpToDate, Web of Science, and Scopus databases by the key words: epicutaneous sensitization, atopic dermatitis, skin barrier impairment, food allergy. Articles were to be in open access and present the most relevant information on the topic. Studies were selected by the largest sample size and the highest citation index. Once publications were identified, they were reviewed by all the authors to select the studies that specifically addressed the theme of the review. A total of 101 publications from 1998–2000 were included in the study.

This review article discusses the data of experimental studies, sets out modern ideas about the hypothesis of a double exposure to an allergen, and presents research data proving the clinical significance of epicutaneous sensitization in relation to food allergy. Knowledge about the mechanisms of epicutaneous sensitization development is necessary to elaborate strategies for prevention of food allergy. One of the modern trends in prevention is the use of emollients, which are supposed to restore the skin response. However, studies on preventive intake of emollients do not present a similar viewpoint.

There is not enough evidence for or against the mechanism of epicutaneous sensitization as an indispensable condition for the formation of food allergies. Further research in this area is required.

Key words: epicutaneous sensitization, skin barrier impairment, food allergy, emollients, atopic dermatitis.

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: Novik G.A., Zhdanova M.V., Demidova A.S. Epicutaneous sensitization. what do we know? *Bulletin of Siberian Medicine.* 2021; 20 (4): 180–192. https://doi.org/10.20538/1682-0363-2021-4-180-192.

Транскутанная сенсибилизация. Все ли мы знаем?

Новик Г.А., Жданова М.В., Демидова А.С.

Санкт-Петербургский государственный педиатрический медицинский университет (СПбГПМУ)
Россия, 194100, г. Санкт-Петербург, ул. Литовская, 2

✉ *Novik Gennady A.,* e-mail: ga_novik@mail.ru

РЕЗЮМЕ

Согласно существующей в настоящее время гипотезе, транскутанная сенсибилизация является одним из ведущих механизмов формирования пищевой аллергии.

Цель: анализ иммунологических механизмов формирования транскутанной сенсибилизации и роли дефекта кожного барьера.

Для написания обзора был проведен поиск полнотекстовых публикаций на английском языке в базах данных PubMed, UpToDate, Web of Science, Scopus по ключевым словам: epicutaneous sensitization, atopic dermatitis, skin barrier defect, food allergy. Статьи должны были находиться в свободном доступе и представлять наиболее актуальную информацию по теме. Исследования отбирались по принципу наибольшей выборки и индекса цитирования. После первичного отбора публикаций авторы изучили их на предмет соответствия информации тематике исследования. В обзор включена 101 публикация за период 1998–2020 гг.

Рассмотрены данные экспериментальных исследований, изложены современные представления о гипотезе двойного воздействия аллергена, приведены данные исследований, доказывающих клиническую значимость транскутанной сенсибилизации в формировании пищевой аллергии. Знание механизмов развития транскутанной сенсибилизации необходимо для выработки стратегий профилактики пищевой аллергии. Одним из перспективных направлений профилактики пищевой аллергии является использование эмолиентов, которые восстанавливают кожный ответ, однако исследования, посвященные профилактическому приему эмолиентов, в настоящее время не дают однозначного ответа.

В настоящее время накоплено недостаточно данных ни «за», ни «против» существования механизма транскутанной сенсибилизации как обязательного условия для формирования пищевой аллергии. Требуется дальнейшее проведение исследований в данном направлении.

Ключевые слова: транскутанная сенсибилизация, дефект кожного барьера, пищевая аллергия, атопический дерматит.

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: Новик Г.А., Жданова М.В., Демидова А.С. Транскутанная сенсибилизация. Все ли мы знаем? *Бюллетень сибирской медицины.* 2021; 20 (4): 180–192. https://doi.org/10.20538/1682-0363-2021-4-180-192.

INTRODUCTION

The term “food allergy” refers to a pathological immune response that develops after a contact with food (usually a meal) [1, 2]. Currently, several types of food allergy are distinguished with differences in their pathophysiological mechanisms (IgE-mediated and non-IgE-mediated) and clinical manifestations.

Atopic dermatitis (AD) is considered to be one of the risk factors for the development of food allergy. About 40% of moderate-to-severe atopic dermatitis cases in children are accompanied by IgE-mediated food allergy, which contributes to significant deterioration in the quality of life of patients [3, 4]. Early onset (less than 3 months of age) and severe course of AD are associated with an increase in the blood level of specific IgE to eggs, milk, and peanuts [5]. Food allergens trigger AD aggravation in 33% of patients with a severe course of AD, 10–20% of patients with moderate AD, and 6% of patients with mild AD [6–8].

The dual-allergen exposure hypothesis suggested by G. Lack et al. (2008) provides a possible explanation for a strong correlation between AD and accompanying food allergy. According to this hypothesis, a sufficient vitamin D level, gut microbiota diversity, and the natural route of food allergen penetration through the gastrointestinal tract induce oral tolerance. Exposure to low doses of food allergens from the environment (on surfaces, hands, and in dust) through the impaired skin barrier, vitamin D deficiency, and decreased gut microbiota diversity lead to the development of sensitization [9].

This review article analyzes in detail the mechanisms of the formation of epicutaneous sensitization according to available modern research data and considers controversial issues that contradict this hypothesis.

DATA SOURCES

The authors analyzed studies on epicutaneous sensitization using the PubMed (https://www.ncbi.nlm.nih.gov/pubmed/) and UpToDate (https://www.uptodate.com/) databases. The review uses original articles published over the period from 1998 to 2020. Preliminarily, the search in PubMed was conducted using the following key words: epicutaneous sensitization, atopic dermatitis, skin barrier defect, food allergy. The search in UpToDate was performed using the following key words: pathogenesis of food allergy, atopic dermatitis pathogenesis, clinical manifestations and diagnosis. The search for studies corresponding to the listed terms was also carried out among the lists of references and citations in the selected publications. 420 publications from the PubMed database were studied. At the stage of publication selection, articles with the absence of the required data in the abstract text were excluded. A total of 289 publications were analyzed. Subsequently, the analysis of full-text publications was carried out. The criteria for eligibility were originality of the article, the English language, publications in journals included in the international citation system Web of Science (www.webofknowledge.com)/Scopus), compliance of the submitted data with the subject of the article. The exclusion criterion was the absence of the required data in the text of the article. Based on the selection results, this article includes data from 101 sources.

EXPERIMENTAL MODELS OF FOOD SENSITIZATION FORMATION

Experimental data now suggest that development of food allergy is closely related to exposure of an allergen through the skin [10]. First attempts to prove the possibility of epicutaneous sensitization were made in experimental models in mice. In the study, applications of ovalbumin (chicken egg protein) to damaged skin caused an increase in the specific IgE. Consequently, when the allergen was administered orally, the mice developed an anaphylactic reaction with an increase in histamine and histological changes in the intestine and lungs [11].

Later, J. Strid et al. (2004) showed that the immune response caused by epicutaneous exposure to an allergen is directed towards the Th2 pathway. This kind of reaction was obtained when various types of mice were exposed to various antigens. [12–16]. In these studies, microdamage to the skin is a model of skin barrier impairment, which is observed in patients with AD. A series of experimental studies showed that preliminary sensitization of mice using the epicutaneous route of allergen penetration through damaged skin leads to inhibition of oral tolerance induction [17, 18].

ROLE OF SKIN BARRIER DEFECTS

One of the important links in the pathogenesis of AD is impairment of the epithelial barrier, which significantly increases the risk of developing food allergies and other allergic diseases. The barrier function of the skin is provided mainly by the stratum corneum, which consists of corneocytes and extracellular matrix. Corneocytes are held together by tight junctions and corneodesmosomes. Loss of function of corneodesmosin, a gene encoding components of corneodesmosomes, in various congenital defects leads to severe defects in the skin barrier, itching, and atopy [19].

FILAGGRIN DEFECT

Currently, the defect in the *FLG* gene encoding filaggrin occupies a prominent place in impairment of the skin barrier. Filaggrin is a major epidermal protein which is crucial for the structure and function of the stratum corneum, which provides a physical barrier [20]. Mutations in the filaggrin-encoding gene are considered some of the most important risk factors for sensitization and development of a number of allergic clinical phenotypes, most likely due to exposure to allergens through the skin [21]. Data from a systematic review and meta-analysis of 24 studies suggest that the presence of an impairment in the skin barrier is fundamental in the development of allergic diseases. Filaggrin defects increase the risk of developing sensitization, atopic eczema, allergic rhinitis, and bronchial asthma in individuals with eczema [22, 23].

Children with a *FLG* gene mutation were 2.4 times more likely to develop a food allergy confirmed by an oral challenge test. Initially, it was shown that children with *FLG* mutations have higher prevalence of sensitization to peanuts, increasing the risk of developing IgE-mediated allergy by more than 5 times [24].

This was also proven in another study in the UK, which was aimed at investigating the effect of mutations in the *FLG* gene on the risk of developing food allergy to peanuts. The amount of peanut antigen in houses where children of the first year of life lived was measured. To identify sensitization and confirm food allergy, skin prick tests and determination of specific IgE to peanut allergens Ara h 1,2,3 in the blood serum by the ImmunoCAP method were used. Oral peanut

test at the age of 8 and 11 years and genotyping were used for six *FLG* gene mutations. The results of the study indicate a significant increase in the risk of sensitization and peanut allergy in children with the *FLG* mutation compared with children without skin barrier impairments, confirming the hypothesis of transcutaneous sensitization [25].

An association between filaggrin mutation and food allergy, not only to peanuts, but also to eggs and milk, as proven by a provocation test, was shown in the genome-wide association study (GWAS), with a significant association observed even in the absence of eczema [26].

Filaggrin gene mutations increase the chances of developing sensitization not only to food, but also to inhaled allergens. In the UK, a population-based cohort study of 1,051 children was conducted, which showed a significant increase in the risk of sensitization to the major cat allergen Fel d1 among children with filaggrin mutations compared with children without them. It was also noted that the risk of developing sensitization to house dust mite with increasing exposure to Der p1 (a major allergen of house dust mites) was consistently higher among children with filaggrin mutations [27, 28].

Other factors that are crucial in disrupting the skin barrier include changes in the structure of the lipid composition of the intracellular matrix, an imbalance between stratum corneum protease and antiprotease activity, tight junction dysfunction of the stratum corneum, microbial colonization, and release of proinflammatory cytokines.

TRANSEPIDERMAL WATER LOSS

The extracellular matrix of the stratum corneum consists of multiple lamellar membranes enriched with ceramides, cholesterol, and free fatty acids.

The study by C. Cole et al. (2014) demonstrated that changes in the lipid composition of the epidermis cause impairment of the epidermal barrier regardless of the presence or absence of the filaggrin gene mutation [29]. A later published meta-analysis revealed that changes in the lipid layer composition in patients with AD occur both in skin lesions and on visually healthy skin [30]. Lipid barrier impairment entails increased transepidermal water loss (TEWL), which is crucial in the pathogenesis of food allergy in newborns. It was discovered that 75% of children suffering from food allergies had high rates of TEWL in infancy, and even in the absence of atopic dermatitis, the risk of developing food allergies was 3.5 times higher [31].

Further studies confirmed these data; it was found that excessive TEWL in the first week of life is an independent risk factor for the development of AD and is associated with higher allergic sensitization.

TIGHT JUNCTION DYSFUNCTION IN THE STRATUM CORNEUM

Tight junctions are transmembrane protein complexes that provide keratinocyte adhesion, thereby creating a permeability barrier for the intercellular space. They regulate paracellular transport of liquids and solutes. This is important because it determines the nature of ion and protein transport and even penetration of Langerhans or dendritic cells. Tight junctions are located directly under the stratum corneum, forming the so-called second barrier in the epidermis [32].

In patients with AD, a congenital deficiency of transmembrane tight junction proteins is observed, which is especially pronounced in the presence of a filaggrin gene mutation. A. De Benedetto et al. (2011) found a decrease in the expression of claudin-1, -4, -23 in patients with AD with undamaged skin [26]. It is important to note that a decrease in the expression of tight junction components was associated with a significant change in the bioelectric characteristics of the epidermis in AD (not damaged and not exposed to the sun) with noticeably lower transepithelial electrical resistance, higher albumin permeability, and associated selective ionic permeability. This is also shown by earlier studies on mice in which the claudin-1 gene was “turned off”, and in the first 24 hours after birth, TEWL was observed, which led to their death [33].

An inverse correlation was also noted between the expression of epidermal claudin-1 and markers of the Th2 response (eosinophilia, total IgE level). This suggests that the Th2 response may inhibit the expression of the key members of the claudin family (e.g., claudin-1, -4, -23) or the other way round. To investigate whether changes in the claudin-1 gene could be associated with AD and its more severe course, two populations (African Americans and Caucasian Americans) were studied. The strongest association was observed in the African American population, with changes in the claudin-1 gene being associated with earlier onset and a more severe course of AD. Weaker associations were observed among Caucasian Americans. It is interesting to note that some defects in the claudin-1 gene are associated with sensitization to contact allergens in the North European population [34].

PROTEASE AND ANTIPROTEASE ACTIVITY OF THE STRATUM CORNEUM

The skin barrier function can also be impaired in cases of genetic disorders with an increased level of chymotrypsin and trypsin enzymes in the stratum corneum. These enzymes cause premature destruction of corneodesmosomes, which leads to disruption of the skin barrier [36].

The *KLK7* gene encoding chymotrypsin was tested for variations in healthy children and children with AD. Defects in the *KLK7* gene were assessed and their possible association with dysregulation of chymotrypsin in humans, leading to thinning of the skin barrier. The strongest association was observed in the subgroup of patients who did not have elevated IgE levels. This association was not significant in the subgroup of patients with high serum IgE [37].

When endogenous proteases are produced excessively, premature desquamation of the stratum corneum occurs, and a thinned skin barrier is formed. This facilitates penetration of allergens, which can further cause AD or its aggravation. External effects, such as washing with detergents and prolonged use of topical corticosteroids can further increase the production of these enzymes in the stratum corneum and impair the skin barrier function [36]. Normally, the activity of proteases involved in epidermal desquamation is regulated by several protease inhibitors co-expressed to balance the rate of stratum corneum degradation.

Genetic mutations have also been identified in genes encoding elements of inhibitors of these proteases. For example, mutations in the *SPINK5* gene, which encodes serine protease lymphoepithelial Kazal-type 5 inhibitor, have been associated with Netherton syndrome. Patients with this syndrome have severe barrier dysfunction, including increased desquamation and impaired keratinization. Several studies showed a link between a defect in the *SPINK5* gene and AD [38–41]. In addition, damaged skin cells can produce endogenous proteases that further impair the skin barrier. These proteases can be considered as a product of an inflammatory response, and their level is proportional to the severity of AD aggravation. Mast cell chymase is a chymotrypsin-like serine protease that is primarily stored in the secretory granules of mast cells. In one study, mast cell chymase level was significantly increased in AD patients in damaged skin compared with intact skin. However, no significant difference was found in the level of mast cell chymase between intact skin in AD patients and healthy individuals, which suggests that increased mast cell chymase activity may be associated with active dermatitis [42].

There is also evidence that mast cell chymase may be involved in the development of chronic dermatitis by inducing eosinophilic infiltration [43]. Different variations in the chymase-encoding gene have been associated with AD in children, the association being the strongest in individuals with low total serum IgE [44].

The skin barrier can also be damaged by exogenous proteases from house dust mites and Staphylococcus aureus [45]. House dust mites are a source of over 30 different proteins that can induce IgE-mediated responses. Some of these proteins are cysteine and serine proteases. Patch tests have shown that two proteins with proteolytic activity derived from house dust mites, Der p 1 and Der p 2, induce skin irritation or immune activation through direct proteolytic activity [46].

ROLE OF STAPHYLOCOCCUS AUREUS IN THE FORMATION OF EPICUTANEOUS SENSITIZATION

At present, there is a large body of scientific data proving the role of *St. aureus* in the pathogenesis of AD [47]. Epicutaneous sensitization with staphylococcal enterotoxin induces local inflammation corresponding to eczema in mice and subjects with normal and atopic skin [48, 49]. Population-based cohort studies report that colonization of the skin or nasopharynx by *St. aureus* precedes the clinical diagnosis of eczema in infancy. In addition, patients with eczema are more prone to colonization with *St. aureus* than healthy controls, and disease severity is associated with colonization of the affected skin with *St. aureus* [50].

St. aureus can cause significant impairment of the skin barrier and thus contribute to the development of food sensitization through epicutaneous exposure to the allergen. Moreover, *St. aureus* causes skin disruption as a result of exotoxins and protease and lipase production.

Exposure to the peanut antigen through the skin in the presence of *St. aureus* enterotoxin significantly enhanced the CD4 + Th2 response in mice, suggesting that *St. aureus* contributes to the development of food allergies. Exposure to *St. aureus* toxin in mice also led to an increase in Th2-mediated responses and a decrease in the regulatory function of T cells, both of these mechanisms having been described in patients with food allergies [51, 52]. In a retrospective study

by A.L. Jones et al., skin cultures from AD patients aged 0–18 years were analyzed. The data obtained indicate the presence of an association between colonization of the skin by *St. aureus* and food allergy to peanuts, egg white, and cow's milk in patients with AD [53]. Later, in the study by O. Tsilochristou et al. (2019), conducted in the age group of 0–6 years, it was shown that, regardless of the severity of eczema, there was an association with sensitization to chicken eggs and peanuts and a weaker association with cow's milk [55].

Thus, various impairments of the skin barrier, including colonization by *St. aureus* lead to immune dysregulation, ultimately contributing to the development of food allergies through local exposure to the allergen [54].

A question arises, how food allergens penetrate the skin. A number of studies showed the presence of food allergens in house dust, not only in the cooking area, but also in children's beds [56, 57]. For example, in Norway, dust samples from 143 houses were found to have fish allergen in 46%, peanuts in 41%, milk in 39%, and egg allergen in 22% of mattress dust samples [58]. Food allergens can be found in cosmetics used for the basic therapy of atopic dermatitis, resulting in direct contact of food proteins with the affected skin [16, 59]. An analysis of data from the study involving 13,971 preschool children showed a significant association of peanut allergy with skin care products containing peanut butter [60]. Despite the proven role of skin barrier impairment in development of epicutaneous sensitization, it is not the only component in its pathogenesis.

ROLE OF IMMUNE MECHANISMS IN THE DEVELOPMENT OF SENSITIZATION

Epidermal exposure to allergens selectively stimulates the Th2 type reaction, leads to an increase in the thickness of the epidermis, a rise in the level of antigen-specific IgE in the blood serum, and production of cytokines, and may contribute to the development of an allergic reaction upon subsequent exposure to the allergen through the gastrointestinal tract, where mast cells accumulate, as evidenced by an increase in the serum level of mast cell protease-1 (MCP-1) [61–63].

The immune response in food allergy includes two phases (Figure). The first phase begins with absorption of antigens by dendritic cells and their transport to the lymph nodes, where the antigen is presented to naive CD4+ T cells. In the lymph nodes, in the presence of interleukin (IL)-4 and cytokines, T cells differentiate into allergen-specific CD4 + T cells, producing high levels of cytokines (IL-4, IL-13) that, in turn, facilitate the production of B cell isotypes – specific IgE memory cells [64].

Due to the facilitated antigen presentation, a very low concentration of allergen can stimulate the formation of a complex between specific IgE, the allergen, and the low-affinity IgE receptor on the surface of antigen-presenting B cells (CD23+ cells). This complex then further stimulates Th2 cell proliferation, leading to further B cell isotype switching and increased IgE production [65, 66].

As B cells mature, they differentiate into plasma cells and produce large amounts of allergen-specific IgE antibodies (sIgE) that bind to high-affinity FceRI receptors on the surface of mast cells and basophils. During this phase, a memory pool of allergen specific B cells and allergen specific CD4+ Th2 cells is generated. Recently, it has been suggested that a subset of Th2 cells (Th2A cells) play an important role in the immune response to allergy. Congenital group 2 innate lymphoid cells (ILC2), which are found on the surface of the lungs, intestines, and skin, serve as key regulators and effectors of immunity and promote tissue repair. They are also found in human skin lesions in AD and are activated by IL-33. ILC2 secrete proallergic cytokines, including IL-5 and IL-13. IL-5 triggers recruitment of eosinophils. IL-13 promotes recruitment of inflammatory cells, alters skin microbiome, and reduces the epidermal barrier.

The effector phase follows the sensitization phase and is triggered when a person encounters a previously sensitizing allergen. This causes cross-linking of the FceRI-bound receptor with sIgE on sensitized mast cells and basophils, resulting in the release of preformed and *de novo* inflammatory mediators. These processes lead to an immediate phase of an allergic reaction and, subsequently, to a late phase of an allergic reaction through activation of allergen-specific Th2 memory cells [64].

Activated Th2 cells produce, among other cytokines, IL-4, IL-5, IL-13. Recent evidence suggests that IL-13 is a key cytokine that stimulates peripheral inflammation in AD, while IL-4 has a more central effect [13, 67]. The primary importance of IL-4 in the development of both sensitization and IgE-mediated food allergy is confirmed by the absence of IgE production in the presence of anti-IL4 antibodies [69]. Eosinophils and basophils are the predominant IL-4 competent cells that accumulate in the skin in response to transepidermal penetration of food allergens

[70]. Cytokines support allergen-specific IgE levels, eosinophilia, mucus production, and recruitment of inflammatory cells in the inflamed tissues, leading to tissue damage.

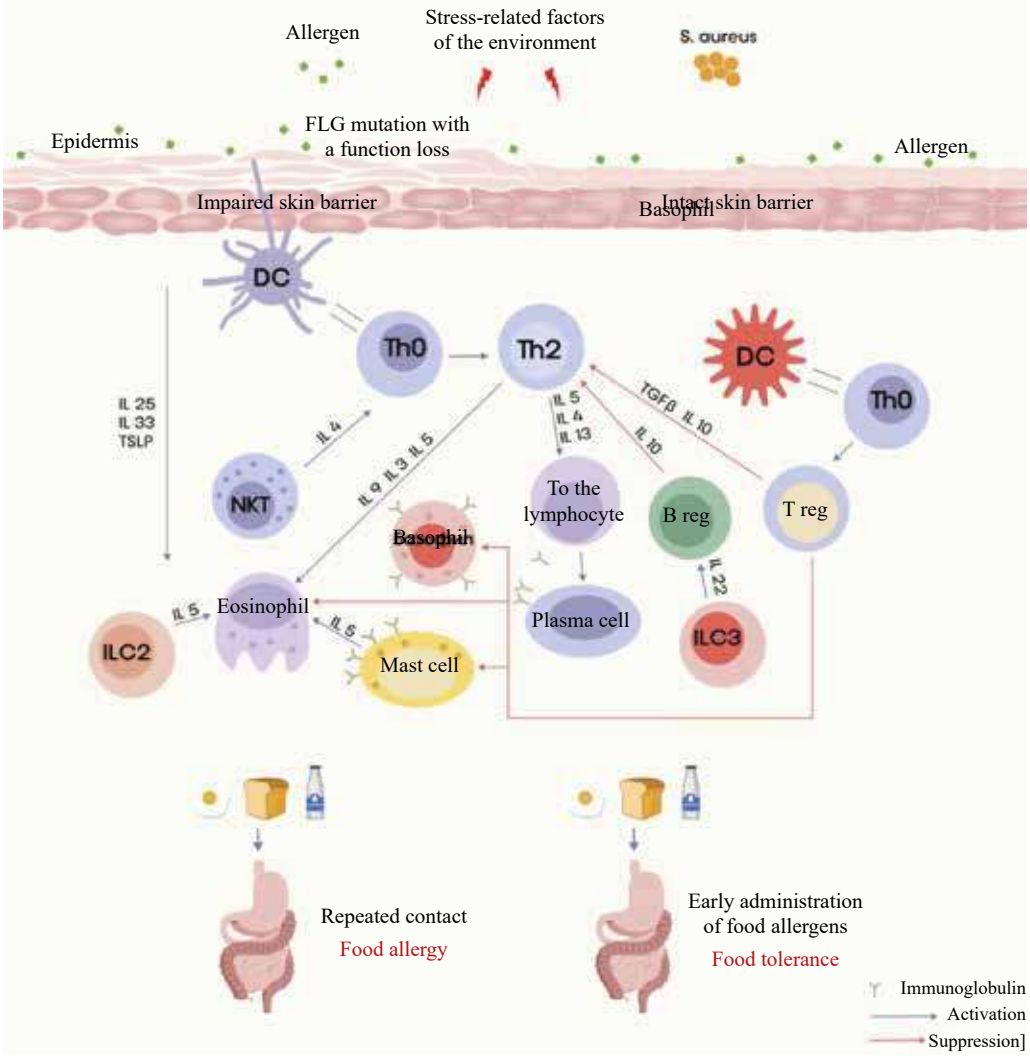


Figure. Immune mechanisms in the development of epicutaneous sensitization: NKT – natural killer cells, B reg – B regulatory cells, ILC2 – congenital group 2 innate lymphoid cells, ILC3 – congenital group 3 innate lymphoid cells

ROLE OF THYMIC STROMAL LYMPHOPOETIN IN THE FORMATION OF EPICUTANEOUS SENSITIZATION

Thymic stromal lymphopoetin (TSLP) is one of the most important cytokines involved in epicutaneous sensitization [68]. This cytokine is elevated in the stratum corneum in patients with AD and correlates with the severity of the disease [71].

TSLP-deficient mice were protected from the development of allergic inflammation of the skin, respiratory tract, and food allergy after exposure to the antigen. These experimental data prove the importance of this cytokine in allergic sensitization [72–75]. The proinflammatory cytokines TNFα and IL-1a, produced in response to skin damage, induce the secretion of TSLP from human keratinocytes.

TSLP expression correlates with maturation of Langerhans cells, regulation of the TSLP receptor on these cells, and their migration to lymph nodes, where they promote the differentiation of naive Th cells into Th2 cells [76]. TSLP induces the migration of Th2 targeted antigen-presenting cells to the mesenteric lymph nodes, thereby promoting the development of allergic reactions in the intestine. These data indicate the association of TSLP with early stages of epicutaneous sensitization [77].

ROLE OF IL-33 IN THE FORMATION OF EPICUTANEOUS SENSITIZATION

Research data also indicate the possible key role of IL-33 in the development of epicutaneous sensitization [45, 78]. IL-33 is a part of the IL-1 cytokine family. It is expressed in epithelial barrier tissues and lymphoid organs and is crucial in the initiation of allergic inflammation after exposure to an allergen [79].

The level of IL-33 was elevated in the affected skin and serum of patients with AD, as well as in experimental models in mice after epicutaneous sensitization with ovalbumin [80, 81]. IL-33 promotes increased secretion of IL-5 and IL-13 by polarized Th2 lymphocytes and is associated with increased serum IgE levels and eosinophilia [82]. A study investigating the role of IL-33 in epicutaneous sensitization in food allergy showed that IL-33 is required to induce IgE-dependent anaphylaxis. IL-33-deficient mice and mice treated with a soluble IL-33 antagonist were protected from oral allergen-induced anaphylaxis [83].

C. Galand et al. (2016) in their work demonstrated that mechanical damage to the skin caused by the removal of the adhesive tape in mice epicutaneously sensitized with ovalbumin induced local and systemic release of IL-33, which led to an increase in IgE-mediated degranulation of mast cells and oral allergen-induced anaphylaxis [84]. Blockade of ST2 (IL-33 receptor) by anti-ST2 monoclonal antibodies led to inhibition of the anaphylactic reaction and suppression of the production of antigen-specific IgE and inflammatory mediators [85]. The existing data suggest that IL-33 plays a key role in epicutaneous sensitization. Neutralization of IL-33 is currently considered a promising strategy for the treatment of food allergy and AD [86, 87]. IL-24 involved in the suppression of filaggrin production in keratinocytes performs an important function in the development of AD [88].

MECHANISM OF FOOD TOLERANCE FORMATION

Several factors, including allergen properties, dose, entry route, genetic factors, and age, contribute to the development of food tolerance or sensitization [89]. In the context of discussing sensitization to food, the entry route of the allergen is the most important. Initial exposure to the food allergen by the extraintestinal route is more likely to lead to sensitization. If the skin barrier is not impaired and the immune system is not primed through the skin, the tolerance mechanisms are triggered [9].

Experimental models show that tolerance is mediated by various mechanisms, such as anergy and deletion of lymphocytes, as well as suppression of sensitization by T regulatory cells [90]. Regulatory T cells are thought to induce tolerance by secreting suppressive cytokines, IL-10, and transforming growth factor (TGF) β (Figure).

The age of exposure is also crucial in the induction of oral allergen tolerance. In the experimental study, feeding newborn mice with albumin led to priming of humoral and cell-mediated responses, while in adults this caused tolerance [91]. Developing tolerance is important in preventing the development of food allergies [92, 93]. Interestingly, countries that have peanut snacks for children have relatively low rates of peanut allergy [94]. It was shown that early introduction of milk, eggs, and peanuts reduces the risk of developing food allergies [95, 96]. Based on the data obtained in 2014, the EAACI consensus was adopted stating that introduction of products during the window of tolerance (the interval between 4–7 months of a child's life) is recommended for all children, regardless of the presence of an atopic predisposition [97].

PREVENTIVE EFFECT OF EMOLLIENTS ON THE FORMATION OF FOOD ALLERGY

According to the available data, a defect in the skin barrier along with immune dysregulation are the leading mechanisms in the formation of epicutaneous sensitization. Various strategies are currently being developed to reduce the risk of formation of AD and food allergy. One of the relevant areas of development is preventive use of emollients in children who have a history of atopy. The idea of using emollients in the context of food sensitization is that, by protecting the skin barrier, they should prevent penetration of the allergen and, as a result, development of sensitization. However, currently, there are many unresolved issues and controversial points regarding the effectiveness of their use.

The study by H. Kenta et al. (2014) showed that the use of emollients in children in the first 32 weeks of life prevented the development of AD in 32% of cases in comparison with the control group [98]. Similar data were also published in 2014, according to which, in children with a high risk of AD, the preventive effect of emollients is independent of the presence of a filaggrin gene defect [99].

At the same time, the study by R.C. Joanne et al. (2020) provided no confirmation of the preventive effect of emollients. The multicenter, randomized study

involved 1,394 children, of whom 693 individuals received emollients (Diprobase cream or Doublebase gel), and 701 children were in the control group. At 2 years of age, eczema was present in 139 (23%) of 598 infants in the emollient group and in 150 (25%) of 612 infants in the control group [100].

Additionally, in the work by E. Dissanayake et al. (2019), which studied the use of emollients in children who were not at high risk of developing AD, no effect from the prophylactic use of emollients was observed [101]. One of the possible reasons for such differences may be the sample of the study population, which was an ordinary-risk group for the development of AD, while in other studies the high-risk group was examined. At the same time, regardless of the obtained effect from the use of emollients to prevent AD, the authors of the works in which the end point of the study was not only AD but also food sensitization agree that emollients do not affect the prevention of food sensitization.

Thus, the aforementioned work by H. Kenta et al. did not find a statistically significant effect on allergic sensitization based on the level of IgE to egg white. However, the level of sensitization was significantly higher in infants with AD [98]. Moreover, no relationship was found between the use of emollients and the development of food allergies; the difference with the control group was only 2% in the work of E. Dissanayake et al. [101]. There are currently insufficient data to draw definitive conclusions for or against this method of preventing food allergy. It should be noted that the effectiveness of the preventive use of emollients must be assessed based on the composition of the specific agent used, therefore further research is required.

CONCLUSION

A large body of data has been accumulated on the possible existence of epicutaneous sensitization, in which priming of immune cells occurs, and, subsequently, food allergy develops. Along with experimental studies confirming the role of epicutaneous sensitization, clinical data were obtained confirming this route of exposure, which opens up ways for possible prevention of food allergies, in particular, early introduction of food allergens, neutralization of IL-33, and preventive use of emollients.

It is assumed that emollients that create a protective film in the case of congenital defects of the skin barrier should prevent penetration of the allergen and, consequently, development of sensitization. However, studies on the preventive effect of emollients have conflicting results: some studies confirm their effectiveness, while others do not. Therefore, additional studies are required on the role, place, and mechanisms of the formation of epicutaneous sensitization in patients with food allergies.

REFERENCES

1. NIAID-Sponsored Expert Panel, Boyce J.A., Assa'ad A. et al. Guidelines for the diagnosis and management of food allergy in the United States: report of the NIAID-sponsored expert panel. *J. Allergy Clin. Immunol.* 2010; 126 (6): S1–58. DOI: 10.1016/j.jaci.2010.10.007.
2. Sampson H.A., Aceves S., Bock S.A. et al. Food allergy: a practice parameter update-2014. *J. Allergy Clin. Immunol.* 2014; 134 (5): 1016–1025.e43. DOI: 10.1016/j.jaci.2014.05.013.
3. Eigenmann P.A., Beyer K., Lack G. et al. Are avoidance diets still warranted in children with atopic dermatitis? *Pediatr. Allergy Immunol*. 2020; 31 (1): 19–26. DOI: 10.1111/pai.13104.
4. Warren C.M. et al. Epidemiology and burden of food allergy. *Curr. Allergy Asthma Rep.* 2020; 20 (2): 6. DOI: 10.1007/s11882-020-0898-7.
5. Hill D.J., Hosking C.S., de Benedictis F.M. et al. Confirmation of the association between high levels of immunoglobulin E food sensitization and eczema in infancy: an international study. *Clin. Exp. Allergy.* 2008; 38 (1): 161–168. DOI: 10.1111/j.1365-2222.2007.02861.x.
6. Eigenmann P.A., Calza A.M. Diagnosis of IgE-mediated food allergy among Swiss children with atopic dermatitis. *Pediatr. Allergy Immunol. 2000;* 11 (2): 95–100. DOI: 10.1034/j.1399-3038.2000.00071.x.
7. Eigenmann .PA., Sicherer S.H., Borkowski T.A. et al. Prevalence of IgE-mediated food allergy among children with atopic dermatitis. *Pediatrics.* 1998; 101 (3): E8. DOI: 10.1542/peds.101.3.e8.
8. Sampson H.A., McCaskill C.C. Food hypersensitivity and atopic dermatitis: evaluation of 113 patients. *J. Pediatr. 1985;* 107 (5): 669–675. DOI: 10.1016/s0022-3476(85)80390-5.
9. Lack G. et al. Epidemiologic risks for food allergy. *J. Allergy Clin. Immunol*. 2008;121 (6): 1331–1336. DOI: 10.1016/j.jaci.2008.04.032.
10. Yu R., Igawa K., Handa Y., Munetsugu T., Satoh T., Yokozeki H. Basophils and mast cells are crucial for reactions due to epicutaneous sensitization to ovalbumin. *Exp. Dermatol*. 2017; 26 (9): 778–784. DOI: 10.1111/exd.13279.
11. Hsieh K.Y., Tsai C.C., Wu C.H., Lin R.H. Epicutaneous exposure to protein antigen and food allergy. *Clin. Exp. Allergy. 2003;* 33 (8): 1067–1075. DOI: 10.1046/j.1365-2222.2003.01724.x.
12. Strid J., Hourihane J., Kimber I. et al. Disruption of the stratum corneum allows potent epicutaneous immunization with protein antigens resulting in a dominant systemic Th2 response. *Eur. J. Immunol. 2004;* 34 (8):2100–2109. DOI: 10.1002/eji.200425196.
13. Benor S., Shani N., Etkin S., Bondar E., Kivity S., Langier S. Epicutaneous exposure to peanut oil induces systemic and pulmonary allergic reaction in mice. *Int. Arch. Allergy Immunol.* 2019;179(3):187–191. DOI: 10.1159/000497382.

14. Glocova I., Brück J., Geisel J., Müller-Hermelink E., Widmaier K., Yazdi A.S. et al. Induction of skin-pathogenic Th22 cells by epicutaneous allergen exposure. *J. Dermatol. Sci.* 2017;87 (3): 268–277. DOI: 10.1016/j.jdermsci.2017.06.006.
15. Koshiba R., Oba T., Fuwa A., Arai K., Sasaki N., Kitazawa G. et al. Aggravation of food allergy by skin sensitization via systemic Th2 enhancement. *Int. Arch. Allergy Immunol.* 2021; 182 (4): 292–300. DOI: 10.1159/000511239.
16. Murakami H., Ogawa T., Takafuta A., Yano E., Zaima N., Moriyama T. Percutaneous sensitization to soybean proteins is attenuated by oral tolerance. *J. Nutr. Sci. Vitaminol.* (Tokyo). 2018; 64 (6): 483–486. DOI: 10.3177/jnsv.64.483.
17. Strid J., Hourihane J., Kimber I. et al. Epicutaneous exposure to peanut protein prevents oral tolerance and enhances allergic sensitization. *Clin. Exp. Allergy. 2005;* 35 (6): 757–766. DOI: 10.1111/j.1365-2222.2005.02260.x.
18. Iwamoto H., Matsubara T., Okamoto T., Matsumoto T., Yoshikawa M., Takeda Y. Ingestion of casein hydrolysate induces oral tolerance and suppresses subsequent epicutaneous sensitization and development of anaphylaxis reaction to casein in mice. *Int. Arch. Allergy Immunol*. 2019; 179 (3): 221–230. DOI: 10.1159/000497410.
19. Oji V., Eckl K.M., Aufenvenne K., Natebus M., Tarinski T., Ackermann K. et al. Loss of corneodesmosin leads to severe skin barrier defect, pruritus, and atopy: unraveling the peeling skin disease. *Am. J. Hum. Genet*. 2010; 87 (2): 274–281. DOI: 10.1016/j.ajhg.2010.07.005.
20. Drislane C., Irvine A.D. The role of filaggrin in atopic dermatitis and allergic disease. *Ann. Allergy Asthma Immunol*. 2020; 124 (1): 36–43. DOI: 10.1016/j.anai.2019.10.008.
21. Irvine A.D. et al. Filaggrin mutations associated with skin and allergic diseases. *N. Engl. J. Med.* 2011; 365 (14): 1315–1327. DOI: 10.1056/NEJMra1011040.
22. Van den Oord R.A., Sheikh A. Filaggrin gene defects and risk of developing allergic sensitisation and allergic disorders: systematic review and meta-analysis. *BMJ.* 2009; 339: b2433. DOI: 10.1136/bmj. b2433.
23. Dębińska A. et al. Filaggrin loss-of-function mutations as a predictor for atopic eczema, allergic sensitization and eczema-associated asthma in Polish children population. *Adv. Clin. Exp. Med*. 2017;26 (6): 991–998. DOI: 10.17219/acem/61430.
24. Brown S.J. et al. Loss-of-function variants in the filaggrin gene are a significant risk factor for peanut allergy. *J. Allergy Clin. Immunol*. 2011; 127 (3): 661–667. DOI: 10.1016/j.jaci.2011.01.031.
25. Brough H.A. et al. Peanut allergy: Effect of environmental peanut exposure in children with filaggrin loss-of-function mutations. *J. Allergy Clin. Immunol*, 2014; 134 (4): 867–875. e1. DOI: 10.1016/j.jaci.2014.08.011.
26. Marenholz I. et al. Genome-wide association study identifies the SERPINB gene cluster as a susceptibility locus for food allergy. *Nat. Commun*. 2017; 8 (1): 1056. DOI: 10.1038/s41467-017-01220-0.
27. Chan A. et al. Filaggrin mutations increase allergic airway disease in childhood and adolescence through interactions with eczema and aeroallergen sensitization. *Clin. Exp. Allergy*. 2018; 48 (2):147–155. DOI: 10.1111/cea.13077.
28. Simpson A. et al. Early-life inhalant allergen exposure, filaggrin genotype and the development of sensitization from infancy to adolescence. *J. Allergy Clin. Immunol*. 2020; 145 (3): 993–1001. DOI: 10.1016/j.jaci.2019.08.041.
29. Cole C., Kroboth K., Schurch N.J., Sandilands A., Sherstnev A., O'Regan G.M. et al. Filaggrin-stratified transcriptomic analysis of pediatric skin identifies mechanistic pathways in patients with atopic dermatitis. *J. Allergy Clin. Immunol*. 2014; 134 (1): 82–91. DOI: 10.1016/j.jaci.2014.04.021.
30. Czarnowicki T., Krueger J.G., Guttman-Yassky E. Novel concepts of prevention and treatment of atopic dermatitis through barrier and immune manipulations with implications for the atopic march. *J.* Allergy Clin. Immunol. 2017; 139 (6): 1723–1734. DOI: 10.1016/j.jaci.2017.04.004.
31. Kelleher M.M. et al. Skin barrier impairment at birth predicts food allergy at 2 years of age. *J. Allergy Clin. Immunol*. 2016; 137 (4): 1111–1116.e8. DOI: 10.1016/j.jaci.2015.12.1312.
32. Leung D.Y. New insights into atopic dermatitis: role of skin barrier and immune dysregulation. *Allergol. Int*. 2013; 62 (2): 151–161. DOI: 10.2332/allergolint.13-RAI-0564.
33. De Benedetto A., Rafaels N.M., McGirt L.Y., Ivanov A.I., Georas S.N., Cheadle C. et al. Tight junction defects in patients with atopic dermatitis. *J. Allergy Clin. Immunol*. 2011; 127 (3):773–86e1-7. DOI: 10.1016/j.jaci.2010.10.018.
34. Furuse M., Hata M., Furuse K. et al. Claudin-based tight junctions are crucial for the mammalian epidermal barrier: a lesson from claudin-1-deficient mice. *J. Cell Biol*. 2002; 156 (6): 1099–1111. DOI: 10.1083/jcb.200110122.
35. Brandner J.M., Zorn-Kruppa M., Yoshida T. et al. Epidermal tight junctions in health and disease. *Tissue Barriers.* 2015; 3 (1–2): e974451. DOI: 10.4161/21688370.2014.974451.
36. Cork M.J., Robinson D.A., Vasilopoulos Y. et al. New perspectives on epidermal barrier dysfunction in atopic dermatitis: gene-environment interactions. *J. Allergy Clin. Immunol.* 2006; 118 (1): 3–21. DOI: 10.1016/j.jaci.2006.04.042.
37. Vasilopoulos Y., Cork M.J., Murphy R., Williams H.C., Robinson D.A, Duff G.W. et al. Genetic association between an AACC insertion in the 3′UTR of the stratum corneum chymotryptic enzyme gene and atopic dermatitis. *J. Invest. Dermatol*. 2004; 123 (1): 62–66. DOI: 10.1111/j.0022-202X.2004.22708.x.
38. Walley A.J. Chavanas S., Moffatt M.F., Esnouf R.M., Ubhi B., Lawrence R. et al. Gene polymorphism in Netherton and common atopic disease. *Nat. Genet.* 2001; 29 (2): 175–178. DOI: 10.1038/ng728.
39. Kato A., Fukai K., Oiso N. et al. Association of SPINK5 gene polymorphisms with atopic dermatitis in the Japanese population. *Br. J. Dermatol.* 2003; 148 (4): 665–669. DOI: 10.1046/j.1365-2133.2003.05243.x.
40. Lan C.C., Tu H.P., Wu C.S. et al. Distinct SPINK5 and IL-31 polymorphisms are associated with atopic eczema and non-atopic hand dermatitis in Taiwanese nursing population. *Exp. Dermatol.* 2011; 20 (12): 975–979. DOI: 10.1111/j.1600-0625.2011.01374.x.
41. Zhao L.P., Di Z., Zhang L. et al. Association of SPINK5 gene polymorphisms with atopic dermatitis in Northeast China. *J. Eur. Acad. Dermatol. Venereol.* 2012; 26 (5): 572–577. DOI: 10.1111/j.1468-3083.2011.04120.x.

42. Badertscher K., Bronnimann M., Karlen S., Braathen L.R., Yawalkar N. Mast cell chymase is increased in atopic dermatitis but not in psoriasis. *Arch. Dermatol. Res.* 2005; 296. (10): 503–506. DOI: 10.1007/s00403-005-0542-3.
43. Tomimori Y., Muto T., Fukami H., Saito K., Horikawa C., Tsuruoka N. et al. Chymase participates in chronic dermatitis by inducing eosinophil infiltration. *Lab. Invest*. 2002; 82 (6): 789–794. DOI: 10.1097/01.lab.0000018827.78602.f4.
44. Mao X.Q., Shirakawa T., Enomoto T., Shimazu S., Dake Y., Kitano H. et al. Association between variants of mast cell chymase gene and serum IgE levels in eczema. *Hum. Hered*. 1998; 48 (1): 38–41. DOI: 10.1159/000022782.
45. Shimura S., Takai T., Iida H., Maruyama N., Ochi H., Kamijo S. et al. Epicutaneous allergic sensitization by cooperation between allergen protease activity and mechanical skin barrier damage in mice. *J. Invest. Dermatol*. 2016; 136 (7): 1408–1417. DOI: 10.1016/j.jid.2016.02.810.
46. Deleuran M., Ellingsen A.R., Paludan K., Schou C., Thestrup-Pedersen K. Purified Der p1 and p2 patch tests in patients with atopic dermatitis: evidence for both allergenicity and proteolytic irritancy. *Acta Derm. Venereol*. 1998; 78(4): 241–243. DOI: 10.1080/000155598441783.
47. Leyva-Castillo J.-M., McGurk A., Raif Geha M.D. Allergic skin inflammation and *S. aureus* skin colonization are mutually reinforcing. *Clinical Immunology*. 2020; 218: 108511. DOI: 10.1016/j.clim.2020.108511.
48. Laouini D., Kawamoto S., Yalcindag A., Bryce P., Mizoguchi E., Oettgen H. et al. Epicutaneous sensitization with superantigen induces allergic skin inflammation. *J. Allergy Clin. Immunol.* 2003; 112 (5): 981–987. DOI: 10.1016/j.jaci.2003.07.007.
49. Skov L., Olsen J.V., Giorno R., Schlievert P.M., Baadsgaard O., Leung D.Y. Application of Staphylococcal enterotoxin B on normal and atopic skin induces up-regulation of T cells by a superantigen-mediated mechanism. *J. Allergy Clin. Immunol*. 2000; 105 (4): 820–826. DOI: 10.1067/mai.2000.105524.
50. Meylan P., Lang C., Mermoud S., Johannsen A., Norrenberg S., Hohl D. et al. Skin colonization by Staphylococcus aureus precedes the clinical diagnosis of atopic dermatitis in infancy. *J. Invest. Dermatol*. 2017; 137 (12): 2497–2504. DOI: 10.1016/j.jid.2017.07.834.
51. Ganeshan K., Neilsen C.V., Hadsaitong A., Schleimer R.P., Luo X., Bryce P.J. Impairing oral tolerance promotes allergy and anaphylaxis: a new murine food allergy model. *J. Allergy Clin*. *Immunol*. 2009; 123 (1): 231–238.e4. DOI: 10.1016/j.jaci.2008.10.011.
52. Forbes-Blom E., Camberis M., Prout M., Tang S.C., Le Gros G. Staphylococcal-derived superantigen enhances peanut induced Th2 responses in the skin. *Clin. Exp. Allergy.* 2012; 42 (2): 305–314. DOI: 10.1111/j.1365-2222.2011.03861.x.
53. Jones A.L., Curran-Everett D., Leung D.Y.M. Food allergy is associated with Staphylococcus aureus colonization in children with atopic dermatitis. *J. Allergy Clin. Immunol.* 2016; 137 (4): 1247–1248.e3. DOI: 10.1016/j.jaci.2016.01.010.
54. Tsilochristou O., du Toit G., Sayre P.H. et al. Association of Staphylococcus aureus colonization with food allergy occurs independently of eczema severity. *J. Allergy Clin. Immunol.* 2019; 144 (2): 494–503. DOI: 10.1016/j.jaci.2019.04.025.
55. Leyva-Castillo J.M., McGurk A., Geha M.D.R. Allergic skin inflammation and S. aureus skin colonization are mutually reinforcing. *Clin. Immunol.* 2020; 218: 108511. DOI: 10.1016/j.clim.2020.108511.
56. Trendelenburg V. et al. Hen's egg allergen in house and bed dust is significantly increased after hen's egg consumption – A pilot study. *Allergy*. 2018; 73 (1): 261–264. DOI: 10.1111/all.13303.
57. Foong R.X., Brough H. The role of environmental exposure to peanut in the development of clinical allergy to peanut. *Clin. Exp. Allergy*. 2017; 47 (10): 1232–1238. DOI: 10.1111/cea.12992.
58. Bertelsen R.J. et al. Food allergens in mattress dust in Norwegian homes – a potentially important source of allergen exposure. *Clin. Exp. Allergy*. 2014; 44 (1): 142–149. DOI: 10.1111/cea.12231.
59. Boussault P. et al. Oatsensitization in children with atopic dermatitis: prevalence, risks and associated factors. *Allergy*. 2007; 62 (11): 1251–1256. DOI: 10.1111/j.1398-9995.2007.01527.x.
60. Lack G. et al. Factors associated wit the development of peanut allergy in childhood. *N. Engl. J. Med.* 2003; 348 (11): 977–985. DOI: 10.1056/NEJMoa013536.
61. Lina T. et al. Epicutaneous sensitization with ovalbumin, staphylococcal enterotoxin B and vitamin D analogue induces atopic dermatitis in mice. *J. Cent. South Univ. (Med. Sci.)* 2017; 42 (9): 1023–1029. DOI: 10.11817/j.issn.1672-7347.2017.09.005.
62. Noti M. et al. Exposure to food allergens through inflamed skin promotes intestinal food allergy through the thymic stromal lymphopoietin-basophil axis. *J. Allergy Clin. Immunol*. 2014; 133 (5): 1390–1399. DOI: 10.1016/j.jaci.2014.01.021.
63. Kawasaki A. et al. Skin inflammation exacerbates food allergy symptoms in epicutaneously sensitized mice. *Allergy*. 2018; 73 (6): 1313–1321. DOI: 10.1111/all.13404.
64. Palomares O., Akdis M., Martin-Fontecha M., Akdis C.A. Mechanisms of immune regulation in allergic diseases: the role of regulatory T and B cells. *Immunol. Rev*. 2017; 278: 219–236. DOI: 10.1111/imr.12555.
65. Holm J., Willumsen N., Wurtzen P.A., Christensen L.H., Lund K. Facilitated antigen presentation and its inhibition by blocking IgG antibodies depends on IgE repertoire complexity. *J. Allergy Clin. Immunol*. 2011; 127 (4): 1029–1037. DOI: 10.1016/j.jaci.2011.01.062.
66. Turcanu V., Stephens A.C., Chan S.M., Rance F., Lack G. IgE-mediated facilitated antigen presentation underlies higher immune responses in peanut allergy. *Allergy.* 2010; 65 (10): 1274–1281. DOI: 10.1111/j.1398-9995.2010.02367.x.
67. Bieber T. Interleukin-13: Targeting an underestimated cytokine in atopic dermatitis. *Allergy*. 2020; 75 (1): 54–62. DOI: 10.1111/all.13954.
68. Brough H.A., Nadeau K.C., Sindher S.B., Alkotob S.S., Chan S., Bahnson H.T. et al. Epicutaneous sensitization in the development of food allergy: What is the evidence and how can this be prevented? *Allergy.* 2020; 75 (9): 2185–2205. DOI: 10.1111/all.14304.
69. Hsieh K.Y. et al. Epicutaneous exposure to protein antigen and food allergy. *Clin. Exp. Allergy*. 2003; 33 (8): 1067–1075. DOI: 10.1046/j.1365-2222.2003. 01724.x.

70. Hussain M. et al. Basophil-derived IL-4 promotes epicutaneous antigen sensitization concomitant with the development of food allergy. *J. Allergy Clin. Immunol*. 2018; 141 (1): 223–234.e5 DOI: 10.1016/j.jaci.2017.02.035.
71. Sano Y., Masuda K., Tamagawa-Mineoka R., Matsunaka H., Murakami Y., Yamashita R. et al. Thymic stromal lymphopoietin expression is increased in the horny layer of patients with atopic dermatitis. *Clin. Exp. Immunol.* 2013; 171 (3): 330–337. DOI: 10.1111/cei.12021.
72. Al-Shami A., Spolski R., Kelly J., Keane-Myers A., Leonard W.J. A role for TSLP in the development of inflammation in an asthma model. *J. Exp. Med*. 2005; 202 (6): 829–839. DOI: 10.1084/jem.20050199.
73. He R., Oyoshi M.K., Garibyan L., Kumar L., Ziegler S.F., Geha R.S. TSLP acts on infiltrating effector T cells to drive allergic skin inflammation. *Proc. Nat. Acad. Sci. USA*. 2008; 105 (33): 11875–11880. DOI: 10.1073/pnas.0801532105.
74. Zhou B., Comeau M.R., De S.T., Liggitt H.D., Dahl M.E., Lewis D.B. et al. Thymic stromal lymphopoietin as a key initiator of allergic airway inflammation in mice. *Nature Immunol.* 2005; 6 (10): 1047–1053. DOI: 10.1038/ni1247.
75. Noti M., Kim B.S., Siracusa M.C., Rak G.D., Kubo M., Moghaddam A.E. et al. Exposure to food allergens through inflamed skin promotes intestinal food allergy through the thymic stromal lymphopoietin-basophil axis. *J. Allergy Clin. Immunol.* 2014; 133: 1390–1399 e1–6. DOI: 10.1016/j.jaci.2014.01.021.
76. Bogiatzi S.I., Fernandez I., Bichet J.C., Marloie-Provost M.A., Volpe E., Sastre X. et al. Cutting edge: Proinflammatory and Th2 cytokines synergize to induce thymic stromal lymphopoietin production by human skin keratinocytes. *J. Immunol*. 2007; 178 (3): 3373–3377. DOI: 10.4049/jimmunol.178.6.3373.
77. Oyoshi M.K., Larson R.P., Ziegler S.F., Geha R.S. Mechanical injury polarizes skin dendritic cells to elicit a T(H)2 response by inducing cutaneous thymic stromal lymphopoietin expression. *J. Allergy Clin. Immunol.* 2010; 126 (5): 976–984. DOI: 10.1016/j.jaci.2010.08.041.
78. Tamari M. et al. The optimal age for epicutaneous sensitization following tape-stripping in BALB/c mice. *Allergology International*. 2018; 67 (3): 380–387. DOI: 10.1016/j.alit.2018.01.003.
79. Cayrol C., Girard J.P. IL-33: an alarmin cytokine with crucial roles in innate immunity, inflammation and allergy. *Curr. Opin. Immunol*. 2014; 31: 31–37. DOI: 10.1016/j.coi.2014.09.004.
80. Savinko T., Matikainen S., Saarialho-Kere U., Lehto M., Wang G., Lehtimaki S. et al. IL-33 and ST2 in atopic dermatitis: expression profiles and modulation by triggering factors. *J. Invest. Dermatol.* 2012; 132 (5): 1392–1400. DOI: 10.1038/jid.2011.446.
81. Tamagawa-Mineoka R., Okuzawa Y., Masuda K., Katoh N. Increased serum levels of interleukin 33 in patients with atopic dermatitis. *J. Am. Acad. Dermatol*. 2014; 70 (5): 882–888. DOI: 10.1016/j.jaad.2014.01.867.
82. Komai-Koma M., Brombacher F., Pushparaj P.N., Arendse B., McSharry C., Alexander J. et al. Interleukin-33 amplifies IgE synthesis and triggers mast cell degranulation via interleukin-4 in naive mice. *Allergy*. 2012; 67 (9): 1118–1126. DOI: 10.1111/j.1398-9995.2012.02859.x.
83. Muto T., Fukuoka A., Kabashima K., Ziegler S.F., Nakanishi K., Matsushita K. et al. The role of basophils and proallergic cytokines, TSLP and IL-33, in cutaneously sensitized food allergy. *Int. Immunol*. 2014; 26 (10): 539–549. DOI: 10.1093/intimm/dxu058.
84. Galand C. et al. IL-33 promotes food anaphylaxis in epicutaneously sensitized mice by targeting mast cells. *J. Allergy Clin Immunol*. 2016; 138 (5): 1356–1366. DOI: 10.1016/j.jaci.2016.03.056.
85. Walker M.T. et al. Mechanism for initiation of food allergy: Dependence on skin barrier mutations and environmental allergen costimulation. *J. Allergy Clin. Immunol*. 2018; 141 (5): 1711–1725.e9. DOI: 10.1016/j.jaci.2018.02.003.
86. Chinthrajah S., Cao S., Liu C., Lyu S.C., Sindher S.B., Long A. et al. Phase 2a randomized, placebo-controlled study of anti-IL-33 in peanut allergy. *JCI Insight*. 2019; 4 (22): e131347. DOI: 10.1172/jci.insight.131347.
87. Chen Y.L., Gutowska-Owsiak D., Hardman C.S., Westmoreland M., MacKenzie T., Cifuentes L. et al. Proof-of-concept clinical trial of etokimab shows a key role for IL-33 in atopic dermatitis pathogenesis. *Sci. Transl. Med*. 2019; (515): eaax2945. DOI: 10.1126/scitranslmed.aax2945.
88. Mitamura Y. et al. IL-24: A new player in the pathogenesis of pro-inflammatory and allergic skin diseases. *Allergology International*. 2020; 69 (3): 405–411. DOI: 10.1016/j.alit.2019.12.003.
89. Vickery B.P., Burks A.W. Immunotherapy in the treatment of food allergy: focus on oral tolerance. *Curr. Opin. Allergy Clin. Immunol.* 2009; 9 (4): 364–370. DOI: 10.1097/ACI.0b013e32832d9add.
90. Pearson R.M., Casey L.M., Hughes K.R., Miller S.D., Shea L.D. *In vivo* reprogramming of immune cells: Technologies for induction of antigen-specific tolerance. *Adv. Drug. Deliver Rev.* 2017; 114: 240–255. DOI: 10.1016/j.addr.2017.04.005.
91. Strobel S., Ferguson A. Immune responses to fed protein antigens in mice. 3. Systemic tolerance or priming is related to age at which antigen is first encountered. *Pediatr. Res*. 1984; 18: 588–594. DOI: 10.1203/00006450-198407000-00004.
92. Matsubara T., Iwamoto H., Nakazato Y., Okamoto T., Ehara T., Izumi H., Takeda Y. Ingestion of partially hydrolyzed whey protein suppresses epicutaneous sensitization to β-lactoglobulin in mice. *Pediatr. Allergy Immunol.* 2018 ;29 (4): 433–440. DOI: 10.1111/pai.12887.
93. Murakami H., Ogawa T., Takafuta A., Yano E., Zaima N., Moriyama T. Percutaneous sensitization to soybean proteins is attenuated by oral tolerance. *J. Nutr. Sci. Vitaminol. (Tokyo).* 2018; 64 (6): 483–486. DOI: 10.3177/jnsv.64.483. PMID: 30606971.
94. Levy Y., Broides A., Segal N., Danon Y.L. Peanut and tree nut allergy in children: role of peanut snacks in Israel? *Allergy*. 2003; 58 (11): 1206–1207. DOI: 10.1046/j.1398-9995.2003.00307.x.
95. Du Toit G., Katz Y., Sasieni P., Mesher D., Maleki S.J., Fisher H.R. et al. Early consumption of peanuts in infancy is associated with a low prevalence of peanut allergy. *J. Allergy Clin. Immunol.* 2008; 122 (5): 984–991. DOI: 10.1016/j.jaci.2008.08.039.

96. Katz Y., Rajuan N., Goldberg M.R., Eisenberg E., Heyman E., Cohen A. et al: Early exposure to cow's milk protein is protective against IgE-mediated cow's milk protein allergy. *J. Allergy Clin. Immunol*. 2010; 126 (1): 77.e1–82. DOI: 10.1016/j.jaci.2010.04.020.
97. Muraro A., Halken S., Arshad S.H., Beyer K., Dubois A.E.J., Du Toit G. et al. EAACI food allergy and anaphylaxis guidelines. Primary prevention of food allergy. *Allergy*. 2014; 69 (5): 590–601. DOI: 10.1111/all.12398.
98. Horimukai K. et al. Application of moisturizer to neonates prevents development of atopic dermatitis. *J. Allergy Clin. Immunol.* 2014; 134 (4): 824–830. DOI: 10.1016/j.jaci.2014.07.060.
99. Simpson E.L., Chalmers J.R., Hanifin J.M., Thomas K.S., Cork M.J., McLean W.H. et al. Emollient enhancement of the skin barrier from birth offers effective atopic dermatitis prevention. *J. Allergy Clin. Immunol*. 2014; 134 (4): 818–823. DOI: 10.1016/j.jaci.2014.08.005.
100. Chalmers J.R., Haines R.H., Bradshaw L.E., Montgomery A.A., Thomas K.S., Brown S.J. et al. Daily emollient during infancy for prevention of eczema: the BEEP randomised controlled trial. *Lancet.* 2020; 395 (10228): 962–972. DOI: 10.1016/S0140-6736(19)32984-8.
101. Dissanayake E., Yumi Tanib Y., Nagaic K. et al. Skin care and synbiotics for prevention of atopic dermatitis or food allergy in newborn infants: A 2 × 2 factorial, randomized, non-treatment controlled trial. *Int. Arch. Allergy Immunol*. 2019; 180 (3): 202–211. DOI: 10.1159/000501636.

Authors information

Novik Gennady A., Dr. Sci. (Med.), Professor, Head of the Department of Children's Diseases named after Professor I.M. Vorontsov, Saint Petersburg, State Pediatric Medical University, St. Petersburg, Russian Federation. ORCID 0000-0002-7571-5460.

Zhdanova Marina V., Cand. Sci. (Med.), Associate Professor, Department of Children's Diseases named after Professor I.M. Vorontsov, Vice Rector for Academic Affairs, Saint Petersburg State Pediatric Medical University, St. Petersburg, Russian Federation. ORCID 0000-0001-7035-010.

Demidova Anastasiya S., Assistant, Department of Children's Diseases named after Professor I.M. Vorontsov, Saint Petersburg State Pediatric Medical University, St. Petersburg, Russian Federation. ORCID 0000-0002-9184-1803.

(✉) **Novik Gennady A.,** e-mail: ga_novik@mail.ru

Received 19.12.2020
Accepted 25.05.2021

УДК 616.12-07:004.032.26
https://doi.org/10.20538/1682-0363-2021-4-193-204

Artificial neural networks in cardiology: analysis of graphic data

Onishchenko P.S.[1, 2], Klyshnikov K.Yu.[1], Ovcharenko E.A.[1]

[1] *Research Institute for Complex Issues of Cardiovascular Diseases*
6, Sosnovi Blv., Kemerovo, 650002, Russian Federation

[2] *Science Institute of Computational Technologies of the Siberian Branch of the Russian Academy of Sciences*
6, Akademika Lavrentieva Av., Novosibirsk, 630090, Russian Federation

ABSTRACT

Aim. To consider application of convolutional neural networks for processing medical images in various fields of cardiology and cardiac surgery using publications from 2016 to 2019 as an example.

Materials and methods. In the study, we used the following scientific databases: PubMed Central, ArXiv, ResearchGate. The cited publications were grouped by the area of interest (heart, aorta, carotid arteries).

Results. The general principle of work of the technology under consideration was described, the results were shown, and the main areas of application of this technology in the studies under consideration were described. For most of the studies, sample sizes were given. The author's view on the development of convolutional neural networks in medicine was presented and some limiting factors for their distribution were listed.

Conclusion. A brief overview shows possible areas of application of convolutional neural networks in the fields of cardiology and cardiac surgery. Without denying the existing problems, this type of artificial neural networks may help many doctors and researchers in the future.

Key words: convolutional neural network, CNN, FFR, cardiology, cardiovascular diseases, stenosis, detection.

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. This work was supported by a comprehensive program of fundamental scientific research of the Siberian Branch of the Russian Academy of Sciences within the framework of the fundamental theme of the Research Institute KPSSZ No. 0419-2021-001 "Development of new pharmacological approaches to experimental therapy of atherosclerosis and complex digital solutions based on artificial intelligence for automated diagnosis of pathologies of the circulatory system and determination of the risk of lethal Exodus "with the financial support of the Ministry of Science and Higher Education of the Russian Federation within the framework of the national project" Science and Universities ".

For citation: Onishchenko P.S., Klyshnikov K.Yu., Ovcharenko E.A. Artificial neural networks in cardiology: analysis of graphic data. *Bulletin of Siberian Medicine.* 2021; 20 (4): 193–204. https://doi.org/10.20538/1682-0363-2021-4-193-204.

Искусственные нейронные сети в кардиологии: анализ графических данных

Онищенко П.С.[1, 2], Клышников К.Ю.[1], Овчаренко Е.А.[1]

[1] *Научно-исследовательский институт комплексных проблем сердечно-сосудистых заболеваний (НИИ КПССЗ)*
Россия, 650002, г. Кемерово, Сосновый бульвар, 6

[2] *Институт вычислительных технологий Сибирского отделения Российской академии наук (ИВТ СО РАН)*
Россия, 630090, г. Новосибирск, пр. Академика Лаврентьева, 6

✉ *Onishchenko Pavel S.,* e-mail: onis.pavel@gmail.com

РЕЗЮМЕ

Рассмотрены области применения сверточных нейронных сетей для обработки медицинских изображений в различных сферах кардиологии и кардиохирургии на примере публикаций с 2016 по 2019 г.

В данной работе использовались следующие базы научных статей: PubMed Central, ArXiv, ResearchGate. Приведенные работы структурировались по области интереса (сердце, аорта, сонные артерии).

Описан общий принцип работы рассматриваемой технологии, показаны результаты и рассмотрены основные области применения данной технологии в анализируемых работах. Для большинства приведенных исследований приведены объемы выборок, авторское видение развития сверточных нейронных сетей в медицине и перечислены некоторые ограничивающие факторы для их распространения.

Показаны возможные сферы применения сверточных нейронных сетей в области кардиологии и кардиохирургии. Не отрицая существующие проблемы, такой тип искусственных нейронных сетей в будущем может стать верным помощником для широкого спектра врачей и исследователей.

Ключевые слова: сверточные нейронные сети, CNN, FFR, кардиология, патология сердечно-сосудистой системы, стеноз, детекция.

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

Источник финансирования. Работа выполнена при поддержке комплексной программы фундаментальных научных исследований СО РАН в рамках фундаментальной темы НИИ КПССЗ № 0419-2021-001 «Разработка новых фармакологических подходов к экспериментальной терапии атеросклероза и комплексных цифровых решений на основе искусственного интеллекта для автоматизированной диагностики патологий системы кровообращения и определения риска летального исхода» при финансовой поддержке Министерства науки и высшего образования Российской Федерации в рамках национального проекта «Наука и университеты».

Для цитирования: Онищенко П.С., Клышников К.Ю., Овчаренко Е.А. Искусственные нейронные сети в кардиологии: анализ графических данных. *Бюллетень сибирской медицины.* 2021; 20 (4): 193–204. https://doi.org/10.20538/1682-0363-2021-4-193-204.

INTRODUCTION

When examining a patient with cardiovascular diseases, a physician receives textual and numerical information (for example, medical history and blood test results), as well as graphic data (the results of computed tomography (CT), magnetic resonance imaging (MRI), echocardiography, scintigraphy, and X-ray), which require long-term analysis and assessment [1, 2]. It takes a highly qualified expert up to 20 minutes to analyze MRI scans of a patient at two time points of the cardiac cycle – the end-diastole and end-systole [3]. It is a tedious and time-consuming process, that could lead to a diagnostic error [4]. However, in addition to the qualitative description, there is another important aspect of the quantitative assessment of images – linear and volumetric measurements for diagnosis, prognosis, treatment monitoring, and research purposes.

With the development of deep learning methods, such as neural networks, which have been used for image segmentation [5], object detection [6], and clinical decision support systems [7, 8], and with their increased availability [9, 10], it became possible to apply these methods in medical imaging [11–13]. In general, neural networks differ significantly from algorithmic approaches, which has been the main reason for their widespread use and implementation in the field of medicine. They have the ability to independently establish a relationship between input and output values via unsupervised training, which results in successful extraction of implicit or multifactorial relationships from data and better image interpretation [14].

Moreover, growth in computing performance, primarily due to graphics processing unit (GPU) computing [15], and availability of open-source neural networks make them accessible to many researchers [16]. Taking into account these factors, as well as the existence of large databases (for example, ImageNet [17], Cardiac CTA [18], ACDC [19]), the task of developing tools for reducing the contribution of the “human” factor to the analysis of medical images

remains relevant. In the period from 2008 to 2018, the number of publications dedicated to the machine learning approaches in medical image analysis increased by 8 times [20]. This paper presents several previous publications on the use of neural networks for medical image processing in various fields of cardiology and cardiac surgery in the period from 2016 to 2019.

THE CONCEPT OF A CONVOLUTIONAL NEURAL NETWORK

The history of neural networks began with the primitive feed-forward artificial neural networks (ANN) (usually known as the perceptron [21]), which were the first and simplest types of ANN. Further development of architectures led to the formation of deep learning ANN, which are characterized by complex topology and larger number of interconnected neurons, compared with perceptrons. These ANN imitate human cognition, making an association based on previous experience with the help of training, during which the probability of accurate object classification increases [22–24]. To date, convolution neural network (CNN) is considered to be the most effective ANN for image recognition. The main feature of this architecture is a convolutional layer. This layer (or set of layers) processes the input image (ANN extracts desired features) and then passes it to subsequent processing, similar to other ANN [25].

Given that CNNs are a type of ANN, they exhibit two main features – a need for training and the ability to switch [1]. To train the ANN, it is necessary to present it with a large number of labelled training data, where experts pre-select the features – similar to training of humans [24]. Therefore, the most important factor affecting the CNN is the quality of input data, primarily accurate segmentation. Another important aspect at the stage of developing CNN architecture is the structure and volume of data: a small sample or insufficient heterogeneity will lead to a large percentage of errors as a result, i.e. to a decrease in the quality of object recognition [1].

The ability of CNN to switch implies the ability to work with similar data. It is possible to conduct pre-training on data from open sources, and then fine-tune it for the target task [26, 27]. Both features make CNN a promising and accessible tool for medical image analysis, and a number of multidisciplinary teams have been conducting research in this field.

MEDICAL IMAGE PROCESSING USING CNN

Heart

Segmentation and quantitative assessment of cardiac and myocardial parameters are important in cardiology for assessing the severity of the initial state of the disease (dilatation, hypertrophy, contractile disorders, anatomical changes, etc.) and monitoring the results of treatment (remodeling, changes in the size of chambers). Despite the achieved progress in this area, this task is still challenging due to wide subject-to-subject anatomical variation. The main research directions in this area are image segmentation and classification.

For example, L. Yu et al. (2016) used CNN for fetal left ventricular (LV) segmentation in echocardiographic sequences [28]. Fetal echocardiography is the primary modality for evaluating prenatal cardiac function due to its low cost, harmless nature, and quick acquisition. A quantitative analysis of fetal echocardiographic images provides important fetal cardiac function parameters for early diagnosis of heart diseases.

The author proposes a dynamic CNN, the training of which includes 2 steps: pre-training and fine-tuning. Pre-training was carried out using images, where the neural network divided each pixel into two categories: a pixel in the region of interest and out of it. The training set consisted of 200,000 samples that were chosen randomly in 10 manually delineated sequences. The validation set consisted of 8,000 samples. It is worth noting that only the first frame of each echocardiographic sequence was segmented manually, which simplified the work of the experts. Thus, the dynamic CNN was fine-tuned by deep tuning to adapt to the first frame and by shallow tuning to fix the latest frame, adapting to the individual features. As a result, the segmentation accuracy was 94.5%. Further work is aimed at carrying out a quantitative analysis of fetal LV functions based on the results obtained using the proposed segmentation method. An example of the results obtained is shown in Fig. 1*a*.

W. Xue et al. (2018) [29] proposed an architecture for a deep multitask relationship learning network (DMTRL) which incorporates CNN for cardiac image representation and two parallel recurrent neural networks (RNN) for temporal dynamic modeling of cardiac sequences. The proposed network quantifies three types of LV parameters (the cavity inside the myocardium, regional wall thicknesses, and a cardiac cycle phase). The authors collected MRI images of

145 individuals (average age was 58.9 years), with 20 frames for each cardiac cycle. Compared with the previous study [30], this ANN demonstrated higher prognosis accuracy with an absolute error of 1.7–10.3% for the studied LV parameters.

J.D. Dormer et al. [31] presented a CNN-based heart chamber segmentation method for 3D CT with 5 classes: left ventricle, right ventricle, left atrium, right atrium, and background. Chest CT images were acquired for 11 patients with the total number of slices ranging from 78 to 154 for each patient, providing a large amount of data. The images were processed into pixel patches of five classes, 2 500 patches from each class for each patient were chosen for CNN training and validation. The results were validated by calculating the overall accuracy of the classification for each segmented region, with the accuracy defined as the number of correctly labeled patches from the total number of patches for the testing dataset. As a result, the accuracy in segmentation of the heart and the overall accuracy were 85.6 ± 6.1% and 87.2 ± 3.3%, respectively. It is worth noting that 11 unique cases resulted in such high accuracy of the network, despite insufficient heterogeneity of data. Nevertheless, this approach seems appropriate only for rare diseases, especially using augmentation of the dataset size due to rotation and scaling without substantial changes [25].

L. Tan et al. (2018) [32] developed a fully automated algorithm for LV segmentation in cardiac MRI. The study utilized the data of 200 subjects with coronary artery disease and regional wall motion abnormalities and 1,140 subjects with a combination of normal and abnormal cardiac functions. The combined training data and the manually labeled data were split 85:15 by the subject for training and cross-validation, respectively (i.e. 26, 069 and 9, 860 unique images). The developed algorithm demonstrated the median Jaccard similarity coefficient of 0.77 ± 0.11. The result of the input data processing is shown in Fig. 1*b*. Contrary to [31], this work has a large sample of input images for both training and validation.

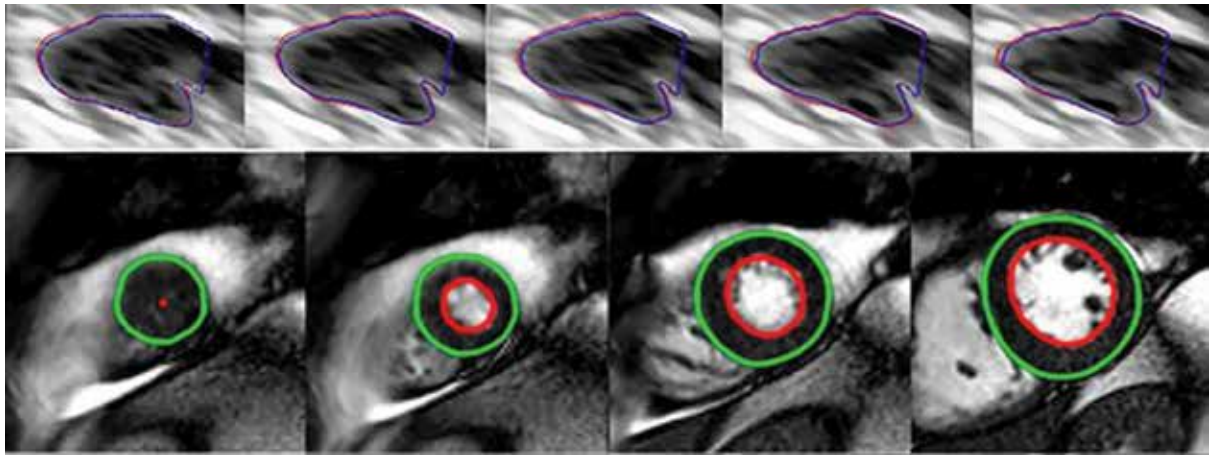


Fig. 1. The segmentation results: *a* – of successive echocardiographic images shown in [28]; *b* – of endo- and epicardium slicing from the apex to the base obtained using CNN [32]

Aorta

Aorta segmentation can be used for reconstructing its geometry, such as 3D models for further numerical analysis and preoperative planning, as well as for detecting pathological changes. Neural networks in this area can be used for assessment and selection of appropriate prostheses for transcatheter aortic valve replacement (TAVR) procedures.

Attempting to solve the problem of aortic segmentation, D. Wang et al. (2018) [33] developed a novel method for CT-MR aortic aneurysm image segmentation. The standard approach to training the CNN incorporates CT and MR images separately. However, this approach is time-consuming and inefficient due computational cost of training the ANN. The novelty of the proposed CNN is fusion of the parts of the model that work with CT and MR images. Such network can undergo end-to-end (complete) training using unlabeled CT and MR images in a shorter time period, since training occurs on two types of input data simultaneously.

Moreover, the fusion model allows for shared representation of CT and MR images showing similar parts of the aorta for all image modalities (Fig. 2*a*). Processing images, the CNN segments them into five different classes, namely, aortic wall, its lumen, thrombus, calcium deposits, and irrelevant parts as background. The validation accuracy of the fusion models is 98.5%, which is 1.2% more than that of other models.

Another study in this area was conducted by P.M. Graffy et al. (2019) [34] (Fig. 2*b*), who used the fully automated Mask R-CNN algorithm [35] for segmentation of aortic calcification. The segmentation algorithm was applied to 9,914 non-contrast CT scans of 9,032 asymptomatic adults, who were screened for conditions not related to cardiovascular diseases [36].

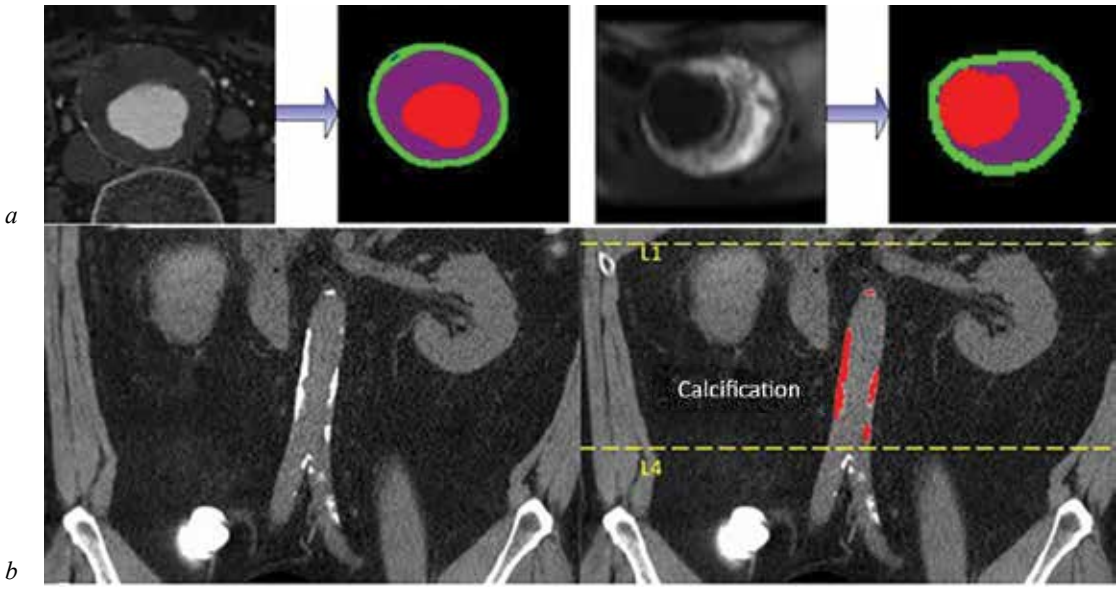


Fig. 2. A: The result of CT (left pair) and MR image (right pair) segmentation into 5 classes [33]: red – lumen of the aortic channel; green – aortic wall; purple – thrombus; blue – calcium; black – background (a). Segmentation of aortic calcification using Mask R-CNN automated algorithm: input image (left); the result of the segmentation (right) presented in [34]; L1 and L4 lines mark the area of algorithm application (b)

The images were used to estimate the abdominal volume and the number of calcifications and assess the Agatston score (showing the extent of coronary artery calcification) [37]. Statistical processing of the results showed that the mean values for the Agatston score were higher in men (924.2 ± 2,066.2 vs. 564.2 ± 1,484.2, $p < 0.001$), the calcium mass was 222.2 ± 526.0 mg vs. 144.5 ± 405.4 mg ($p < 0.001$), and the abdominal volume – 699.4 ± 1,552.4 ml vs. 426.9 ± 1,115.5 ml ($p < 0.001$). The mean score increased with age by 10% per year for the entire cohort. Compared with women, men (age 40–60 years) had higher calcium scores (91.2% vs. 75.1%, $p < 0.001$) and significantly higher mean Agatston score (age 50–80, $p < 0.001$). Thus, in combination with statistical methods, CNN allows researchers to quickly obtain large amounts of quantitative data and measurements and process them with standard methods of medical statistics, making this combination a necessary tool for scientific research. The authors noted, that this study is only the first step towards creating a clinical tool for detecting calcifications in the aortic wall.

CORONARY ARTERIES

Diseases of the coronary arteries (CA) may result in critical conditions [38, 39], primarily coronary artery disease, which is the most common cause of death worldwide. CNN has the potential to become a valuable tool for locating and determining the degree of pathological changes in the arteries, especially in multivessel diseases.

X-ray coronary angiography is a primary imaging technique for diagnosing coronary diseases, consisting of consecutive projection images. E. Nasr-Esfahani et al. (2016) [40] used convolutional ANN to find and extract CA in X-ray coronary angiography

images. However, low quality resolution and image noise complicated processing of such images. Initially, an input angiogram was preprocessed to enhance its contrast. Afterwards, the image was evaluated using patches of pixels, and the network determined the CA and background regions and extracted them. A set of 1,040,000 patches was used for deep CNN learning, which were obtained from 44 X-ray angiography images. The large sample allowed for high accuracy of CA and background region identification – 93.5% and specificity of 97%. Fig. 3 shows the ANN work, compared with manually annotated images.

It is impossible to assess the coronary bed from images using one projection angle. A 3D model provides more information, so research in this area would be promising. Hence, J.M. Wolterink et al. (2019) [41] proposed a method for coronary artery centerline extraction in cardiac CT angiography using a CNN-based orientation classifier (Fig. 4). Starting from a single seed point placed manually or automatically anywhere in the coronary artery, a tracker follows the vessel centerline in two directions using the predictions of the CNN. Tracking is terminated when no direction can be identified with high certainty. The CNN is trained using manually annotated centerlines in test images.

Evaluation was performed using a test set consisting of 24 coronary CT angiography (CCTA) test images in which 96 centerlines were extracted. The extracted centerlines had an average overlap of 93.7% with manually annotated reference centerlines. This study was a part of the Rotterdam Coronary Artery Evaluation Framework, which allows for the evaluation of algorithms for coronary artery centerline extraction.

Intravascular optical coherence tomography (OCT) is an optical imaging modality commonly used in the assessment of coronary artery diseases during percutaneous coronary intervention (PCI). Y.L. Yong et al (2017) [42] proposed a linear-regression CNN to automatically perform vascular lumen segmentation in OCT. The study used the total of 64 pullbacks acquired from 28 patients (25% / 75% male / female, the average age 59.71 (± 9.61) years) using Dragonfly™ Duo Imaging Catheter. These pullbacks were randomly split into a training and a test set in the ratio of 7:3. Benchmarking the results against the gold standard for manual segmentation, the proposed algorithm demonstrated the average CA wall location accuracy of 22 microns and the Dice coefficient and Jaccard similarity coefficient of 0.985 and 0.970, respectively. The mean absolute error in luminal area estimation was 1.38%.

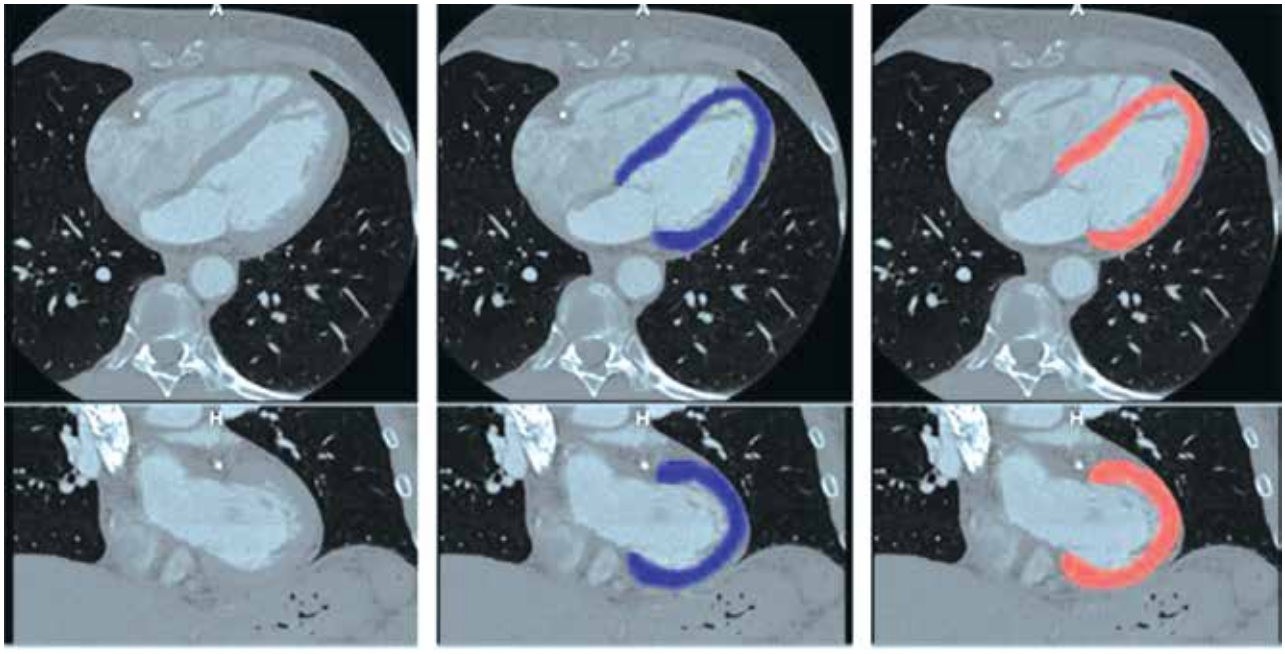


Fig. 3. Results of the ANN work: blue – manual annotation, red – automatic segmentation [40]

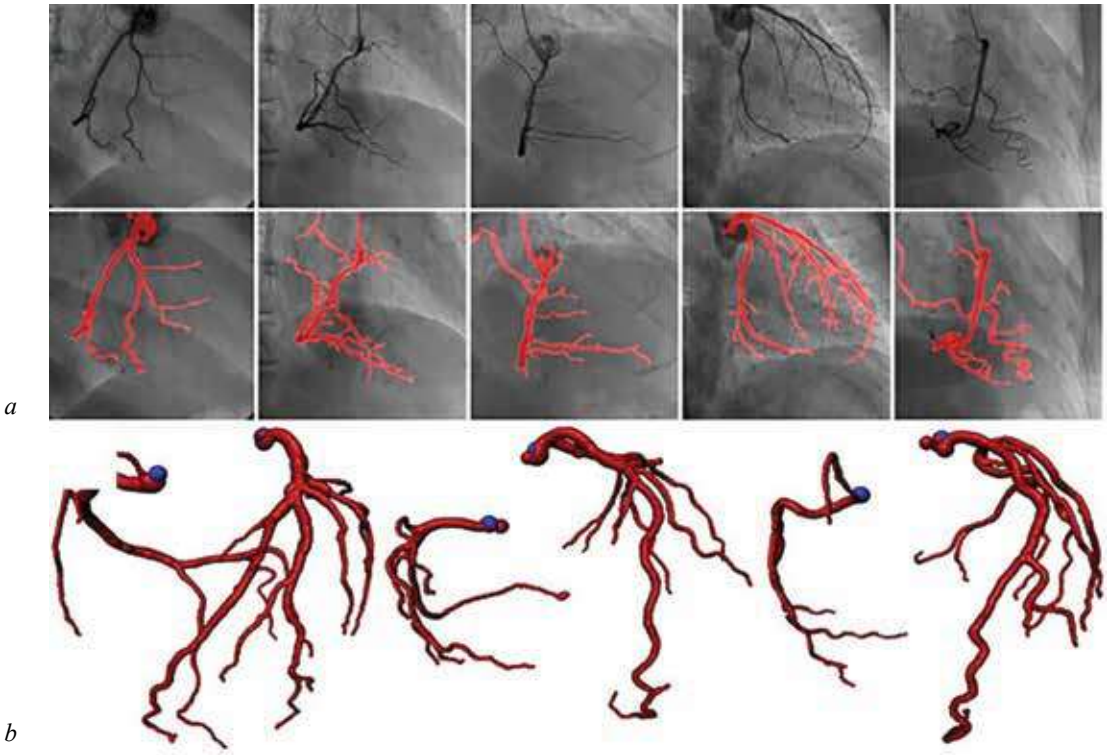


Fig. 4. Fully automatic centerline extraction: *a* – input images (upper row) and segmentation by the ANN (lower row); *b* – blue spheres indicate the starting points of the algorithm predicting the most likely direction and radius of the artery [41]

Assessment of the fractional flow reserve (FFR) [45] is a special form of CNN application in the field of medical image processing. After detecting regions of blocked CA during angiography, an interventional cardiologist, following the guidelines, makes a decision on FFR application based on the percentage of lumen diameter reduction. However, such intervention may be excessive in some cases, since stenosis could be hemodynamically insignificant, despite the occlusion. Therefore, there is a tendency for defining FFR as a functional parameter of CA stenosis. The FFR is defined as a distal / proximal pressure ratio in the stenosed segment [46].

These parameters are measured during invasive coronary angiography. To reduce the number of invasive procedures, M. Zreik et al. (2018) [47] presented a method for automatic identification of patients with functionally significant coronary artery stenoses, employing deep learning analysis of the LV myocardium at rest using CCTA. The automatic analysis of the LV myocardium was used to assess the FFR in the study. The analysis incorporated manual annotations of the LV myocardium (Fig. 3) and traditionally measured FFR parameters (n = 156) with the values of 0.79 ± 0.10. The neural network was tasked with classifying patients into those with functionally significant stenosis (FFR < 0.78) and those without it (FFR > 0.78). Quantitative evaluation of the segmentation performed on the 20 test scans resulted in a Dice coefficient of 91.4 ± 2.1% [43, 44]. However, the sensitivity was 0.60–0.80 with the corresponding specificity of 0.77–0.59, depending on the CNN settings. These results cannot be properly transferred into clinical practice as a classification model, although the network helps noninvasively estimate FFR. The subsequent work of this team following the same principle did not demonstrate a significant increase in the quality of classification despite changing the FFR cut-off values for functionally significant stenosis (FFR ≤ 0.8) and adjusting the input data (n = 136) [48].

L. Itu et al. [49] proposed an efficient method for determining FFR in 2016. Researches trained CNN directly on CT scans of the CAs, i.e. associated geometric features with hemodynamic significance. The input data were multislice computed tomography (MSCT) scans of 87 patients with 125 stenosed regions. The researchers manually annotated arteries, reconstructed 3D models of the coronary vascular bed, and performed numerical modeling of the fluid dynamics, assessing the pressure gradient.

The model was validated, and the diagnostic accuracy for the detection of functionally significant CAD was shown to be 75–85%. Then, 12,000 coronary geometries were generated to artificially increase the sample, so the model could assess the FFR. These data were used to train CNN. Thus, the researchers increased the training set from 87 to 12,000 objects (by 138 times). The results of the proposed neural network were 99.7% consistent with the results of the numerical analysis ($R = 0.9998$, $p < 0.001$). The trained CNN was tested using input clinical MSCT data ($n = 87$) with the resulting sensitivity of 81.6% and specificity of 83.9%. This result is largely due to the imperfection of the computational fluid dynamics algorithms. Nevertheless, this study seems to be the most promising, since FFR estimation is based primarily on the CA anatomy. Perhaps, by combining two studies described above, a synergistic effect could be achieved by incorporating both LV and CA geometry analysis to improve FFR estimation. It should be noted that the method proposed in [41], allowing for the reconstruction of 3D representation of CA, can be combined with the method in [49], which can lead to a breakthrough in the field of noninvasive FFR estimation.

Detection of surgical devices

For TAVR procedures, the task of detecting catheters remains urgent, as it could assist in determining the optimal implant positioning.

The authors (2017) in [50] attempted to detect guidewires using datasets comprised of X-ray images. Overall, 22 image sequences were used in the study. The testing task of the region proposal network was divided into three steps. At the first step, 256 region proposals of guide-wires were generated from a test image as input data. At the second step, all the proposals were classified by the region, the region proposal was considered as the target, if its corresponding score was larger than the threshold value. Finally, the detected proposals with the highest score were selected. Following this algorithm, a total of 5, 092 images were obtained from the 22 original X-ray images. Then, researches divided 22 sequences into two sets, one – for training (19 sequences) and the rest – for testing (3 sequences). The detection accuracy reached 89.2%. The detection results are shown in Fig. 5, *a*.

In 2019, H. Yang et al. [51] developed a method for catheter segmentation in 3D ultrasound images (Fig. 5, *b*) intending to use it during minimally invasive interventions. Since it was a pilot study, four data sets from four porcine hearts were used as the study samples. The whole algorithm was divided into three steps: 1) extracting the discriminating features from each voxel; 2) classifying voxels into catheter-like and non-catheter voxels using the CNN; 3) employing cubic spline interpolation to identify the catheter in the images. The proposed method can localize the catheter with the mean error of 2.1 mm while scanning the images for 10 seconds. With the increase in the computing power and optimization of the algorithm, this method would be able to instantly process datasets.

In the same year, a team of researchers led by H. Lee [52] used CNN to track and detect a peripherally inserted central catheter (PICC) and its tip. A total of 600 DICOM images from 600 different patients containing the keyword “PICC” were used in the study. The authors randomly selected 50 cases from the entire cohort to be used as a validation dataset and 150 cases to be used as a test dataset. The remaining cases were utilized to train fully convolutional networks (FCN) [53]. The neural networks developed by this team obtained absolute distances from ground truth with the mean of 3.10 mm, a standard deviation of 2.03 mm, and a root mean square error of 3.71 mm per 150 test cases. Despite the fact that all the images have a different angle and image noise, the CNN is able to accurately segment PICC line, ECG sensors, various objects (any additional structures), and threads (Fig. 5, *c*).

PROMISING DIRECTIONS FOR CNN

ANN have proved useful in the field of graphic data analysis: from medical image segmentation to assessing and predicting the development of pathologies in experimental and pilot studies. With the increasing availability of high-performance systems, ANN could find application in the form of commercial products, but this would require solving a number of problems related to clinical data and interaction between ANN and infrastructures.

Besides training, ANN requires a sufficient number of heterogenic data. The existing databases of annotated MSCT, CT, and MR images used for CNN training are limited. They usually include 100–300 images [19], while training often requires 1,000–10,000 samples. Various methods of artificial data generation and augmentation used in the studies have their own limitations and, due to their nature, contribute to the accuracy of CNN performance. Hence, collecting, standardizing, and annotating medical data can result in a promising project, especially concerning the development of multicenter databases.

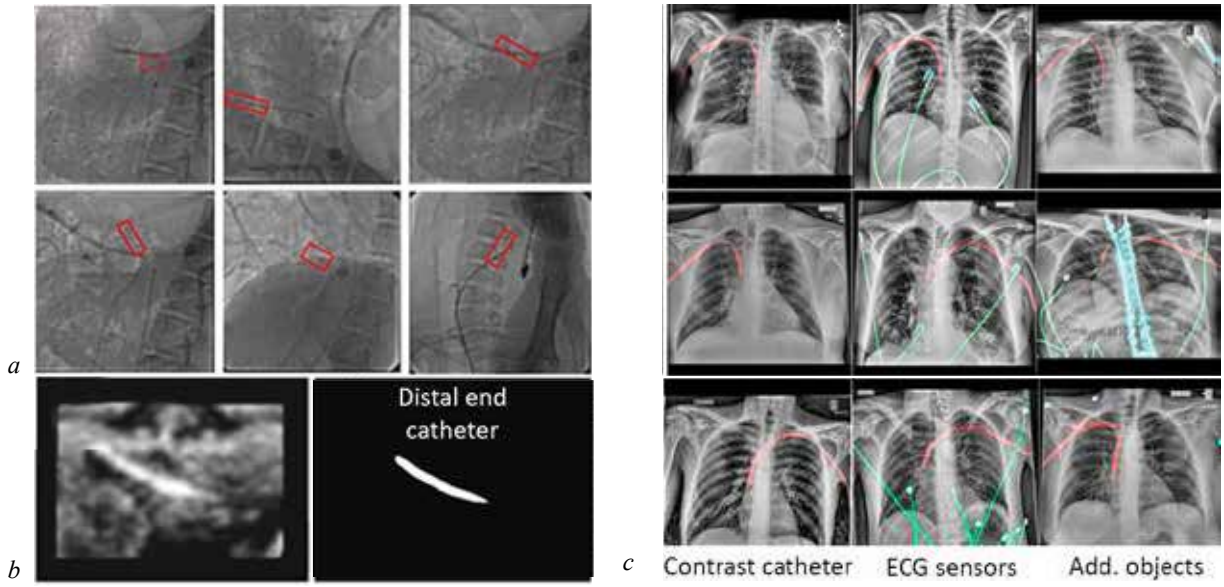


Fig. 5. Results of CNN performance in detecting medical devices: *a* – when detecting a catheter in the CA; the tip of the catheter is marked with a square [50]; *b* – successful segmentation (left) and its original image (right) [51]; *c* – when segmenting PICC (red), ECG sensors (green), and various objects (dark cyan) [52]

Another feature is heterogeneity of the neural network architectures. Research teams have been using their own models of ANN, never combining them with other CNNs for a cumulative effect. The different networks mentioned in this review could have been fused into a comprehensive system of neural network analysis to increase accuracy of the results. However, in practice this rarely happens. Perhaps, it is due to incompatibility of input data or neural network architecture. The prospect of combining multidirectional approaches for medical image segmentation or disease prediction, i.e., development of an integrated approach to neural network performance, can significantly increase the sensitivity and specificity of the results.

Finally, despite the development of computing hardware and image processing, many performance problems persist. The main calculations, such as selection of weight coefficients at the CNN training stage, happen on the graphic core of high-performance video cards (GPU). Compared with training on the central processing unit, GPU accomplishes the task much faster. However, a real-time image analysis (detection, segmentation) is usually carried out on less sophisticated machinery. The development of cloud computing in combination with CNN optimization algorithms should significantly simplify practical implementation of such systems by reducing the requirements to the PC computing power.

CONCLUSION

Over the past few years, ANN has been incorporated in many areas of our lives – from entertainment (applications for photo processing in smartphones, etc.) to engineering design systems (for example, generative technologies). The wide spread of machine learning methods in everyday life occurred due to the growth of computing power, both in stationary and in wearable devices. Medical field is no exception – ANN have proven effective in a wide range of tasks, including graphic data processing. Despite the advances in this field, the development of ANN has been slow for a number of reasons, several of which are described above. In this brief review, the possible applications of CNN in the field of cardiology and cardiac surgery have been shown. Although there is room for improvements, the network could become a reliable assistant for practitioners and researchers in the future.

Data availability presents the main problem to the implementation of CNN in healthcare. Thus, the question remains open, whether it would be possible to collect enough annotated data to train the ANN. Recent studies have shown that the more data there is, the better the results will be. However, it is not known how the big data can be used.

The above-mentioned studies have demonstrated that deep learning methods assist in the research process, but due to the uniqueness of the used data,

researchers must search for other complex methods that would allow efficient analysis of clinical data. It can be concluded that the prospects of ANN application in healthcare have no limitations.

REFERENCES

1. Shen D., Wu G., Suk H.-I. Deep Learning in Medical Image Analysis. *Annu Rev Biomed Eng*. 2017; 19: 221–248. DOI: 10.1146/annurev-bioeng-071516-044442.
2. Smith B.J., Adhami R.R. Medical imaging. *IEEE Potentials*. 2000; 17 (5): 9–12. DOI: 10.1109/45.730965.
3. Bai W., Sinclair M., Tarroni G., Oktay O., Rajchl M., Vaillant G. et al. Automated cardiovascular magnetic resonance image analysis with fully convolutional networks 08 Information and Computing Sciences 0801 Artificial Intelligence and Image Processing. *J Cardiovasc. Magn. Reson*. 2018; 20 (1): 65. DOI: 10.1186/s12968-018-0471-x.
4. Caterini A.L., Chang D.E. Recurrent neural networks. *Springer Briefs Comput. Sci*. 2018; 59–79.
5. Nie D., Wang L., Gao Y., Sken D. Fully convolutional networks for multi-modality isointense infant brain image segmentation. *Proc - Int Symp Biomed Imaging*. 2016; 2016: 1342–5. DOI: 10.1109/ISBI.2016.7493515.
6. Thaha M.M., Kumar K.P.M., Murugan B.S., Dhanasekeran S., Vijayakarthick P., Selvi A.S. Brain tumor segmentation using convolutional neural networks in MRI images. *J. Med. Syst*. 2019; 43 (9): 1240–1251. DOI: 10.1007/s10916-019-1416-0.
7. Suk H.I., Lee S.W., Shen D. Latent feature representation with stacked auto-encoder for AD/MCI diagnosis. *Brain Struct. Funct*. 2015; 220 (2): 841–859. DOI: 10.1007/s00429-013-0687-3.
8. Suk H.-I., Shen D. Deep learning in diagnosis of brain disorders. *Recent. Prog. Brain Cogn. Eng.* Springer. 2015; 203–213. DOI: 10.1007/978-94-017-7239-6_14.
9. Ronneberger O., Fischer P., Brox T. U-net: Convolutional networks for biomedical image segmentation. *Lect. Notes Comput. Sci. (including Subser. Lect. Notes Artif. Intell. Lect. Notes Bioinformatics)*. 2015; 9351: 234–241.
10. Milletari F., Navab N., Ahmadi S.A. V-Net: Fully convolutional neural networks for volumetric medical image segmentation. Proc. - 2016 4th Int. Conf. 3D Vision, 3DV 2016. *IEEE.* 2016; 565–571.
11. Szegedy C., Toshev A., Erhan D. Deep Neural Networks for object detection. *Adv. Neural Inf. Process. Syst*. 2013; 2553–2561.
12. Taigman Y., Yang M., Ranzato M., Wolf L. DeepFace: Closing the gap to human-level performance in face verification. Proc. *IEEE Comput. Soc. Conf. Comput. Vis. Pattern Recognit*. 2018; 1701–1708. DOI: 10.1109/CVPR.2014.220.
13. Silver D., Huang A., Maddison C.J., Guez A., Sifre L., van den Driessche G. et al. Mastering the game of Go with deep neural networks and tree search. *Nature*. 2016; 529 (7587): 484–489. DOI: 10.1038/nature16961.
14. Razzak M.I., Naz S., Zaib A. Deep learning for medical image processing: Overview, challenges and the future. *Lect. Notes Comput. Vis. Biomech*. 2018; 26: 323–350.
15. Smistad E., Falch T.L., Bozorgi M., Elster A.C., Lindseth F. Medical image segmentation on GPUs - A comprehensive review. *Med. Image Anal*. 2015; 20 (1): 1–18. DOI: 10.1016/j.media.2014.10.012.
16. Zhou T., Ruan S., Canu S. A review: Deep learning for medical image segmentation using multi-modality fusion. *Array*. 2019;3–4:100004. DOI: 10.1016/j.array.2019.100004.
17. Russakovsky O., Deng J., Su H., Krause J., Satheesh S., Ma S., Huang Z., Karpathy A., Khosla A., Bernstein M., Berg A.C., Fei-Fei L. ImageNet large scale visual recognition challenge. *Int. J. Comput. Vis*. 2015; 115 (3): 211–252. DOI: 10.1007/s11263-015-0816-y.
18. Moeskops P., Wolterink J.M., van der Velden B.H., Gilhuijs K.G., Leiner T., Viergever M.A., Išgum I. Deep learning for multi-task medical image segmentation in multiple modalities. *Lect. Notes Comput. Sci. (including Subser. Lect. Notes Artif. Intell. Lect. Notes Bioinformatics)*. 2016; 9901 LNCS: 478–486. DOI: 10.1007/978-3-319-46723-8_55.
19. Baumgartner C.F., Koch L.M., Pollefeys M., Konukoglu E. An exploration of 2D and 3D deep learning techniques for cardiac MR image segmentation. *Lect. Notes Comput. Sci. (including Subser. Lect. Notes Artif. Intell. Lect. Notes Bioinformatics*. 2018; 10663 LNCS: 111–119. DOI: 10.1007/978-3-319-75541-0_12.
20. Pesapane F., Codari M., Sardanelli F. Artificial intelligence in medical imaging: threat or opportunity? Radiologists again at the forefront of innovation in medicine. *Eur. Radiol.* Exp. 2018; 2 (1): 35. DOI: 10.1186/s41747-018-0061-6.
21. Bryukhomitskiy Yu.A. Neural network models for information security systems. Taganrog: TRTU, 2005: 160 (in Russ.).
22. Kim M., Yun J., Cho Y., Shin K., Jang R., Bae H., Kim N. Deep learning in medical imaging. *Neurospine*. 2019;16 (4): 657–668. DOI: 10.14245/ns.1938396.198.
23. Krittanawong C., Tunhasiriwet A., Zhang H.J., Wang Z., Aydar M., Kitai T. Deep learning with unsupervised feature in echocardiographic imaging. *J. Am. Coll. Cardiol*. 2017; 69 (16): 2100–2101. DOI: 10.1016/j.jacc.2016.12.047.
24. Zhao Y., Xia X., Togneri R. Applications of deep learning to audio generation. *IEEE Circuits Syst. Mag*. 2019; 19 (4): 19–38. DOI: 10.1109/MCAS.2019.2945210.
25. LeCun Y., Bengio Y., Hinton G. Deep learning. *Nature*. 2015; 521 (7553): 436–444. DOI: 10.1038/nature14539.
26. Gupta A., Ayhan M.S., Maida A.S. Natural image bases to represent neuroimaging data. 30th Int. Conf. Mach. Learn. *ICML* 2013. 2013; 2024–2031.
27. Brosch T., Tam R. Initiative for the Alzheimers Disease Neuroimaging. Manifold Learn brain MRIs by Deep Learning Med. *Image Comput. Assist. Interv*. 2013; 16 (2): 633–640. DOI: 10.1007/978-3-642-40763-5_78.
28. Yu L., Guo Y., Wang Y., Yu J., Chen P. Segmentation of fetal left ventricle in echocardiographic sequences based on dynamic convolutional neural networks. *IEEE Trans. Biomed. Eng*. 2017; 64 (8): 1886–1895. DOI: 10.1109/TBME.2016.2628401.
29. Xue W., Brahm G., Pandey S., Leung S., Li S. Full left ventricle quantification via deep multitask relationships learning. *Med. Image Anal*. 2018; 43: 54–65. DOI: 10.1016/j.media.2017.09.005.
30. Xue W., Lum A., Mercado A., Landis M., Warrington J., Li S. Full quantification of left ventricle via deep multitask

learning network respecting intra- and inter-task relatedness. *Lect. Notes Comput. Sci. (including Subser. Lect. Notes Artif. Intell. Lect. Notes Bioinformatics)*. 2017; 10435 LNCS: 276–284. DOI: 10.1007/978-3-319-66179-7_32.

31. Dormer J.D., Fei B., Halicek M., Ma L., Reilly C.M., Schreibmann E. Heart chamber segmentation from CT using convolutional neural networks. Med. Imaging 2018 Biomed. Appl. Mol. Struct. Funct. Imaging, vol. 10578. International Society for Optics and Photonics. 2018; 100. DOI: 10.1117/12.2293554.
32. Tan L.K., McLaughlin R.A., Lim E., Abdul Aziz Y.F., Liew Y.M. Fully automated segmentation of the left ventricle in cine cardiac MRI using neural network regression. *J. Magn. Reson. Imaging*. 2018; 48 (1): 140–152. DOI: 10.1002/jmri.25932.
33. Wang D., Zhang R., Zhu J., Teng Z., Huang Y., Spiga F., Du M.H.-F., Gillard J.H., Lu Q., Liò P. Neural network fusion: a novel CT-MR aortic aneurysm image segmentation method. *Med. Imaging 2018 Image Process*. 2018; 10574: 75. DOI: 10.1117/12.2293371.
34. Graffy P.M., Liu J., Pickhardt P.J., Burns J.E., Yao J., Summers R.M. Deep learning-based muscle segmentation and quantifcation at abdominal CT: Application to a longitudinal adult screening cohort for sarcopenia assessment. *Br. J. Radiol*. 2019; 92 (1100): 2921–2928. DOI: 10.1259/bjr.20190327.
35. He K., Gkioxari G., Dollár P., Girshick R. Mask r-cnn. Proc. IEEE Int. Conf. Comput. *Vis.* 2017; 2961–2969. DOI: 10.1109/ICCV.2017.322.
36. Pickhardt P.J. Imaging and screening for colorectal cancer with CT colonography. *Radiol. Clin. North Am*. 2017; 55 (6): 1183–1196. DOI: 10.1016/j.rcl.2017.06.009.
37. Neves P.O., Andrade J., Monção H. Escore de cálcio coronariano: Estado atual. *Radiol Bras*. 2017; 50 (3): 182–189. DOI: 10.1590/0100-3984.2015.0235.
38. Segal B.L. The pathology of coronary heart disease. *Can. Med. Assoc. J*. 1962; 87 (26): 1387–1390.
39. Van der Wal A.C. Coronary artery pathology. *Heart*. 2007; 93 (11): 1484–1489. DOI: 10.1136/hrt.2004.038364.
40. Nasr-Esfahani E., Samavi S., Karimi N., Soroushmehr S.R., Ward K., Jafari M.H., Felfeliyan B., Nallamothu B., Najarian K. Vessel extraction in X-ray angiograms using deep learning. *Proc. Annu. Int. Conf. IEEE Eng. Med. Biol. Soc.* 2016; 2016: 643–646. DOI: 10.1109/EMBC.2016.7590784.
41. Wolterink J.M., Hamersvelt R.W., Viergever M.A., Leiner T., Išgum I. Coronary artery centerline extraction in cardiac CT angiography using a CNN-based orientation classifier. *Med. Image Anal*. 2019; 51: 46–60. DOI: 10.1016/j.media.2018.10.005.
42. Yong Y.L., Tan L.K., McLaughlin R.A., Chee K.H., Liew Y.M. Linear-regression convolutional neural network for fully automated coronary lumen segmentation in intravascular optical coherence tomography. *J. Biomed. Opt*. 2017; 22 (12): 1–9. DOI: 10.1117/1.jbo.22.12.126005.
43. Dice L.R. Measures of the amount of ecologic association between species. *Ecology*. 1945; 26 (3): 297–302. DOI: 10.2307/1932409.
44. Zou K.H., Warfield S.K., Bharatha A., Tempany C.M.C., Kaus M.R., Haker S.J., Wells W.M., Jolesz F.A., Kikinis R. Statistical validation of image segmentation quality based on a spatial overlap index. *Acad. Radiol*. 2004; 11 (2): 178–189. DOI: 10.1016/S1076-6332(03)00671-8.
45. Pijls N.H., De Bruyne B., Peels K., van der Voort P.H., Bonnier H.J.R.M., Bartunek J., Koolen J.J. Measurement of fractional flow reserve to assess the functional severity of coronary-artery stenoses. *N. Engl. J. Med*. 1996; 334 (26): 1703–1708. DOI: 10.1056/NEJM199606273342604.
46. Stegehuis V.E., Wijntjens G.W., Piek J.J., van de Hoef T.P. Fractional flow reserve or coronary flow reserve for the assessment of myocardial perfusion: Implications of FFR as an imperfect reference standard for myocardial ischemia. *Curr. Cardiol. Rep*. 2018; 20 (9): 77. DOI: 10.1007/s11886-018-1017-4.
47. Zreik M., Lessmann N., van Hamersvel R.W., Wolterink J.M., Voskuil M., Viergever M. A., Leinerb T., Išgum I. Deep learning analysis of the myocardium in coronary CT angiography for identification of patients with functionally significant coronary artery stenosis. *Med. Image Anal*. 2018; 44: 72–85. DOI: 10.1016/j.media.2017.11.008.
48. Van Hamersvelt R.W., Zreik M., Voskuil M., Viergever M.A., Išgum I., Leiner T. Deep learning analysis of left ventricular myocardium in CT angiographic intermediate-degree coronary stenosis improves the diagnostic accuracy for identification of functionally significant stenosis. *Eur. Radiol*. 2019; 29 (5): 2350–2359. DOI: 10.1007/s00330-018-5822-3.
49. Itu L., Rapaka S., Passerini T., Georgescu B., Schwemmer C., Schoebinger M., Flohr T., Sharma P., Comaniciu D. A machine-learning approach for computation of fractional flow reserve from coronary computed tomography. *J. Appl. Physiol*. 2016; 121 (1): 42–52. DOI: 10.1152/japplphysiol.00752.2015.
50. Wang L., Xie X.L., Bian G.B., Hou Z.G., Cheng X.R., Prasong P. Guide-wire detection using region proposal network for X-ray image-guided navigation. *Proc. Int. Jt. Conf. Neural Networks.* 2017; 2017: 3169–3175. DOI: 10.1109/IJCNN.2017.7966251.
51. Yang H., Shan C., Kolen A.F., de With P.H.N. Catheter localization in 3D ultrasound using voxel-of-interest-based ConvNets for cardiac intervention. *Int. J. Comput. Assist. Radiol. Surg*. 2019; 14 (6): 1069–1077. DOI: 10.1007/s11548-019-01960-y.
52. Lee H., Mansouri M., Tajmir S., Lev M.H., Do S. A deep-learning system for fully-automated peripherally inserted central catheter (PICC) tip detection. *J. Digit. Imaging*. 2018; 31 (4): 393–402. DOI: 10.1007/s10278-017-0025-z.
53. Shelhamer E., Long J., Darrell T. Fully convolutional networks for semantic segmentation. *IEEE Trans. Pattern Anal. Mach. Intell*. 2017; 39 (4): 640–651. DOI: 10.1109/TPAMI.2016.2572683.

Authors information

Onishchenko Pavel S., Junior Researcher, Research Institute for Complex Issues of Cardiovascular Diseases, Kemerovo; Post-Graduate Student, Laboratory of New Biomaterials, Science Institute of Computational Technologies of the Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russian Federation. ORCID 0000-0003-2404-2873.

Klyshnikov Kirill Yu., Researcher, Laboratory of New Biomaterials, Science Institute of Computational Technologies of the Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russian Federation. ORCID 0000-0003-3211-1250.

Ovcharenko Evgeny A., Cand. Sci. (Technical Sciences). Head of the Laboratory of New Biomaterials, Science Institute of Computational Technologies of the Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russian Federation. ORCID 0000-0001-7477-3979.

(✉) **Onishchenko Pavel S.,** e-mail: onis.pavel@gmail.com

Received 14.07.2020
Accepted 28.12.2020

УДК 616.132-007.271-007.21-06:616.12-008.331.1]-055.2-053.81
https://doi.org/10.20538/1682-0363-2021-4-205-210

A clinical case of secondary hypertension in a young woman with coarctation of the aorta

Manukyan M.A., Falkovskaya A.Yu., Mordovin V.F., Saushkin V.V., Ryabova T.R.

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

ABSTRACT

The article presents a case of diagnosis and treatment of coarctation of the aorta in a 20-year-old woman, who previously received follow-up care with the diagnosis of hypertension. This case demonstrates the importance of qualitative examination of young patients with hypertension, including tonometry in the lower extremities and transthoracic echocardiography. The peculiarities of this clinical case encompass a rarer, isolated type of coarctation of the aorta and high physical fitness of the patient, which reduced doctors' alertness regarding this anomaly. After surgical correction, a significant decrease in the blood pressure was achieved; however, such patients need long-term follow-up in order to detect complications, such as aneurysms, restenosis, or residual stenosis.

Key words: coarctation of the aorta, secondary hypertension, aortic diseases.

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: Manukyan M.A., Falkovskaya A.Yu., Mordovin V.F., Saushkin V.V., Ryabova T.R. A clinical case of secondary hypertension in a young woman with coarctation of the aorta. *Bulletin of Siberian Medicine.* 2021; 20 (4): 205–210. https://doi.org/10.20538/1682-0363-2021-4-205-210.

Клинический случай симптоматической артериальной гипертонии у молодой женщины с коарктацией аорты

Манукян М.А., Фальковская А.Ю., Мордовин В.Ф., Саушкин В.В., Рябова Т.Р.

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

РЕЗЮМЕ

Представлен случай диагностики и лечения коарктации аорты у женщины в возрасте 20 лет, ранее наблюдавшейся с диагнозом «артериальная гипертония» (АГ). Приведенный клинический пример демонстрирует важность качественного обследования молодых больных с АГ, включающего тонометрию на нижних конечностях и трансторакальную эхокардиографию. Особенностями данного клинического случая являются более редкий, изолированный тип коарктации аорты и высокая физическая тренированность пациентки, что снижало настороженность врачей в отношении этой аномалии. После хирургической

✉ *Manukyan Musheg H.,* e-mail: manukyan.muscheg@yandex.ru

коррекции порока достигнуто существенное снижение артериального давления, однако в дальнейшем такие пациенты нуждаются в длительном наблюдении с целью возможного выявления таких осложнений, как аневризмы, рестенозы и резидуальные стенозы.

Ключевые слова: коарктация аорты, вторичная артериальная гипертензия, заболевания аорты.

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: Манукян М.А., Фальковская А.Ю., Мордовин В.Ф., Саушкин В.В., Рябова Т.Р. Клинический случай симптоматической артериальной гипертонии у молодой женщины с коарктацией аорты. *Бюллетень сибирской медицины.* 2021; 20 (4): 205–210. https://doi.org/10.20538/1682-0363-2021-4-205-210.

INTRODUCTION

Coarctation of the aorta (CoA) is a relatively rare anomaly, accounting for 5–8% of all congenital heart defects. Its prevalence is 2.5–4 cases per 10,000 infants, with predominance in males (2:1 ratio) [1]. Despite the traditional division into preductal (infantile type) and postductal (adult type) depending on the location with respect to the arterial duct, congenital CoA is essentially periductal, and the age of its manifestation depends on the degree of aortic stenosis and the association with other defects [2]. There is currently no consensus on the etiopathogenesis of CoA; the most common theories are the abnormal genetic mutation theory [3], the flow theory (A. Rudolf, 1972), and the loop theory (J. Skoda, 1855). It should be recognized that in most cases (up to 83%), CoA is combined with various congenital anomalies, most commonly bicuspid aortic valve (up to 60%) and hereditary diseases, such as Turner syndrome and other anomalies [3]. In the past, CoA was regarded as a local anatomical narrowing, but is now considered to be a diffuse aortopathy [4].

The hemodynamic essence of CoA involves reduced blood supply to the lower body. At this time, renal hypoperfusion activates the renin – angiotensin – aldosterone system, which leads to persistent arterial hypertension (AH) of the upper body, development of left ventricular (LV) hypertrophy, pre- and post-stenotic aortic dilatation, and formation of collateral circulation due to anastomosed intercostal, internal mammary, and scapular arteries [5].

Proximal hypertension increases the risk of aneurysms in the cerebral arteries [6] and thoracic aorta, as well as early coronary artery disease. Depending on the degree of aortic constriction and compensatory capacity, the clinical presentation can range from critically severe heart failure in infants to asymptomatic hypertension in adults [7]. The main manifestations of CoA are upper-limb systolic hypertension and lower-limb hypotension, with the arterial pressure (AP) gradient between the upper and lower extremities > 20 mmHg. In the natural course of the disease, the average life expectancy of patients, according to M. Campbell, is about 34 years, and the leading causes of death according to 304 autopsies were congestive heart failure (25.5%), aortic rupture (21%), bacterial endocarditis (18%), and intracranial hemorrhage (11.5%) [8]. In the meantime, surgical correction of the defect can dramatically change the fate of patients [9–11].

As CoA is mostly well diagnosed in childhood, it is one of the rare causes of arterial hypertension in adults, in whom its distinguishing feature is resistance to pharmacotherapy. Transthoracic echocardiography verifies the diagnosis of CoA, while computed tomography (CT) or magnetic resonance imaging (MRI) are useful tools in selecting an individual therapy, detecting complications, and managing the defect.

CLINICAL CASE

Patient R., 20 years old, born in 1999, was admitted to the Department of Arterial Hypertension of the Cardiology Research Institute, Tomsk NRMC on April 13, 2020 with the following complaints: 1) stable elevation of AP up to 160–170 /80–90 mmHg, with episodes of elevation up to 220 / 120 mmHg, accompanied by intense headache in the occipital region, dizziness, and generalized weakness; 2) shortness of breath, discomfort in the left side of the chest during rapid walking up to 300 m; 3) leg weakness during prolonged walking.

It is known from anamnesis that the patient has been actively involved in sports since childhood (basketball, biathlon), and her first elevation of AP to 160 / 100 mmHg was recorded at the age of 16 years (2015) during a physical examination. Due to her good health, the patient had not consulted a doctor until 2018 and had not taken any antihypertensive medications. In January 2018, leg weakness, headache, and dizziness appeared for the first time with a rise in AP to 220 / 120 mmHg. During the examination at the local district hospital, renovascular and endocrine hypertension were excluded and angiotensin-converting enzyme inhibitors, beta-blockers, and diuretics were prescribed to correct the AP. The patient was referred to the Cardiology Research Institute (Tomsk NRMC) for further examination and treatment in April 2020 due to failure to achieve the target AP values. In addition, it is known that the patient was the second child in the family, mother's pregnancy had no special features. Her menstruation had been regular since the age of 14. She denies the presence of chronic diseases, bad habits, aggravated heredity for cardiovascular diseases and malformations.

Upon physical examination: satisfactory general condition, clear consciousness, regular physique. Body mass index – 27.1 kg / m^2. The left border of the relative cardiac dullness is along the midclavicular line. Heart sounds are clear and rhythmic, second heart sound has accentuation over the aorta, heart rate is 76 beats per minute. A coarse systolic murmur at the Botkin – Erb's point, is noted passing to the vessels of the neck. Blood pressure in the arms: left – 203 / 115 mmHg, right – 206 / 115 mmHg; blood pressure in the legs: right and left – 140 / 90 mmHg. Other organs and systems had no clinically significant abnormalities. No significant abnormalities were detected according to the laboratory tests. ECG revealed Cornell voltage criteria for LV hypertrophy (R in aLV + S in V3 = 26 mm). According to transthoracic echocardiography, ejection fraction was 67%, heart chambers were of normal size, concentric LV hypertrophy was observed (LV myocardial mass 204 g, LV myocardial mass index 114 g/m^2), valves were without evident structural changes, aortic regurgitation up to grade I was noted, the remaining systems function normally. Systolic pressure in the right ventricle was normal. No interchamber shunts were detected. CoA with a gradient of 80 / 32 mmHg was documented at the transition of the arch to the descending aorta (Fig. 1, 2).

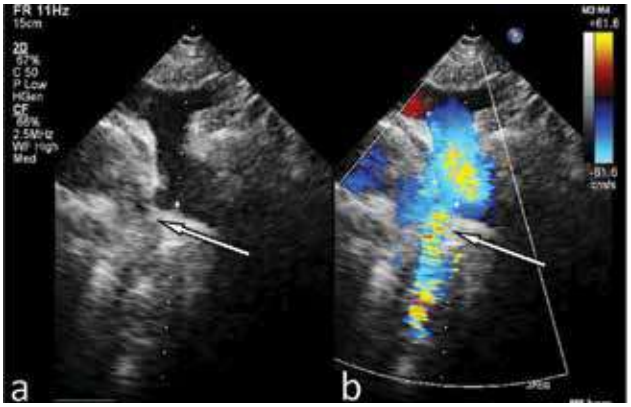


Fig. 1. Patient R., 20 years old: transthoracic echocardiography. Suprasternal position along the long axis: *a* – B-mode; *b* – color Doppler; white arrow indicates the site of stenosis

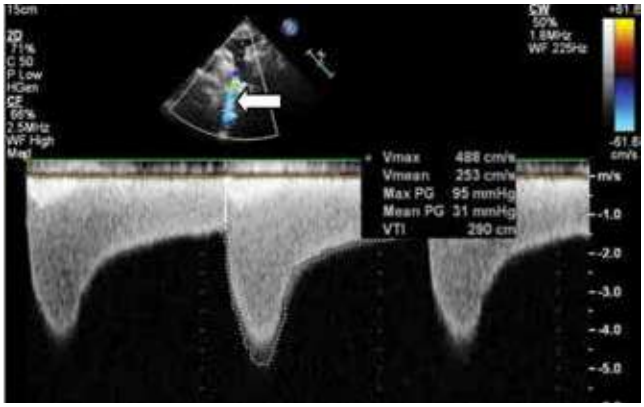


Fig. 2. Patient R., 20 years old: suprasternal position along the long axis. Continuous-wave Doppler; the arrow shows stenotic flow at the site of stenosis

MSCT aortography findings on Fig. 3 and Fig. 4 confirmed CT signs of aortic coarctation in the typical location, as well as collateral blood flow through the dilated intercostal and mammary arteries.

On the basis of the comprehensive clinical and instrumental examination, a diagnosis of congenital heart disease was made with aortic coarctation at the transition of the arch to the descending aorta, gradient 80/32 mmHg. Symptomatic arterial hypertension was detected. Left ventricular hypertrophy, risk group 4. One week after admission to the hospital, surgical repair of aortic coarctation was performed, the arch and descending aorta were repaired with 20 mm Gore-Tex 20 suture, the aortopulmonary window was dissected. The postoperative period was without complications, the patient was discharged 10 days after the intervention with the recommendations of continuous administration of metoprolol 100 mg twice a day, torasemide

5 mg /day. The entire hospital stay, including surgery, took 17 days. In 3 months after discharge from hospital, AP stabilized at 130 / 80 mmHg, and torasemide was discontinued due to a dramatic drop in AP. Taken into account tachycardia, a beta-blocker was preferred for further treatment.

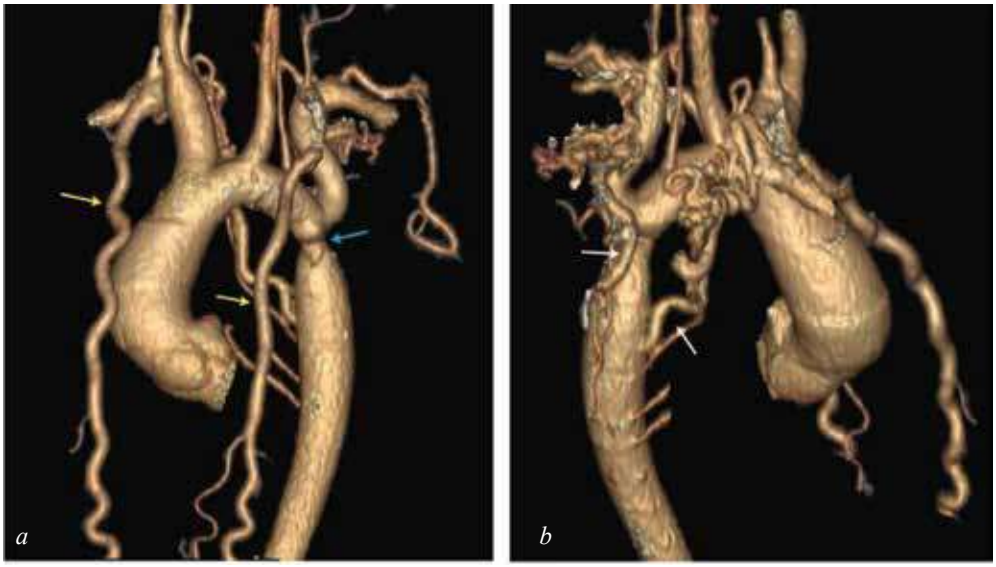


Fig. 3. Patient R., 20 years old: 3D reconstructions of the aorta in the anterior left oblique projection (*a*) and in the posterior right oblique projection (*b*). The blue arrow shows coarctation of the aorta; dilated tortuous intercostal arteries (white arrows) depart from the descending aorta; dilated mammary arteries depart from the subclavian arteries (yellow arrows).

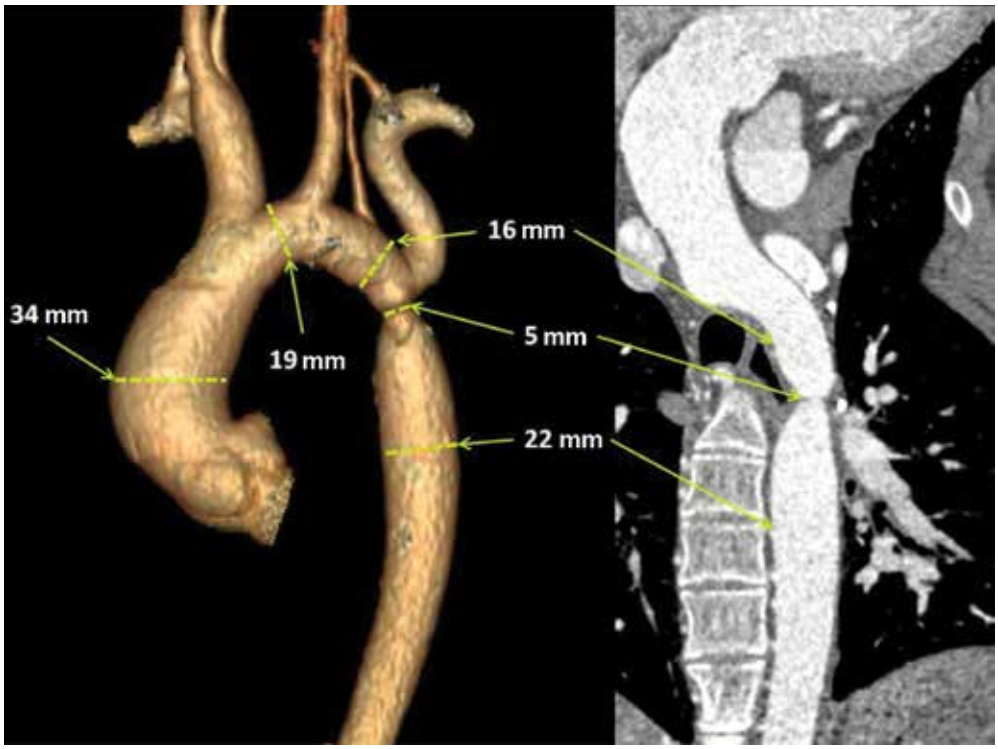


Fig. 4. Patient R., 20 years old: 3D reconstruction (left) and curvilinear reconstruction of the thoracic aorta with coarctation of the aorta. Severe narrowing of the aorta is identified distal to the origin of the left subclavian artery. Stenosis of the lumen is 70%. Poststenotic dilatation of the aorta is detected distal to the narrowing. The root and ascending thoracic aorta are dilated. The left vertebral artery branches off the aortic arch and its orifice is proximal to the left subclavian artery

CONCLUSION

A rarer, isolated type of aortic coarctation, asymptomatic course of the anomaly till the age of 16, and high physical fitness of the patient were the peculiarities of this clinical case, which disoriented primary care physicians during the examination, including that after manifestations of AH. Nevertheless, given the characteristic complaints and auscultation findings, tonometry on the lower extremities would have greatly facilitated the diagnosis. Referral of the patient to transthoracic echocardiography with obligatory inspection of the aorta from suprasternal access using Doppler color flow mapping and continuous-wave Doppler, as well as assessment of the abdominal blood flow would allow for timely diagnosis of this type of pathology.

It seems quite prominent that in this case, the diagnosis and correction of the defect in a specialized heart center took less than 3 weeks. However, despite a successful intervention, patients with aortic coarctation require long-term follow-up to monitor blood pressure, exclude the development of late aneurysms due to prolonged exposure of the vascular wall to increased hemodynamic pressure, and identify possible long-term postoperative complications.

REFERENCES

1. Hoffman J.I., Kaplan S. The incidence of congenital heart disease. *Journal of the American College of Cardiology.* 2002; 39 (12): 1890–1900. DOI: 10.1016/s0735-1097(02)01886-7.
2. Fox E.B., Latham G.J., Ross F.J., Joffe D. Perioperative and Anesthetic Management of Coarctation of the Aorta. *Seminars in Cardiothoracic and Vascular Anesthesia*. 2019; 23 (2): 212–224. DOI: 10.1177/1089253218821953.
3. Teo L.L.S., Cannell T., Babu-Narayan S. V., Hughes M., Mohiaddin, R. H. Prevalence of associated cardiovascular abnormalities in 500 patients with aortic coarctation referred for cardiovascular magnetic resonance imaging to a tertiary center. *Pediatric Cardiology*. 2011; 32 (8): 1120–1127. DOI: 10.1007/s00246-011-9981-0.
4. Kim Y. Y., Andrade L., Cook S. C. Aortic Coarctation. *Cardiology Clinics*. 2020; 38 (3): 337–351. DOI: 10.1016/j.ccl.2020.04.003.
5. Agasthi P., Pujari S.H., Tseng A., Graziano J.N., Marcotte F., Majdalany D., Mookadam F., Hagler D.J., Arsanjani R. Management of adults with coarctation of aorta. *World Journal of Cardiology*. 2020; 12 (5):167–191. DOI: 10.4330/wjc.v12.i5.167.
6. Donti A., Spinardi L., Brighenti M., Faccioli L., Leoni C., Fabi M., Trossello M.P., Gargiulo G.D., Bonvicini M. Frequency of intracranial aneurysms determined by magnetic resonance angiography in children (mean age 16) having operative or endovascular treatment of coarctation of the aorta (mean age 3). *The American Journal of Cardiology.* 2015; 116 (4): 630–633. DOI: 10.1016/j.amjcard.2015.05.030.
7. Ganigara M., Doshi A., Naimi I., Mahadevaiah G. P., Buddhe S., Chikkabyrappa S.M. Preoperative Physiology, Imaging, and Management of Coarctation of Aorta in Children. *Seminars in Cardiothoracic and Vascular Anesthesia*. 2019; 23 (4): 379–386. DOI: 10.1177/1089253219873004.
8. Torok R.D., Campbell M. J., Fleming G. A., Hill K. D. (2015). Coarctation of the aorta: Management from infancy to adulthood. *World Journal of Cardiology*. 2015; 7(11): 765–775. DOI: 10.4330/wjc.v7.i11.765.
9. Abugov S.A., Averina T.B., Axelroth B.A., Akchurin R.S., Alekian B.G., Arakelyan V.S. Clinical guidelines for the diagnosis and treatment of aortic diseases (2017). Cardiology and Cardiovascular Surgery. 2018; 1: 5-67 (in Russ.).
10. Kozlov B.N., Panfilov D.S., Saushkin V.V., Kuznetsov M.S., Nasrashvili G.G., Shipulin V.M. Reconstructive surgery of aortic arch rupture in adults. *Surgery. Journal named after N.I. Pirogov.* 2016; (5): 13-16 (in Russ.). DOI: 10.17116/hirurgia2016513-16.
11. Ilyinov V.N., Krivoschekov E.V., Shipulin V.M. Surgical treatment of aortic coarctation combined with arch hypoplasia. *Siberian Medical Journal*. 2014; 29 (3):80-86 (in Russ.).

Acknowledgements

The authors would like to express their gratitude to E.V. Tsymbaluk, Dr. Sci. (Med.), Head of the Department of Arterial Hypertension at Cardiology Research Institute, Tomsk NRMC, and E.V. Krivoshchekov, Dr. Sci. (Med.), Head of the Cardiovascular Surgery Department at Cardiology Research Institute, Tomsk NRMC, for their help in preparation of clinical material.

Authors contribution

Manukyan M.A. – drafting of the manuscript, preparation of the manuscript for publication. A.Y. Falkovskaya drafting of the manuscript, conception, critical revision of the manuscript for important intellectual content. V.F. Mordovin – advisory assistance, critical revision of the manuscript for important intellectual content. V.V. Saushkin. – carrying out of MSCT aortography, illustrations for the manuscript, consultative assistance, critical revision of the manuscript for important intellectual content. Ryabova T.R. – carrying out of ultrasound examination, illustrations for the manuscript, critical revision of the manuscript for important intellectual content.

Authors information

Manukyan Musheg H., Post-Graduate Student, Department of Arterial Hypertension, Cardiology Research Institute, Tomsk NRMC, Tomsk, Russian Federation. ORCID 0000-0003-3577-1895.

Falkovskaya Alla Yu., Cand. Sci. (Med.), Senior Researcher, Acting Head of the Department of Arterial Hypertension, Cardiology Research Institute, Tomsk NRMC, Tomsk, Russian Federation. ORCID 0000-0002-5638-3034.

Mordovin Viktor F., Dr. Sci. (Med.), Leading Researcher, Department of Arterial Hypertension, Cardiology Research Institute, Tomsk NRMC, Tomsk, Russian Federation. ORCID 0000-0002-2238-4573.

Ryabova Tamara R., Cand. Sci. (Med.), Senior Researcher, Department of Functional and Laboratory Diagnostics, Cardiology Research Institute, Tomsk NRMC, Tomsk, Russian Federation. ORCID 0000-0001-8573-5695.

Saushkin Victor V., Cand. Sci. (Med.), Senior Researcher, Laboratory for Radionuclide Methods, Cardiology Research Institute, Tomsk NRMC, Tomsk, Russian Federation. ORCID 0000-0001-5564-3802.

(✉) **Manukyan Musheg H.,** e-mail: manukyan.muscheg@yandex.ru

Received 18.08.2020
Accepted 28.12.2020

УДК 616.895.8-02:616.89-008.43-06
https://doi.org/10.20538/1682-0363-2021-4-211-217

The effect of antipsychotic-induced extrapyramidal disorders on patient's compliance with schizophrenia (a clinical case)

Rahim E.G.[1], Kornetova E.G.[1, 2], Goncharova A.A.[1], Kornetov A.N.[2], Semke A.V.[1]

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

[2] Siberian State Medical University (SSMU)
2, Moscow Trakt, Tomsk, 634050, Russian Federation

ABSTRACT

Extrapyramidal disorders are common adverse events in antipsychotic therapy. However, their diagnosis is difficult due to broad differential diagnosis, and often their specific clinical variant is not recognized, and timely intervention is not performed, which leads to severe patient suffering. This affects the quality of life of patients with schizophrenia and leads to their refusal to receive therapy, which aggravates the course of the disease. The article presents a clinical case of a 33-year-old patient at a psychiatric hospital with schizophrenia combined with such rare severe extrapyramidal disorders as antipsychotic-induced tardive dyskinesia and tardive dystonia.

The diagnosis was carried out in accordance with the criteria of the International Classification of Diseases, Tenth Revision (ICD-10). The intensity of clinical manifestations was assessed using the Positive and Negative Syndrome Scale (PANSS), the Abnormal Involuntary Movement Scale (AIMS), and the Barnes Akathisia Rating Scale (BARS). Compliance was assessed using the Method for Measuring Medication Adherence in Psychiatry. Detailed differential diagnosis of tardive dyskinesia and tardive dystonia with akathisia and Huntington's disease was presented. Substantiated treatment strategy and positive clinical dynamics with increased compliance were described.

Key words: tardive dyskinesia, tardive dystonia, schizophrenia, compliance, akathisia.

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

Source of funding. The study was funded by the Russian Foundation for Basic Research within the research project No.19-315-90010.

Conformity with the principles of ethics. The research was approved by the Ethics Committee at Mental Health Research Institute, Tomsk NRMC (Protocol No. 103 of 23.10.2017).

For citation: Rahim E.G., Kornetova E.G., Goncharova A.A., Kornetov A.N., Semke A.V. The effect of antipsychotic-induced extrapyramidal disorders on patient's compliance with schizophrenia (a clinical case). *Bulletin of Siberian Medicine.* 2021; 20 (4): 211–217. https://doi.org/10.20538/1682-0363-2021-4-211-217.

✉ *Goncharova Anastasia A.,* e-mail: goncharanastasya@gmail.com

Влияние экстрапирамидных антипсихотик-индуцированных нарушений на комплаенс пациента с шизофренией (клинический случай)

Рахим Е.Г.[1], Корнетова Е.Г.[1, 2], Гончарова А.А.[1], Корнетов А.Н.[2], Семке А.В.[1]

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

[2] Сибирский государственный медицинский университет (СибГМУ)
634050, г. Томск, Московский тракт, 2

РЕЗЮМЕ

Экстрапирамидные нарушения являются частыми нежелательными явлениями терапии антипсихотиками. Однако с учетом широкого круга и сложностей дифференциальной диагностики часто не распознается их конкретный клинический вариант и не проводится своевременное вмешательство, что приводит к выраженным страданиям пациента. Это влияет на качество жизни больных с шизофренией и приводит к отказу от приема терапии, что усугубляет течение заболевания. В статье представлен клинический случай 33-летнего пациента психиатрического стационара с шизофренией, сочетанной с такими редкими, но тяжелыми экстрапирамидными нарушениями, как тардивная дискинезия и тардивная дистония, индуцированными приемом антипсихотиков.

Диагностика проводилась в соответствии с критериями Международной классификации болезней десятого пересмотра (МКБ-10). Выраженность клинических проявлений оценивалась с помощью Шкалы позитивных и негативных симптомов (PANSS), Шкалы патологических непреднамеренных движений (AIMS), Шкалы оценки акатизии Барнса (BARS). Проведена оценка комплаенса с помощью Метода прогнозирования медикаментозного комплаенса в психиатрии. Представлена развернутая дифференциальная диагностика тардивной дискинезии и поздней дистонии с акатизией и болезнью Гентингтона. Описана мотивированная врачебная тактика и положительная клиническая динамика с повышением комплаенса.

Ключевые слова: тардивная дискинезия, тардивная дистония, шизофрения, комплаенс, акатизия.

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

Источник финансирования. Исследование выполнено при финансовой поддержке РФФИ в рамках научного проекта № 19-315-90010.

Соответствие принципам этики. Исследование одобрено этическим комитетом НИИ психического здоровья, Томский НИМЦ (протокол № 103 от 23.10.2017).

Для цитирования: Рахим Е.Г., Корнетова Е.Г., Гончарова А.А., Корнетов А.Н., Семке А.В. Влияние экстрапирамидных антипсихотик-индуцированных нарушений на комплаенс пациента с шизофренией (клинический случай). *Бюллетень сибирской медицины.* 2021; 20 (4): 211–217. https://doi.org/10.20538/1682-0363-2021-4-211-217.

INTRODUCTION

Patient noncomliance with therapy is a common problem in the treatment of schizophrenia, leading to relapses, readmissions, and aggravating long-term events [1, 2]. Treatment noncompliance is one of the reasons for transferring patients to therapy with long-acting antipsychotics, which are mostly conventional neuroleptics [3]. The average rate of noncompliance among patients with schizophrenia is 47% [4]. 32 % of patients discontinue seeing a psychiatrist upon discharge from hospital; and 31% of patients who continue regular sessions with a psychiatrist in the outpatient setting are found to have noncompliance or complete refusal of therapy [5].

Within the first 18 months of treatment, 5% of patients discontinue it completely [6]. About 250 factors affecting compliance have been identified

[7]. A key factor is multiple adverse effects of antipsychotics [8], which cause about half of patients to refuse from antipsychotic therapy [9]. Tardive dyskinesia, dystonia, and akathisia occupy a special place among specific motor side effects of antipsychotics. They aggravate the course of schizophrenia, cause treatment failure, and worsen the quality of life [10].

Tardive dyskinesia is a persistent movement disorder that occurs 1–2 years [11] after taking antipsychotics. It represents chaotic movements of different parts of the body. The specifics of the movements and the part of the body vary and may change over time. The first symptoms of tardive dyskinesia usually include repetitive, uncontrolled, chaotic movements of lips, tongue, and facial mimic muscles. Movements of the extremities, head, neck, and soma, difficult swallowing, and forced body postures may also be present [12]. The quality of life of patients with tardive dyskinesia is reduced by 12.3% relative to patients without it [13], and the presence of tardive dyskinesia complicates treatment of schizophrenia and increases the financial burden on the patient's family [14].

Tardive dystonia is an extrapyramidal disorder, one of the most disabling variants of tardive dyskinesia. It occurs several years after the start of antipsychotic therapy and persists after its withdrawal. In less than 20% of cases, it occurs within the first year of treatment [15]. Tardive dystonia begins in the muscles of face or neck. Generalization of dystonia to the extremities and trunk is observed in a small number of cases. The most frequent form of tardive dystonia is oromandibular dystonia. All of this affects patients' quality of life and leads to refusal of therapy, aggravates the course of the underlying disease, and requires increasing doses of antipsychotics: a pathological circle is formed [15].

One of the types of tardive akathisia is persistent akathisia, which develops 3 months after withdrawal of antipsychotic therapy or when a second neuroleptic drug is added to the treatment [16]. Motor restlessness with predominant localization in the lower extremities (repetitive movements such as crossing and uncrossing the legs, and constant shifting from one foot to the other, walking along a single line) is typical of akathisia and is defined as its objective component. The subjective component is represented in the form of an imperative need for movement, restlessness, and tension. The emergence of tardive akathisia, especially when its severity increases, can be mistakenly interpreted as aggravation of the mental state, and can be the reason for the formation of suicidal tendencies [16]. Timely diagnosis and correction of adverse motor events of antipsychotic therapy are necessary to improve the quality of life, increase compliance, and form long-term remission [17].

The aim of the study was to diagnose and describe the effect of combined antipsychotic-induced extrapyramidal disorders, such as tardive dyskinesia and tardive dystonia, on patient's compliance with schizophrenia.

We used clinical-psychopathological and clinical-catamnestic methods with the analysis of medical records of the patient undergoing inpatient treatment at the Department of Endogenous Disorders of Mental Health Research Institute clinics. The patient was examined and treated according to the national clinical guidelines [18]. Schizophrenia was diagnosed according to ICD-10 criteria [19]. The severity of pathopsychological symptoms was assessed according to the Positive and Negative Syndrome Scale (PANSS) [20]. The severity of pathological involuntary movements was assessed according to the Abnormal Involuntary Movement Scale (AIMS) [21]. The Barnes Akathisia Rating Scale (BARS) was used to diagnose akathisia [22]. Compliance was assessed using the Method for Measuring Medication Adherence in Psychiatry [23].

CLINICAL CASE

Patient A., 33 years old, was admitted to the hospital in January 2020 for managing adverse effects of antipsychotic therapy.

Anamnesis. The patient was born following normal pregnancy and delivery and was not behind his peers in his early development. Since preschool age, the patient had not been socially active and could not adapt in a group of peers. He was often ridiculed and physically bullied at school, then he was transferred to homeschooling. Since the age of 13, he could hardly comprehend tasks and could understand simple instructions only after several repetitions. He could not talk for days, looking at geographical atlas for a long time, freezing over one page. He was first examined by a psychiatrist following teachers' recommendations, but was not subjected to full examination and treatment.

At the age of 15, he began to have fear of men and refused to go out unaccompanied. On the eve

of the school year, he made numerous razor cuts on his forearm and neck. He never sought medical care. At the age of 16, he barely completed grade 11, after which he refused to continue his education. Patient's behavior changed, he stiffened while sitting on a chair in one position, could walk quickly around the room from time to time, raised his legs high, squeezed his hands in front of him with effort, strained his neck and moved his lower jaw forward, and was moody. With suicidal intentions, he took a blister pack of zopiclone, after which he was taken to the Accident and Emergency Unit. Having returned home, he learned of his brother's death; he did not react emotionally, went outside, and ran around the house in silence; he had not thought of his brother since.

He took the recommended haloperidol for 1 month on an outpatient basis, then stopped taking it. He was angry and tense; he declared to his parents that he had no future. At the age of 17, he was first hospitalized to Mental Health Research Institute clinics. His mental state was characterized by behavioral disorders against a background of the flat effect, alogia, and hypobulia. He took zuclopenthixol 25 mg/day, clozapine 237.5 mg/day, carbamazepine 800 mg/day, and trihexyphenidyl 10 mg/day. In the outpatient setting, he was irregularly treated with zuclopenthixol 5 mg/day. Nevertheless, he graduated from a law college.

He did not work in his specialization, he was a mine laborer for about 1 year, constantly complaining of fatigue. At the age of 26, the patient was hospitalized to a psychiatric hospital for medical and social assessment. He was diagnosed with paranoid schizophrenia with a continuous course; the second disability group was registered. As of the moment of admission, visual hallucinations were present. After discharge, he irregularly took the recommended risperidone 6 mg/day for 3 years and lived in parental care. About 2 years ago, he completely withdrew the treatment, his condition worsened again: he became restless, wrung his hands, his statements were stereotypical.

One year before the current hospitalization, the patient began to complain of many thoughts in his head, stereotypically repeating the same phrases. He was repeatedly hospitalized in the clinics of Mental Health Research Institute. His mental condition was characterized by the presence of psychic automatism, expressed by schizophrenia-specific disorders in all spheres of mental activity. However, he refused of therapy on the first day, as the doctor suggested a physical examination. He was discharged with a recommendation to take risperidone 6 mg/day.

While taking risperidone, the patient complained of stiffness in the lower jaw. After 3 months of taking it, involuntary movements in the lower half of the face and mouth appeared for the first time, occurring spontaneously or provoked by a speech act. The patient's speech became indistinct. His mother noticed improvement in speech in the morning after a night sleep. Fearing further deterioration of the patient's condition, his mother reduced the dose of the antipsychotic by half. The patient was prescribed biperiden 2 mg/day by the district psychiatrist. Involuntary movements did not decrease. The patient's mother discontinued all medications and initiated a consultation with a neurologist at Scientific Center of Neurology, where amantadine 200 mg was prescribed. It did not have the expected therapeutic effect: involuntary movements increased. The mental state of the patient aggravated, he became more withdrawn, looked gloomy, refused to leave the house due to a fear of being hit on the head, and kept silent while staring out of the window. He would stand up for no reason, shift from one foot to the other, and the involuntary movements of the mouth persisted. Accompanied by his mother, he was admitted to the Department of Endogenous Disorders of Mental Health Research Institute.

Mental status. The patient looked tidy and neat due to constant care of his mother, who changed his clothes and washed him. The patient was tense and seemed withdrawn and not interested in talking to the doctor. The facial expression was hypomimic. Motor skills were slow, movements lacked plasticity and were robotic in nature. During the conversation, he sometimes swayed his trunk back and forth rhythmically. Gestures were absent. Targeted contact was difficult to establish. Speech was indistinct due to involuntary movements in the mouth area, which were provoked by the speech act. To facilitate articulation, the patient touched his lower jaw, sometimes quickly pulled out his tongue, involuntary eye movements in the form of excessive blinking and eyebrow frown were also noted. He answered questions monosyllabically and stereotypically: "yes", "no", "maybe".

According to information received from his mother, the patient lost 9 kg due to poor appetite, became moody, did not sleep well, feared a hit on

his head, and spent a long time in one pose. He complained of a lot of thoughts in his head; episodically saw pictures and cartoon characters before his eyes; experienced fragmentary sensations of imposing, other people's thoughts. Mood was low. He did not actively express suicidal thoughts and intentions and did not exhibit any suicidal tendencies in his behavior. In the ward, he stayed in his bed or walked back and forth across the ward, raising his legs high, stopping and shifting from one foot to the other.

Data of laboratory tests and examinations by somatic specialists. According to electroencephalography, focal changes, stable interhemispheric asymmetry, epileptiform activity, or paroxysmal activity were not found. According to the magnetic resonance imaging, focal or diffuse changes were not found. The therapist's conclusion: chronic cholecystitis, remission stage. Examination by a neurologist: involuntary opening of the mouth, curvature of the lips and cheeks, forced smile during a conversation; squeezing the eyes or frowning the eyebrows, running the hands across the clothes, stroking the hair, shifting the feet periodically while sitting on a chair. Conclusion: combined manifestation of oromandibular dystonia and tardive dyskinesia.

Psychometric examination data: upon admission, according to PANSS, the total score was 113; total AIMS score was 20; according to BARS, no akathisia was found; compliance score was 29.

Differential diagnosis and substantiation of the diagnosis. Involuntary forced movements [24] had a chronic nature; they appeared against the background of the therapy with the antipsychotic and persisted after its withdrawal. There were no other causes for the condition (it was not a secondary disease of the underlying disease), there was no hereditary burden of dementia in Huntington's disease. Motor acts had a typical localization with involvement of the facial muscles in the form of clenching of the jaws and forced smile, observed during the speech act, sometimes with the use of corrective gestures (touching the jaw).

The patient had stereotyped repetitive tongue movements and expressive movements in the form of excessive blinking and motor acts involving the lower extremities (shifting from one foot to the other, walking back and forth, imitating walking while standing still, jactation). Progression of these manifestations is closely associated with receiving antipsychotic therapy for more than 3 months, and the movements persist even after withdrawal of the drug. There are no organic causes of the disorders [25].

Despite the presence of restless movements of the lower extremities, resembling the objective component of akathisia, the key factor is a lack of the subjective component, namely feelings of inner restlessness, tension, an imperative need for movement, and fatigue associated with it [26]. Irregular antipsychotic therapy, violation of the dosage regime and the frequency of intake, and an increase in the negative symptom complex act as both predisposing factors and aggravating factors for the condition. Progression of negative disorders, among other things, is a consequence of low compliance.

In this case, there is a combined presence of adverse antipsychotic-induced movement disorders: tardive dyskinesia and tardive oromandibular dystonia. There is hereditary burden of schizophrenia on the father's side. The patient's schizoid features became more pronounced in adolescence, apathy and absence of purposefulness in learning increased with the emergence of depressive experiences and subsequent suicide attempt, development of hallucinations and delusions, and mental automatism with microcatatonic symptoms. Further, in addition to hallucinations and delusions, progression of decreased energy potential and social withdrawal with the formation of an emotional-volitional defect were observed.

Definitive clinical diagnosis: paranoid schizophrenia, a continuous course. Combined manifestation of adverse antipsychotic-induced motor disorders: tardive dyskinesia and tardive oromandibular dystonia.

Treatment strategy. According to the modern understanding [27], intake of aripiprazole is less likely to cause tardive dyskinesia compared with other antipsychotics. A daily dose of aripiprazole of 7.5 mg was the drug of choice in this clinical case. The patient was also prescribed a cycle of sessions with a speech – language pathologist and recommended a course of physiotherapy in order to restore speech skills. The patient refused of physiotherapy, as any touching caused him bodily discomfort and mental suffering.

Changes in the condition. Following the sessions with a speech – language pathologist, patient's speech became more distinct, involuntary movements in the oral cavity were provoked only by a

speech act. Upon discharge, the overall PANSS score decreased to 95, the overall AIMS score – to 12, and the Medicaid Compliance Scale score increased to 37. Therefore, positive changes with a decrease in the psychopathological symptoms and pathological involuntary movements and an increase in adherence to the therapy were observed.

CONCLUSION

The clinical case presented a situation in which untimely detection and inadequate attempts to correct antipsychotic-induced tardive dyskinesia and tardive dystonia resulted in a treatment failure, up to withdrawal. Prolonged interruptions in maintenance therapy led to mental deterioration and rehospitalizations, worsened the patient's quality of life, and placed a burden on the family. Diagnosis and correction of combined tardive dyskinesia and tardive dystonia by individual selection of antipsychotics allowed to reduce the severity of their manifestations, increase compliance, and improve the patient's mental state.

REFERENCES

1. Dmitrieva E.G., Danilenko O.A., Kornetova E.G., Semke A.V., Lobacheva O.A., Gutkevich E.V., Katkova M.N. Compliance and its influence on adaptation of patients with schizophrenia. *Sibirskij Vestnik Psihiatrii i Narkologii.* 2014; (3):18–23 (in Russ.).
2. Semke A.V., Fedorenko O.Yu., Lobacheva O.A., Rakhmazova L.D., Kornetova E.G., Smirnova L.P., Mikilev F.F., Shchigoreva Yu.G. Clinical, epidemiological, and biological prerequisites of the adaptation of schizophrenic patients as the basis for a personalized approach to antipsychotic therapy. *Sibirskij Vestnik Psihiatrii i Narkologii.* 2015; (3):19–25 (in Russ.).
3. Ivanov M.V., Kosterin D.N. Efficacy of long-acting antipsychotic therapy in patients with schizophrenia in the long term. *Sibirskij Vestnik Psihiatrii i Narkologii.* 2017; (3): 84–91 (in Russ.). DOI: 10.26617/1810-3111–2017-3(96)-84-91.
4. Lacro J.P., Dunn L.B., Dolder C.R., Leckband S.G., Jeste D.V. Prevalence of and risk factors for medication nonadherence in patients with schizophrenia: A comprehensive review of recent literature. *Journal of Clinical Psychiatry*. 2002; 63(10): 892–909. DOI: 10.4088/jcp.v63n1007.
5. Chandra I.S., Kumar K.L., Reddy M.P., Reddy C.M. Attitudes toward Medication and Reasons for Non-Compliance in Patients with Schizophrenia. *Indian Journal of Psychological Medicine.* 2014; 36(3): 294–298. DOI: 10.4103/0253-7176.135383.
6. Keefe R.S., Bilder R.M., Harvey P.D., Davis S.M., Palmer B.W., Gold J.M., Meltzer H.Y., Green M.F., Miller D.D., Canive J.M., Adler L.W., Manschreck T.C., Swartz M., Rosenheck R., Perkins D.O., Walker T.M., Stroup T.S., McEvoy J.P., Lieberman J.A. Baseline neurocognitive deficits in the CATIE schizophrenia trial. *Neuropsychopharmacology.* 2006; 31(9): 2033–2046. DOI: 10.1038/sj.npp.1301072.
7. Phillips E. L. Patient compliance: New light on health delivery systems in medicine and psychotherapy. Hogrefe & Huber Publishers, 1988: 20.
8. Dubrovskaya V.V., Kornetova E.G., Semke A.V., Ivanova S.A., Arzhanik M.B., Golovakha N.E. Clinical dynamics and social adaptation in patients with schizophrenia and concomitant metabolic syndrome. *Modern Therapies for Mental Health Problems.* 2019; (2):17–21 (in Russ.).
9. Higashi K., Medic G., Littlewood K.J., Diez T., Granström O., De Hert M. Medication adherence in schizophrenia: factors influencing adherence and consequences of nonadherence, a systematic literature review. *Therapeutic Advances in Psychopharmacology.* 2013; 3 (4): 200–218. DOI: 10.1177/2045125312474019.
10. Ivanova S.A. Pharmacogenetics of antipsychotic-induced tardive dyskinesia in schizophrenic patients. *Mental Health.* 2018; 16(4): 19–21 (in Russ.). DOI: 10.25557/2074-014X.2018.04.19-21.
11. Klawans H.L., Tanner C.M., Goetz C.G. Epidemiology and pathophysiology of tardive dyskinesia. *Advances in Neurology.* 1988; 49: 185–197.
12. Meyer J.M. Forgotten but not gone: new developments in the understanding and treatment of tardive dyskinesia. *CNS Spectrums.* 2016; 21 (S1):13–24. DOI: 10.1017/S1092852916000730.
13. Rana A.Q., Chaudry Z.M., Blanchet P.J. New and emerging treatments for symptomatic tardive dyskinesia. *Drug Design Development & Therapy.* 2013; 7 (7): 1329–1340. DOI: 10.2147/DDDT.S32328.
14. Charlson F.J., Ferrari A.J., Santomauro D.F., Diminic S., Stockings E., Scott J.G., McGrath J.J., Whiteford H.A. Global Epidemiology and Burden of Schizophrenia: Findings From the Global Burden of Disease Study. *Schizophrenia Bulletin.* 2018; 44 (6): 1195–1203. DOI: 10.1093/schbul/sby058/
15. Browne S., Roe M., Lane A., Gervin M., Morris M., Kinsella A., Larkin C., Callaghan E.O. Quality of life in schizophrenia: relationship to sociodemographic factors, symptomatology and tardive dyskinesia. *Acta Psychiatrica et Neurologica Scandinavica.* 1996; 94 (2): 118–124. DOI: 10.1111/j.1600-0447.1996.tb09835.x.
16. Cunningham Owens D.G. A Guide to the Extrapyramidal Side-Effects of Antipsychotic Drugs. University of Edinburgh, 2014: 383.
17. Stacy M., Jankovic J. Tardive tremor. *Movement Disorders.* 1992; 7 (1): 53–57. DOI: 10.1002/mds.870070110.
18. Psychiatry: national guidelines; ed. by Yu.A. Alexandrovsky, N.G. Neznanov; 2nd ed. Moscow: GEOTAR-Media, 2018: 1008 (in Russ.).
19. World Health Organization: The ICD-10 Classification of Mental Disorders – Diagnostic Criteria for Research. Geneva: WHO, 1993.
20. Kay S.R., Fiszbein A., Opler L.A. The positive and negative syndrome scale (PANSS) for schizophrenia. *Schizophrenia Bulletin.* 1987; 13 (2): 261–276. DOI: 10.1093/schbul/13.2.261.

21. Guy W. ECDEU Assessment Manual for Psychopharmacology. Washington DC: Us Department of Health, Education and Welfare: 1976.
22. Barnes T. R. A rating scale for drug-induced akathisia. *The British Journal of Psychiatry*. 1989; 154 (5): 672–676. DOI: 10.1192/bjp.154.5.672.
23. Lutova N.B., Bortsov A.V., Weed V.D., Wooks A. Method for predicting medication compliance in psychiatry: guidelines. St. Petersburg, 2007: 26 (in Russ.).
24. Burke R.E., Fahn S., Jankovic J., Marsden C. D., Lang A. E., Gollomp S., Ilson J. Tardive dystonya: Late-onset and persistent dystonya caused by antipsychotic drugs. *Neurology.* 1982; 32 (12): 1335–1346. DOI: 10.1212/wnl.32.12.1335.
25. Dayalu P., Chou K.L. Antipsychotic-induced extrapyramidal symptoms and their management. *Expert Opinion on Pharmacotherapy*. 2008; 9 (9): 1451–1462. DOI: 10.1517/14656566.9.9.1451.
26. Goncharova A.A., Kornetova E.G., Kornetov A.N., Semke A.V. Is there a constitutional and morphological predisposition to akathisia in schizophrenic patients receiving antipsychotic therapy? *Bulletin of Siberian Medicine*. 2019; 18 (4): 36–43 (in Russ.). DOI: 10.20538/1682-0363-2019-4-36-43.
27. Ostroumova T.M., Tolmacheva V.A., Ostroumova O.D., Parfenov V.A. Drug-induced tardive dyskinesia. *Neurology, Neuropsychiatry, Psychosomatics.* 2020; 12 (1): 81–86 (in Russ.). DOI: 10.14412/2074-2711-2020-1-81-86.

Authors contribution

Rahim E.G. – examination and management of the patient, drafting of the article. Kornetova E.G. – development of case management strategy, drafting of an article. Goncharova A.A. – participation in the examination and treatment of the patient, drafting of the article. A.N. Kornetov – drafting of the article and critical revision of the manuscript for important intellectual content. Semke A.V. – final approval of the manuscript for publication.

Authors information

Rakhim Ekaterina G., Cand. Sci. (Med.), Researcher, Department of Endogenous Disorders, Mental Health Research Institute, Tomsk NRMC, Tomsk, Russian Federation. ORCID 0000-0002-7261-3434.

Kornetova Elena G., Dr. Sci. (Med.), Principal Researcher, Department of Endogenous Disorders, Mental Health Research Institute, Tomsk NRMC, Tomsk; Consulting Psychiatrist, SSMU, Tomsk, Russian Federation. ORCID 0000-0002-5179-9727.

Goncharova Anastasia A., Junior Researcher, Department of Endogenous Disorders, Mental Health Research Institute, Tomsk NRMC, Tomsk, Russian Federation. ORCID 0000-0001-5260-5245.

Kornetov Alexander N., Dr. Sci. (Med.), Head of the Division of Fundamental Psychology and Behavioral Medicine, SSMU, Tomsk, Russian Federation. ORCID 0000-0002-2342-7504.

Semke Arkady V., Dr. Sci. (Med.), Professor, Deputy Director for Scientific and Medical Work, Mental Health Research Institute, Tomsk NRMC, Tomsk, Russian Federation.ORCID 0000-0002-8698-0251.

(✉) **Goncharova Anastasia A.,** e-mail: goncharanastasya@gmail.com

Received 28.12.2020
Accepted 25.05.2021

УДК 616.831-005.1-005.4-005.6-089.87-052:347.463
https://doi.org/10.20538/1682-0363-2021-4-218-224

Work of a stroke team: experience of transferring ischemic stroke patients from district hospitals to Krasnoyarsk Regional Vascular Center for thrombectomy

Shniakin P.G.[1, 2], Isaeva N.V.[1, 2], Kuznetsov V.Y.[2], Protopopov A.V.[1, 2], Korchagin E.E.[1, 2], Dovbish N.Y.[1, 2], Litvinyuk N.V.[2]

[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

Modern high-technology methods for ischemic stroke treatment (systemic thrombolysis, mechanical thrombectomy, thrombaspiration, stenting of cerebral arteries) can improve the rehabilitation potential and survival of patients. Important tasks here are selection for reperfusion and its performance on the greatest possible number of peracute patients. Mechanical thrombectomy combined with systemic thrombolysis is the most effective reperfusion strategy in the therapeutic window, but the availability of endovascular methods is limited to highly specialized centres. One way to solve this problem is to organize effective logistics with stroke patients, which will provide high-tech care for patients living far from large treatment centers due to regulated interaction between institutions at different levels.

The aim of the study was to improve emergency interaction related to transfer of peracute stroke patients from primary vascular units and district hospitals of the Krasnoyarsk region to Krasnoyarsk Regional Vascular Center for thrombectomy.

Key words: ischemic stroke, stroke team, logistics of patients with stroke, mechanical thrombectomy, thrombolysis.

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: Shniakin P.G., Isaeva N.V., Kuznetsov V.Y., Protopopov A.V., Korchagin E.E., Dovbish N.Y., Litvinyuk N.V. Work of a stroke team: experience of transferring ischemic stroke patients from district hospitals to Krasnoyarsk Regional Vascular Center for thrombectomy. *Bulletin of Siberian Medicine.* 2021; 20 (4): 218–224. https://doi.org/10.20538/1682-0363-2021-4-218-224.

Работа Stroke team: опыт перевода пациентов с ишемическим инсультом на тромбэкстракцию из межрайонных больниц в Региональный сосудистый центр г. Красноярска

Шнякин П.Г.[1, 2], Исаева Н.В.[1, 2], Кузнецов В.Ю.[2], Протопопов А.В.[1, 2], Корчагин Е.Е.[1, 2], Довбыш Н.Ю.[1, 2], Литвинюк Н.В.[2]

[1] Красноярский государственный медицинский университет (КрасГМУ) имени профессора В.Ф. Войно-Ясенецкого
Россия, 660022, г. Красноярск, ул. Партизана Железняка, 1

✉ *Kuznetsov Vladimir Yu.,* e-mail: vykuznetsov@list.ru

[2] *Краевая клиническая больница (ККБ)*
Россия, 660022, г. Красноярск, ул. Партизана Железняка, 3а

РЕЗЮМЕ

Современные высокотехнологичные методы лечения ишемического инсульта (системный тромболизис, механическая тромбэкстракция, тромбаспирация, стентирование церебральных сосудов) позволяют улучшить реабилитационный потенциал и выживаемость пациентов. Важной задачей является отбор и проведение реперфузии в острейшем периоде наибольшему количеству больных. Тромбэкстракция в сочетании с системным тромболизисом является наиболее эффективной стратегией реперфузии в терапевтическом окне, однако доступность эндоваскулярных методов ограничена высокоспециализированными центрами. Одним из путей решения этой задачи является организация эффективной логистики пациентов с острым нарушением мозгового кровообращения (ОНМК), что позволит оказывать высокотехнологичную помощь пациентам, проживающим на удалении от крупных лечебных центров, благодаря отрегулированному взаимодействию между учреждениями разного уровня.

Цель исследования: совершенствование экстренного взаимодействия по вопросам перевода пациентов в острейшем периоде ишемического инсульта из первичных сосудистых отделений и межрайонных больниц Красноярского края в Региональный сосудистый центр г. Красноярска для проведения тромбэкстракции.

Ключевые слова: ишемический инсульт, Stroke team, логистика пациентов с ОНМК, тромбэкстракция, тромболизис.

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: Шнякин П.Г., Исаева Н.В., Кузнецов В.Ю., Протопопов А.В., Корчагин Е.Е., Довбыш Н.Ю., Литвинюк Н.В. Работа Stroke team: опыт перевода пациентов с ишемическим инсультом на тромбэкстракцию из межрайонных больниц в Региональный сосудистый центр г. Красноярска. *Бюллетень сибирской медицины.* 2021; 20 (4): 218–224. https://doi.org/10.20538/1682-0363-2021-4-218-224.

INTRODUCTION

Hyperacute ischemic stroke treatment with implementation of high-tech methods into clinical practice sets new goals in healthcare management and patient routing. An important issue is to improve the availability of a full range of medical services, combining methods of therapeutic (systemic thrombolysis) and surgical (mechanical thrombectomy, thrombaspiration, stenting of cerebral vessels) reperfusion for the largest number of patients [1–5]. In Krasnoyarsk, endovascular methods for ischemic stroke are successfully applied at the premises of three largest clinics: Krasnoyarsk Regional Clinical Hospital (chief physician – Egor E. Korchagin), Karpovich Krasnoyarsk Regional Clinical Emergency Hospital (chief physician – Sergey V. Grebennikov), and Krasnoyarsk Regional Hospital No. 20 (chief physician – Vladimir A. Fokin).

At the same time, healthcare institutions in remote areas of the Krasnoyarsk region have limitations in relation to the use of such methods, and making them available for patients from remote areas is relevant. The solution to this problem may be to improve the logistics for ischemic stroke patients and their emergency transportation to healthcare institutions with necessary resources for high-tech medical care [6, 7].

RESULTS

Currently, the Regional Vascular Disease Center (RVDC) of Krasnoyarsk Regional Clinical Hospital (KRCH) (the head of the RVDC is A.V. Protopopov, Dr. Sci. (Med.), Professor), interacts and cooperates on a round-the-clock basis with primary vascular departments (PVDs) and interdistrict hospitals located within a 100 km radius from the Krasnoyarsk Regional Clinical Hospital: Clinical Hospital No. 51 of the Federal Medical Biological Agency of the Russian Federation (62.4 km) and Divnogorsk Interdistrict Hospital (45 km) (Fig. 1).

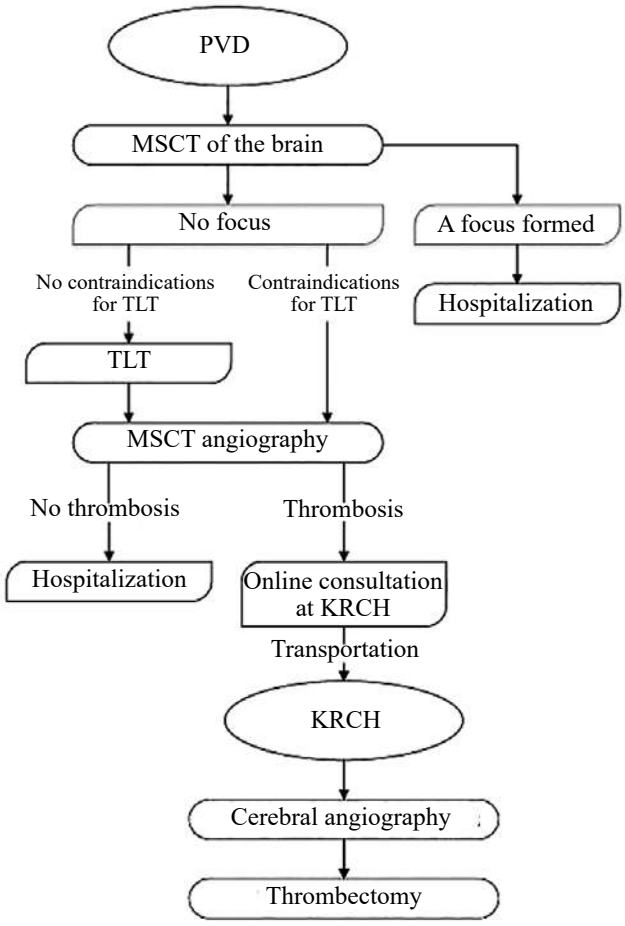


Fig. 1. The current system of interaction between the Krasnoyarsk Regional Vascular Disease Center and other treatment facilities in the region in selecting ischemic stroke patients for emergency thrombectomy

The problem of organizing emergency interaction between medical and prevention institutions in Krasnoyarsk and the Krasnoyarsk region is solved with various tools. Along with the traditional method of inter-hospital interaction using telephone communication, modern technologies have been introduced in the Krasnoyarsk region. Telemedicine consultations are performed through the RCH Center of X-ray Consultations with the possibility to upload neuro-visualization images (multi-slice computed tomography (MSCT), magnetic resonance imaging (MRI)) to the unified database for their immediate evaluation by radiologists of the center from any remote station. Another important tool for assessing the patient's condition and determining further management strategy is the neuromonitoring program [8], with which a consulting neurologist can establish a two-way communication with doctors of all hospitals in the Krasnoyarsk region, receiving full information on the patient with the ability to make timely decisions on treatment correction or transfer the patient to the RVDC. Taking into consideration wide capabilities of telemedicine, it is now possible to apply high-tech methods of treating hyperacute ischemic stroke in patients living outside the city of Krasnoyarsk.

In 2019, 10 patients with stroke received treatment in the Neurology Department of RVDC after transfer from PVDs with subsequent endovascular surgery (mechanical thrombectomy, stenting of cerebral vessels).

CLINICAL CASE 1

Patient B, 63 years old. Transferred from PVD of Zheleznogorsk Clinical Hospital No. 51 of FMBA of the Russian Federation with the clinical presentation of right-sided hemiparesis, motor aphasia; the patient was alert (Modified Rankin scale score: 3, NIHSS: 5). On the emergence of symptoms, the patient was admitted to a local PVD with a suspected stroke. A standard neurological examination involving scaling, laboratory data evaluation, as well as native brain MSCT was performed. The diagnosis of ischemic stroke was confirmed. Taking into consideration admission within the therapeutic window and lack of contraindications, systemic thrombolytic therapy (TLT) was immediately started in the MSCT room in the PVD [9–11]. According to MSCT angiography of cerebral and brachiocephalic vessels, symptomatic thrombosis was revealed: a left-sided occlusion of the internal carotid artery (ICA).

The Regional Clinical Hospital has developed a standard regulating the selection of patients admitted with the diagnosis of stroke for transfer to the RVDC for mechanical reperfusion [3]. The prerequisites are admission within the therapeutic window, the consciousness level of over 9 according to the Glasgow Coma Scale, ASPECTS scale score of no less than 6 (MSCT of the brain), the Modified Rankin Scale score for the degree of disability before stroke of no more than 2. Patient B. met all the listed criteria.

Considering the presence of symptomatic ICA occlusion, required transportation time (approximately one hour), and the evacuable state of the patient (the level of consciousness no lower than obtundation, unassisted breathing, stable hemodynamics), the neurologist at the PVD informed the neurologist at the RVDC by phone about the anamnesis data, neurological status, and MSCT results, indicating the time of TLT onset. The neurologist and radiologist at the RVDC assessed the MSCT scans of the patient's brain

and cerebral vessels, which were presented to the Center of X-ray Consultions.

This clinical examination complied with all the conditions for a possible thrombectomy [12–15], and a decision was made to urgently transfer the patient to the RVDC. Meanwhile, the RCH neurologist informed the interventional radiologist and the anesthesiology team about the forthcoming arrival of the patient who was a candidate for thrombectomy. The endovascular surgeon began preparing the X-ray-equipped operating room for emergency intervention.

The patient was delivered to the Krasnoyarsk RVDC by the ambulance team. In the admission unit at the RCH, the patient was examined at runtime by the attending neurologist, MSCT of the brain was performed (the ASPECTS score of 8) to exclude major irreversible ischemia and intracranial hemorrhage (Fig. 2).

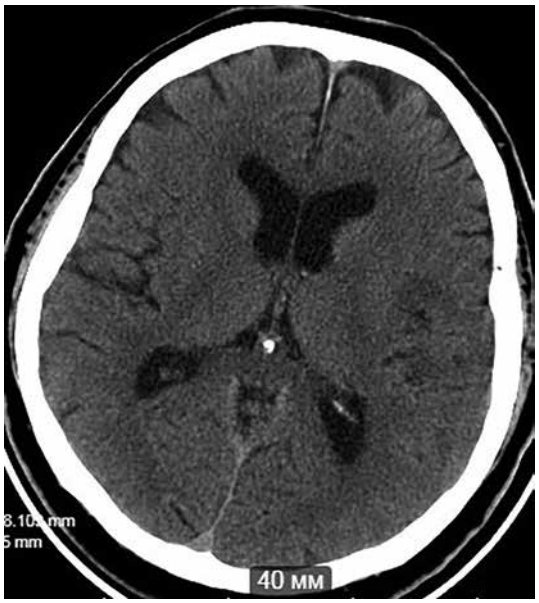


Fig. 2. Computed tomography of the brain of patient B., 63 years old, after thrombolysis before thrombectomy

Since the therapeutic window period was about to end, cerebral perfusion MSCT was performed in order to assess the core – penumbra area ratio. The core was within 1/3 of the left middle cerebral artery (MCA) circulation.

Indications for emergency thrombectomy were evaluated in the admission unit by a multidisciplinary team including a neurologist, an interventional radiologist, and a radiologist. All parameters met the criteria for thrombectomy. The patient was admitted to the X-ray-equipped operating room, where selective cerebral angiography was performed to resolve the issue of further treatment strategy. Mechanical thrombectomy was carried out on the left ICA circulation, and the blood flow was restored according to grade 3 of the mTICI scale. According to the data of MSCT of the brain at runtime, a small ischemic area formed in the left temporoparietal zone after 24 hours without decrement in the neurological status (Fig. 3).

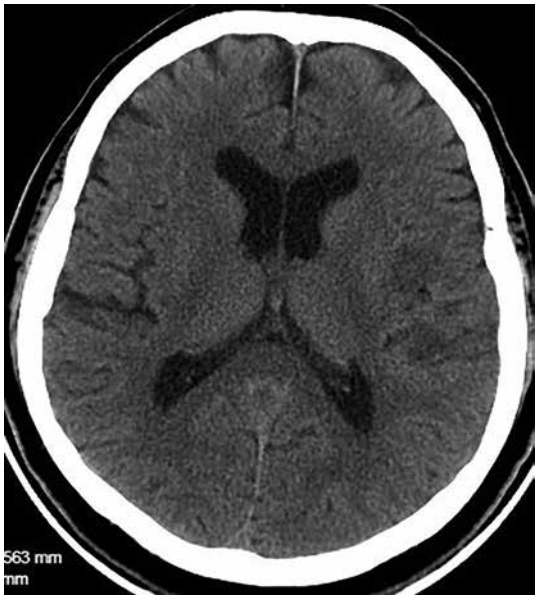


Fig. 3. Computed tomography of the brain of patient B., 63 years old, after thrombectomy of the internal carotid artery on the left

The patient received standard baseline and neurometabolic therapy and underwent early rehabilitation. Positive changes in the patient's condition were observed, hemiparesis regressed, moderate speech disorders persisted, manifested through efferent motor and dynamic aphasia. The motor component of the speech improved (Modified Rankin Scale: 2, NIHSS: 1). The patient was discharged in a satisfactory condition for follow-up rehabilitation of speech disorders.

The choice of stroke treatment methods and organization of transportation should be made as quickly as possible. The feature of the following clinical case is that a stroke patient was transported twice between healthcare institutions before admission to the RVDC. In the meantime, the patient was delivered within the therapeutic window.

CLINICAL CASE 2

Patient R., 57 years old, was urgently taken to the Divnogorsk Interdistrict Hospital due to acute

development of focal neurologic deficit with suspected stroke. Considering the absence of CT equipment in the interdistrict hospital, according to the routing, the patient was initially transferred to the private healthcare institution "Clinical hospital "RZD-Medicine", Krasnoyarsk, where MSCT of brain structures and MSCT angiography of cerebral arteries were performed, M1 segment occlusion of the left MCA was diagnosed, and systemic thrombolysis was initiated (Fig. 4). Taking into account the duration of the therapeutic window, the patient was transported for the next stage to the RVDC. Upn admission, the patient was fully alert, with focal neurologic deficit in the form of right-sided hemiparesis, hemihypesthesia, and motor aphasia. Modified Rankin Scale score was 4, NIHSS score was 13.

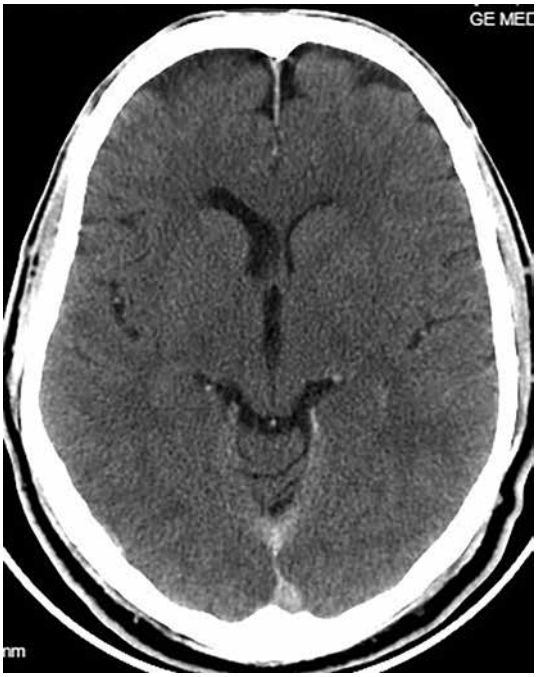


Fig. 4. Computed tomography of patient R., 57 years old, after TLT, before the start of thrombectomy

At the RVDC, thrombectomy was performed on the M1 segment of the left MCA with full perfusion restoration, the mTICI scale score was 3. A small ischemic area in the left MCA circulation was revealed using dynamic MSCT of the brain (Fig. 5). During the inpatient treatment, the patient's condition showed positive changes: muscle strength and sensitivity in the right limbs were restored, overall mental activity and physical performance increased. Voluntary attention and memory enhanced, self-service ability improved. Upon discharge, speech disorders corresponded to dynamic aphasia in the form of systemic perseverations.

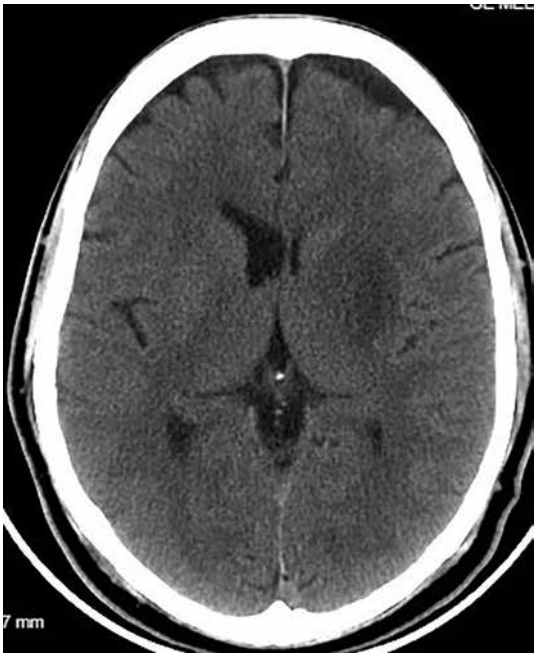


Fig. 5. Computed tomography of the brain of patient R., 57 years old, after thrombectomy of the M1 segment of the left middle cerebral artery. Formation of a small ischemic area without neurological status deterioration

Upon discharge, the score on the Modified Rankin Scale was, on NIHSS – 1. The patient was referred to follow-up treatment: stage 3 of speech rehabilitation.

This article presents clinical cases of a small sample of patients to show the possibility of increasing the availability of endovascular methods for treating hyperacute ischemic stroke patients who live outside large cities with specialized vascular centers.

The analysis of time intervals in 10 clinical cases of patients from the initial ischemic stroke symptoms in the area of residence to thrombectomy at the Krasnoyarsk RVDC in 2019 showed that the duration of each period largely depended on the promptness of the stroke team actions. The key elements of medical aid provision at each stage are as follows: reaction to the call for an ambulance team, stroke diagnosis at the PVD where the patient was initially transported to and where the decision to transfer the patient to the RCH was made, and promptness of actions of the RVDC team. It was determined that the average time between the stroke onset and admission to the RCH was 245 [198.25; 257.25] minutes, while thrombolysis started after 155 [140; 180] minutes, beginning at the PVD and continuing in the ambulance during transportation to the RCH. The time between entering the

hospital and starting the operation was 37.5 [28.25; 47.5] minutes. Thrombectomy was initiated after an average of 247 minutes [237.5; 293.5] from the first stroke symptoms. Recanalization had been completed by 388 [300.5; 408.25] minutes from the stroke onset (Fig. 6).

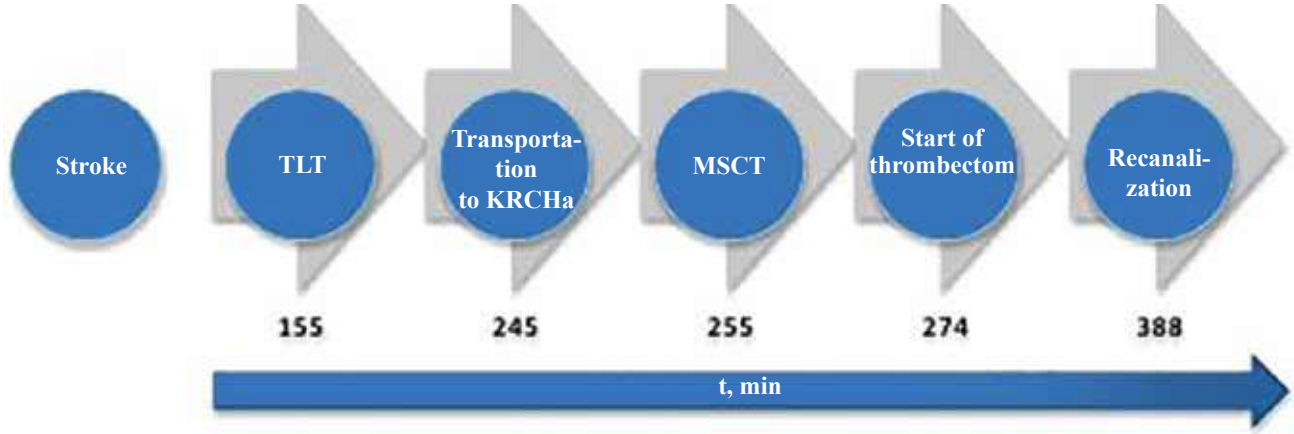


Fig. 6. Time values of working with hyperacute ischemic stroke patients ($n = 10$) transferred from interdistrict hospitals of the region to the Regional Vascular Disease Center for emergency thrombectomy (*Me* [p_{25}; p_{75}])

It is obvious that each region may develop its own system of emergency logistics for stroke patients taking into account its territorial and administrative peculiarities. In the Krasnoyarsk region, a system of interaction between interdistrict hospitals, PVDs, and the RVDC allows to make timely decisions on transfer of patients for thrombectomy, which increases availability of high-quality care for hyperacute ischemic stroke patients. Coordinated teamwork of specialists from healthcare institutions at different levels allows to start thrombectomy within the therapeutic window. Therefore, mechanical thrombectomy in all patients was started within 4.5 hours, and successful recanalization was performed within 6.5 hours from the stroke onset. There were no lethal cases in the group of patients observed after thrombectomy. Good recovery of the neurologic deficit was observed in all the patients.

CONCLUSION

Maximum efficacy of treatment for hyperacute ischemic stroke patients requires:

– a well-developed system for interaction between emergency room specialists, neurologists, radiologists, and intervention radiologists within healthcare institutions providing high-tech medical care for ischemic stroke patients;

– improving the quality of interaction between the RVDC and healthcare institutions at primary and secondary levels by introducing modern technology, such as continuous X-ray consultation and neuromonitoring;

– well-managed work of the ambulance and its interaction with PVDs and the RVDC.

Therefore, well-coordinated teamwork of stroke team specialists at different levels of care for hyperacute ischemic stroke patients contributes to an increase in the availability of high-tech methods for treating patients who live outside the regional center, which makes it possible to preserve the maximum possible amount of nervous tissue, reduce mortality, increase the rehabilitation potential, and improve functional outcomes.

REFERENCES

1. On the approval of the procedure for the provision of medical care to patients with acute cerebrovascular accidents: order of the Ministry of Healthcare of the Russian Federation No. 928н dated 15.11.2012 (in Russ.).
2. Order of the Ministry of Healthcare of the Krasnoyarsk Krai No. 859-орг dated 14.09.2018 (in Russ.). 3. The procedure for the provision of emergency medical care to patients with acute cerebrovascular accident: the standard of Krasnoyarsk Regional Clinical Hospital Order No. 232 dated 17.05.2019, the standard of the 18.10.2019, 3rd ed. (in Russ.).
4. Lukyanchikov V.A., Udodov E.V. Possibilities of surgical correction of internal carotid artery thrombosis in patients with acute ischemic stroke. *Russian Sklifosovsky Journal "Emergency Medical Care"*. 2017; 6(2): 110–117 (in Russ.). DOI: 10.23934/2223-9022-2017-6-2-110-117.
5. Shamalov N.A., Khasanova D.R., Stakhovskaya L.V., Voznyuk I.A., Yanishevsky S.N., Vinogradov O.I., Kharitonova T.V., Alekyan B.G., Kandyba D.V., Savello A.V.,

Volodyukhin M.Yu. Reperfusion therapy for ischemic stroke. Clinical protocol. M., 2019: 80 (in Russ.).
6. Saver J.L., Goyal M., Bonafe A., Diener H.-Ch., Levy E.I., Pereira V.M. et al. Stent-retriever Thorombectomy after intravenous t-PA vs. t-PA alone in stroke. *N. Engl. J. Med*. 2015; 372 (24): 2285–2295. DOI: 10.1056/NEJMoa1415061.
7. Saver J.L., Goyal M., Aad van der Lugt A., Menon B.K., Majoie C.B.L.M., Dippel D.W. et al. Time to treatment with endovascular thorombectomy and outcomes from ischemic stroke: a meta-analysis. *JAMA*. 2016; 316 (12): 1279–1288. DOI: 10.1001/jama.2016.13647.
8. Shnyakin P.G., Demko I.V., Korchagin E.E., Protopopov A.V., Samokhvalov E.V., Nemik D.B., Tyumentsev N.V., Gordeeva N.V. Monitoring of population-significant diseases (experience of the Krasnoyarsk Krai). Krasnoyarsk: Versona, 2018: 99 (in Russ.).
9. Domashenko M.A., Maksimova M.Yu. Personification of the approaches to reperfusion therapy for ischemic stroke. *Annals of Neurology*. 2017; 11 (1): 7–13 (in Russ.).
10. Berkhemer O.A., Fransen P.S.S., Beumer D., van den Berg L.A., Lingsma H.F., Yoo A.J. et al. A randomized trial of intraarterial treatment for acute ischemic stroke. *N. Engl. J. Med*. 2015; 372 (1): 11–20. DOI: 10.1056/NEJMoa1411587.
11. Powers W.J., Rabinstein A.A., Ackerson T., Adeoye O.M., Bambakidis N.C., Becker K. et al. 2018 Guidelines for the Early Management of Patients With Acute Ischemic Stroke: A Guideline for Healthcare Professionals From the American Heart Association/American Stroke Association. *Stroke*. 2018; 49(3): 46–110. DOI: 10.1161/STR.0000000000000158.
12. Protopopov A.V., Shnyakin P.G. Litvinyuk N.V., Evdokimova E.Yu., Tyumentsev N.V. Thrombectomy in acute ischemic stroke: methodological recommendations for doctors. Krasnoyarsk: Verso, 2019: 100 (in Russ.).
13. Campbell B.C.V., Mitchell P.J., Kleinig T.J., Dewey H.M., Churilov L., Yassi N. et al. Endovascular therapy for ischemic stroke with perfusion-imaging selection. *N. Engl. J. Med.* 2015; 372(11): 1009–1018. DOI: 10.1056/NEJMoa1414792.
14. Saver J.L. Time is brain – quantified. Stroke. 2006; 37(1): 263–266. DOI: 10.1161/01.STR.0000196957.55928.ab.
15. Goyal M., Demchuk A.M., Menon B.K., Eesa M., Rempel J.L., Thornton J. et al. Randomized assessment of rapid endovascular treatment of ischemic stroke. *N. Engl. J. Med.* 2015; 372(11): 1019–1030. DOI: 10.1056/NEJMoa1414905.

Authors contribution

Isaeva N.V. – conception and design. Kuznetsov V.Y., Dovbysh N.Yu., Litvinyuk N.V. – analysis and interpretation of data. Shnyakin P.G. – substantiation of the manuscript and critical revision for important intellectual content. Protopopov A.V., Korchagin E.E. – final approval of the manuscript for publication.

Authors information

Shnyakin Pavel G., Dr. Sci. (Med.), Associate Professor, Deputy Head of the Regional Vascular Disease Center, Krasnoyarsk Regional Clinical Hospital, Krasnoyarsk, Russian Federation. ORCID 0000-0001-6321-4557.

Isaeva Natalya V., Dr. Sci. (Med.), Professor, V.F. Voino-Yasenetsky Krasnoyarsk State Medical University, Krasnoyarsk, Russian Federation. ORCID 0000-0002-8323-7411.

Kuznetsov Vladimir Y., Cand. Sci. (Med.), Head, Neurology Department for patients with stroke, Krasnoyarsk Regional Clinical Hospital, Krasnoyarsk, Russian Federation. ORCID 0000-0002-3042-0925.

Protopopov Aleksey V., Dr. Sci. (Med.), Professor, Rector of V.F. Voino-Yasenetsky Krasnoyarsk State Medical University; Head of Regional Vascular Disease Center, Krasnoyarsk Regional Clinical Hospital, Krasnoyarsk, Russian Federation. ORCID 0000-0001-5387-6944.

Korchagin Egor E., Chief Physician, Krasnoyarsk Regional Clinical Hospital, Krasnoyarsk, Russian Federation. ORCID 0000-0002-4153-9585.

Dovbysh Nikolay Yu., Head of the Department of Neuroresuscitation, Krasnoyarsk Regional Clinical Hospital, Krasnoyarsk, Russian Federation. ORCID 0000-0001-7222-9224.

Litvinyuk Nikita V., Head of the Department of X-ray Surgical Methods of Diagnosis and Treatment, Krasnoyarsk Regional Clinical Hospital, Krasnoyarsk, Russian Federation. ORCID 0000-0002-0630-7244.

(✉) **Kuznetsov Vladimir Yu.,** e-mail: vykuznetsov@list.ru

Received 04.06.2020
Accepted 28.12.2020

K

L

M

N

O

P

R

S

T

U

V

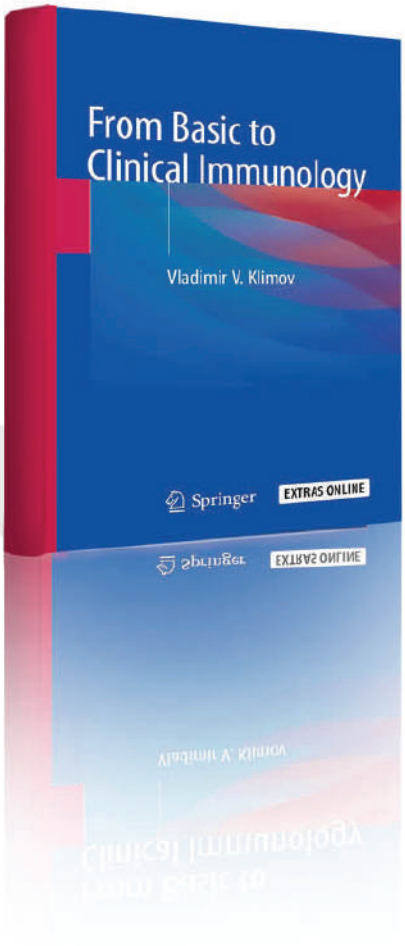
Y

Z

В издательстве «Springer» готовится к печати рукопись новой книги ALLERGEN TOLERANCE профессора Сибирского государственного медицинского университета Владимира Климова

Создание книги и подписание контракта были инициированы издательством «Springer» (Швейцария). в связи с успешным продвижением и высоким международным рейтингом первой книги заведующего кафедрой иммунологии и аллергологии СибГМУ профессора В.В. Климова «From Basic to Clinical Immunology» (2019), включенной в каталог Библиотеки Конгресса США и каталог 32 лучших книг по иммунологии и аллергологии последних 35 лет рейтингового агентства «BookAuthority (CNN/Forbes Inc)» (США).

Новая рукопись написана автором досрочно, что получило одобрение одного из редакторов издательства «Springer», который отметил профессионализм и пунктуальность профессора В.В. Климова и достойное представление им своего университета и своей страны в международном научно-образовательном сообществе. Проект успешно прошел внешнее рецензирование.



Рукопись состоит из 10 глав, написана на 379 страницах, включает 67 авторских рисунков, 20 аудио, 11 видео. Список литературы составляет 955 наименований статей и книг последних лет. Написана в интерфейсе клинической аллергологии, фундаментальной иммунологии и нейробиологии.

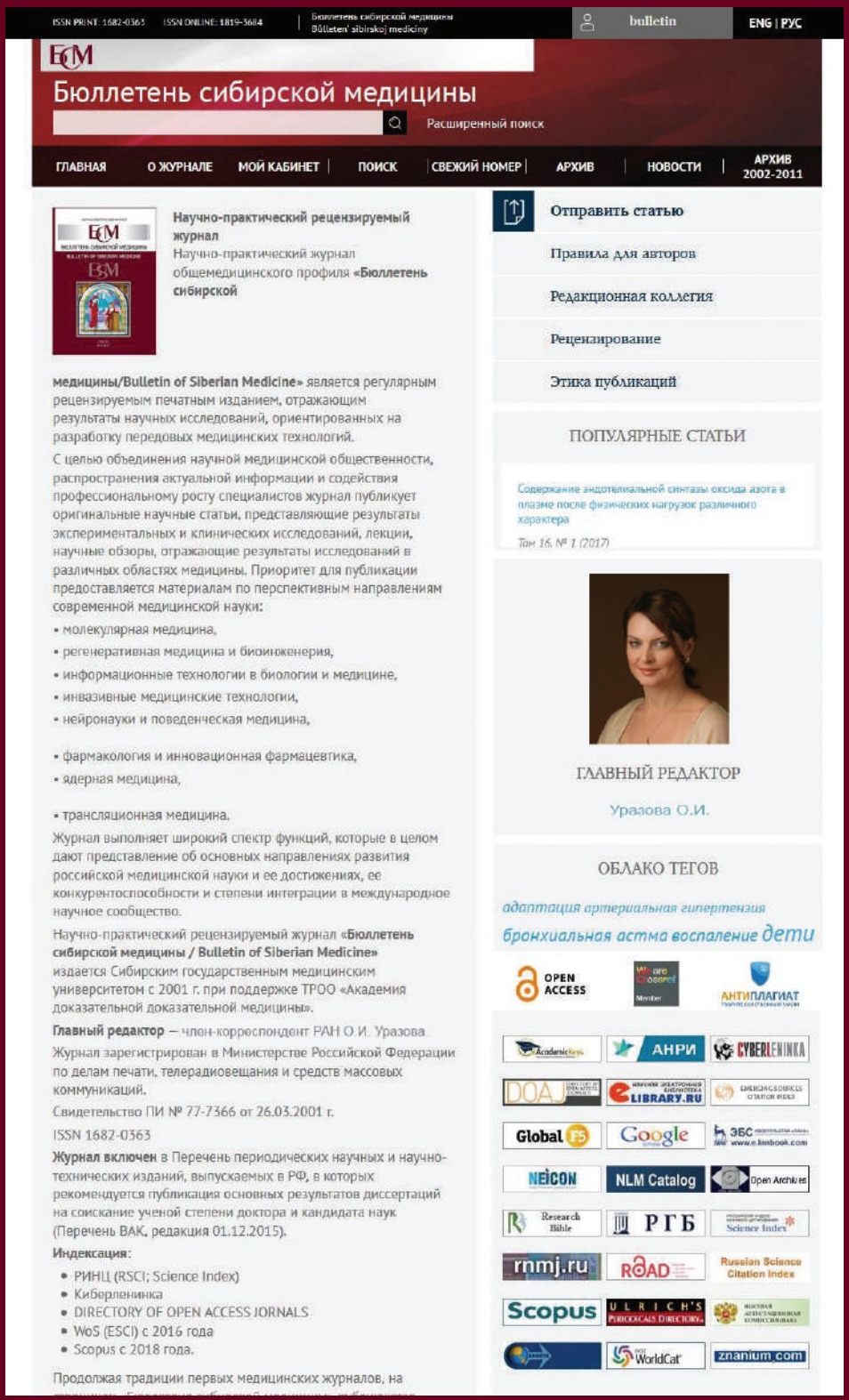
Для студентов на последипломном уровне обучения, научных работников, врачей и всех интересующихся данными направлениями в медико-биологических науках.

Содержание книги "Allergen Tolerance":

1. Antigens and Allergens
2. Immune Tolerance at a Glance
3. Immune-Derived Maintenance of Allergen Tolerance
4. Role of the Neuroimmune Network in Allergic Inflammation
5. Conventional Atopic Diseases
6. Local Atopic Disorders in the Unified Airway
7. Food Allergies and Oral Tolerance
8. Allergen-Specific Immunotherapy (AIT)
9. Allergen Tolerance in the Genitourinary Tract
10. Allergies in COVID-19 and Post-COVID Syndrome

Ориентировочный срок выхода книги в свет – весна–лето 2022 г.

bulletin.tomsk.ru



ISSN 1682-0363 (print)
ISSN 1819-3684 (online)
БЮЛЛЕТЕНЬ СИБИРСКОЙ МЕДИЦИНЫ
2021. Т. 20. № 4. 1–228
Подписной индекс 10650